Prevalence and Multidrug Resistance Profile of *Klebsiella Pneumoniae* Isolated from Poultry Farms in Abakaliki, Ebonyi State Nigeria

Ugbo E N[1], Ukpai E G[2], Ukhureigbe O M[3], Nwojiji E C[2], Ugbo A I[2], Ewah C M[4].

[1]Department of Applied Microbiology, Faculty of Science, Ebonyi State University, P.M.B. 053, Abakaliki, Ebonyi State, Nigeria.

[2]Department of Microbiology and Parasitology, Faculty of Allied Health Sciences, David Umahi Federal University of Health Sciences, P. M. B. 211 Uburu, Ebonyi, Nigeria.

[3]Department of Microbiology, Federal University, Oye Ekiti, Ekiti State, Nigeria.

[4]Department of Biochemistry, Faculty of Science, Ebonyi State University, P.M.B. 053, Abakaliki, Ebonyi State, Nigeria.

Corresponding author:
Dr. Ugbo, Emmanuel Nnabuike. Email: ugbonuel2001@yahoo.com
or emmanuel.ugbo@ebsu.edu.ng

Abstract

Background: *Klebsiella pneumoniae* is one of the prominent foodborne pathogens found in livestock causing infectious diseases such as pneumonia, septicaemia, and diarrhea. This study determined the prevalence of multidrug resistant *Klebsiella pneumoniae* in poultry farms in Abakaliki.

Methods: A total of 135 samples were collected from three poultry farms, 45 each from drinkers, chicken transport cages and faecal droppings within Abakaliki metropolis. The samples were analyzed bacteriologically using standard microbiological techniques for isolation and identification. Antibiotics susceptibility testing of *Klebsiella pneumoniae* was done against different classes of antibiotics using Kirby-Bauer disk diffusion method and Multiple Antibiotic Resistance Index was determined. Statistical analysis was done using Statistical Products and Service Solutions version 20.0.

Results: Out of 135 samples analyzed, the overall prevalence of *Klebsiella pnuemoniae* was 24 (17 .8%). However, drinkers harbored 6(13.3%), faecal droppings had 10(22.2%) and transport cages harbored 8(17.8%). *Klebsiella pnuemoniae* isolates showed moderate levels of resistance to tetracycline, ampicillin and erythromycin (16.7% to 33.3%). *Klebsiella pnuemoniae* isolates were 83.3% to 100% susceptible to ciprofloxacin and gentamicin. Multidrug resistant *Klebsiella pnuemoniae* isolates were detected; 6(25.0%). There was no statistically significant association between the areas of poultry tested and the occurrence of MDR *K. pneumoniae* ($p < 0.05$).

Conclusion: Multidrug resistant *Klebsiella pneumoniae* was reported in poultry farms as 25.0%. This high prevalence of MDR *K. pnuemoniae* in the poultry birds is probably due to unregulated use of antibiotics. We recommend stringent antibiotic administration policies for poultry birds and surveillance on the emergence of antimicrobial resistance in poultry farms.

Keywords: Prevalence, Multidrug resistance, *Klebsiella pnuemoniae,* Public health

Introduction

Klebsiella pneumoniae is one of the prominent zoonotic conditionally pathogenic microorganisms in the *Enterobacteriaceae* family.[1] Most of their members live in the intestines of poultry birds such as chickens and in humans, and have been widely studied due to their obvious impact on human and animal health as well as agricultural practices.[2,3] They are one of the important members of the *Enterobacteriaceae* family that cause food poisoning, *gastroenteritis,* enteric fever, pneumonia and plague.[4] *Klebsiella* species are found in the mucosa of the upper respiratory, gastrointestinal, and urogenital tracts of humans and animals, and cause *pneumonia*, nasal infection, and urinary tract infection in birds and biogenic infection in men. *Klebsiella pneumoniae* and *Klebsiella oxytoca* are opportunistic pathogens associated with severe nosocomial infections. *Klebsiella pneumoniae* multidrug resistance (MDR) is worrying, given the risk of food-borne pathogens spreading to humans through the food chain and the emergence of super-resistant bacteria.[5]

Livestock is important for generating revenue, offering job opportunities, maintaining food security, providing services, adding assets, social, cultural, and environmental values, and sustaining livelihoods.[6] Poultry is one of the world's livestock, with many people involved in the industry. About 71.6% of poultry farmers reside in developing countries, producing 67,718,544 metric tons of chicken meat and 57,861,747 metric tons of hen eggs every year.[7] The poultry industry provides a chance to feed the world's rapidly rising population while also providing revenue to resource-limited farmers. In many parts of the modern world, poultry is regarded as the primary source of not just inexpensive animal protein but also high-quality human food. The primary goal of chicken rearing in all production systems is egg and meat production for income creation and home consumption.[8]

Antibiotics are often utilized as prophylactics, growth promoters, and medications in poultry birds to maintain high production.[9] The widespread use of an antibiotics has contributed in triggering the rise of antibiotic resistance not only in humans but in animals.[10] It has been documented that chicken products and the overall environment contain antibiotic-resistant bacteria.[11] Consumption of microbial contaminated chicken carcasses or eggs may upset the normal balance of bacteria in the human digestive tract since these bacteria are resistant to antibiotics and are commonly found in the digestive systems of poultry birds.[12]

The increase in the prevalence of infections caused by MDR bacteria belonging to the *Enterobacteriaceae* group poses a great concern since these are common natural inhabitants of the microbiome.[13] There is a serious risk to international health because of the rise of antibiotic-resistant bacterial strains in the food chain.[4] Gram-negative bacteria have developed multidrug resistance by developing enzymes that can destroy or degrade antibiotics, efflux pumping, by-passing metabolic pathways, and by changing receptor sites for antimicrobial agents[14], which is an alarming situation in the field of human medicine and animal production.[15] However, current data is scarce on the status of multidrug resistance *Klebsiella pneumoniae* in poultry farms. Thus, the need to determine the prevalence and multidrug resistance profiles of *Klebsiella pneumoniae* in poultry farms at Abakaliki.

Methodology

The study area, Abakaliki in Ebonyi State Nigeria falls within longitude 6^0 45'N and latitude 8^0 65'E in the tropical rainforest of southeast agro-ecological zone. A cross sectional descriptive study was carried out on privately owned poultry farms in Abakaliki. The Cochran's formula $\{n = [Z^2 (pq)] / e^2\}$, was used to calculate the sample size and a total of 135 samples was obtained based on the prevalence reported by a previous study.[20] Three privately owned poultry farms were selected using simple random sampling technique in Abakaliki and fresh chicken faecal droppings and drinker and transport cage swabs of layer chickens from poultry farms were tested.

Sample collection

Three poultry farms of layer birds were selected from between April to June 2024 and 45 samples were collected from each poultry farm. In each farm, 15 samples each were taken from faecal droppings, transport cages and drinkers, totaling one hundred and thirty-five samples. The samples were taken aseptically using sterile swab stick by stabbing methods for faecal droppings and surface scrubbing for the drinker and transport cages and the samples were immersed into test tubes containing approximately 10 ml buffered peptone water. The test tubes were properly labeled and stored in an icebox. The samples were transported to the Department of Applied Microbiology laboratory of Ebonyi State University, Abakaliki within an hour of collection for bacteriological analysis.

Isolation and identification of *Klebsiella pneumoniae* organisms

The collected fresh chicken faecal droplets, drinkers, and transport cage swabs were inoculated into 5 mL of nutrient broth for pre-enrichment and incubated overnight. A sterile wire loop was used to collect a loopful of the overnight grown organisms for culturing purpose. The bacterial suspension on nutrient broth medium was then cultured on Eosin Methylene Blue agar (Sigma Aldrich, German) and MacConkey agar (Sigma Aldrich, German) media, and incubated aerobically for 24 hours at 37°C. The bacterial cultures were further purified using streak plate method onto the agar media. The sterilized inoculating wire loop was used to collect a distinct colony from the spread plate and the wire loop was dragged over the surface of another plate in a zigzag motion and incubated at 37°C to get pure culture and stored for further studies. The colonies of suspected *Klebsiella pneumoniae* isolates were further analyzed and confirmed using their phenotypic characteristics based on colony morphology, where the organisms were identified to be pink and hypermucoid on MacConkey agar and showed red purplish/mucoid on EMB agar. Gram staining and biochemical characteristics which include catalase test, carbohydrate fermentation (sucrose, mannitol, lactose, and glucose), IMVIC test (indole, methyl red, Voges-Proskauer, indole motility, and citrate), TSIA, and urease were used to confirm the isolates.[16,17]

Antimicrobial susceptibility testing

Antimicrobial susceptibility assay was conducted using the following antibiotics: ampicillin (AMP-10 µg); gentamicin (CN-500 µg), erythromycin (E-15 µg), ciprofloxacin (C-5 µg), and tetracycline (TE-30 µg) (Oxoid, UK) which are commonly used by poultry farms in the treatment of their birds. The antibiotic sensitivity of the *Klebsiella pneumoniae* isolates was determined using the Kirby-Bauer disc diffusion method. The Mueller Hinton Agar (MHA) (Oxoid, UK) was prepared according to the manufacturer's standard. The 0.5 McFarland standardized equivalent of the *Klebsiella pneumoniae* isolates was seeded onto Mueller Hinton agar using a sterile swab stick. Thus, five different antibiotic paper disks were carefully placed onto Mueller Hinton agar prepared in 90 mm Petri dishes. The antibiotic disks were placed at a distance of 30 mm away from each other and 15 mm measured from the edge of the plate. The plates with the antibiotic disk were allowed to stay 10 to 15 minutes before it was inverted and incubated at 37°C for 18 to 24 hours. After incubation, the zone of inhibition was determined. The caliper was used to measure the diameter of zone of inhibition and was interpreted as susceptible or resistant using CLSI guidelines.[18]

Ethical consideration

Oral consent was obtained from the poultry farm owners before sample collections.

Data analysis

Statistical analysis was performed using SPSS version 20.0 software. The frequencies of bacteria isolates from the farms were calculated and compared using one-way ANOVA. Results were considered statistically significant where p value was less than 0.05.

Results

The overall prevalence of *Klebsiella pneumoniae* isolates from the poultry farms was 24(17.8%). However, the prevalence was 6(13.3%) among drinkers, 10(22.2%) among faecal droppings and 8(17.8%) among transport cages (Table 1). The *Klebsiella pneumoniae* isolates showed high level of resistance to tetracycline and ampicillin ranging from 25.0% to 30.0% among the isolates from drinkers, faecal droppings and transport cage swabs. Average resistances were observed among the isolates to erythromycin. All the *Klebsiella pneumoniae* isolates reported in this study were 87.5% to 100% susceptible to ciprofloxacin and gentamicin (Table 2). Multiple antibiotic resistance was reported among the *Klebsiella pneumoniae* isolates; 6(25.0%) from the poultry birds within the farms at Abakaliki. The MDR *Klebsiella pneumoniae* isolates from transport cages had a prevalence of **25.0%, drinker had 16.1% and faecal droplets had 30.0% (Table 3).**

Table 1: Prevalence of *Klebsiella pneumoniae* isolates from poultry farms

Sample source	Numbers of samples Collected	Number positive for *K. pneumoniae*	Prevalence (%)
Transport Cage Swab			
Farm A	15	2	13.3
Farm B	15	4	26.7
Farm C	15	2	13.3
Total	45	8	17.8
Drinkers Swab			
Farm A	15	1	6.7
Farm B	15	3	20.0
Farm C	15	2	13.3
Total	45	6	13.3
Faecal Droppings			
Farm A	15	2	13.3
Farm B	15	5	33.3
Farm C	15	3	20.0
Total	45	10	22.2
Overall Total	135	24	17.8

Table 2 : Antimicrobial resistance profiles of *Klebsiella pneumoniae* isolates from poultry farms

Antibiotics	Transport Cage; n = 8		Drinker Swabs; n = 6		Faecal droppings; n= 10	
	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)
E	2 (25.0)	6 (75.0)	1 (16.7)	5 (83.3)	3 (30.0)	7 (70.0)
CIP	1 (12.5)	7 (87.5)	1 (16.7)	5 (83.3)	1 (10.0)	9 (90.0)
AMP	2 (25.0)	6 (75.0)	2 (33.3)	4 (66.7)	3 (30.0)	7 (70.0)
TE	2 (25.0)	6 (75.0)	2 (33.3)	4 (66.7)	3 (30.0)	7 (70.0)
CN	0 (0.0)	8 (100.0)	0 (0.0)	6 (100.0)	1 (10.0)	9 (90.0)

Key: E = Erythromycin, CIP =Ciprofloxacin, AMP = Ampicillin, CN = Gentamicin, TE = Tetracycline

Table 3: Multidrug resistance profile *Klebsiella pneumoniae* isolates from poultry farms

Sample source	Numbers of samples Collected	Number positive for *K. pneumoniae*	No of MDR *K. pneumoniae*	(%) prevalence
Transport Cage	45	8	2	25.0
Drinkers Swab	45	6	1	16.1
Faecal Dropings	45	10	3	30.0
Total	135	24	6	25.0

P-value = 0.47

Discussion

The current state of MDR bacterial conditions in the poultry farms in Abakaliki was studied in order to implement appropriate precautionary measures. *Klebsiella pneumoniae* was identified from the poultry faecal dropings and equipment used in poultry farms in Abakaliki. *Klebsiella pneumoniae* is an important pathogen that causes infection in the respiratory system of animals and humans.[16] Poultry is a highly significant food industry, accounting for approximately 90 billion tons of chicken meat produced annually worldwide.[4] For greater yield of poultry products, many countries rely on a diverse range of antimicrobials, some of which are considered essential in human medicine. Antimicrobial resistance *Klebsiella pneumoniae* was reported from this study. The antimicrobials used in animal production is contributing to the rise of antimicrobial resistance in both commensal and pathogenic microbes. Also, antimicrobial resistance characteristics of pathogenic bacteria are continuously changing every year. This alarming situation can lead to treatment failures, financial losses, and the potential transmission of resistant genes to humans. Moreover, the presence of antibiotic residues in poultry products such as meat, eggs, and other animal products raises concerns about human health.[19] The overall prevalence of *Klebsiella pnuemoniae* isolates from poultry farms was reported to be about one in every five. [20]A study identified presence of *Klebsiella pneumoniae* from chicken and is in agreement with the present study. The prevalence of *Klebsiella* species has previously been reported from chicken farms[21], although it was lower than that being reported from the present study. Another study reported prevalence of *Klebsiella pneumoniae* of two in every three from poultry farms.[22] A recent study identified *Klebsiella pneumoniae* isolates from poultry farms in closed-house system cages and open house system cages.[16]

The *Klebsiella pneumoniae* isolates were observed to show high level of resistance to tetracycline and ampicillin ranging from one in four to one in three among the isolates from the poultry farms. High levels of resistance to antibiotics such as tetracycline, cloxacillin, and erythromycin (Nigeria)[24], amoxycillin (Uganda)[25], and ampicillin (Ethiopia) had been reported in poultry.[20] However, there was a variation in the observation of the present study when compared to the reports by previous researches. All the *Klebsiella pneumoniae* isolates reported in this study showed a high level of susceptibility to gentamicin, ciprofloxacin and erythromycin. The sensitivity of *Klebsiella pnuemoniae* isolates to ciprofloxacin, chloramphenicol was high and the reported antibiotic resistance in *K. pneumoniae* isolated from poultry farms was also high.[16] *Klebsiella pnuemoniae* isolates resistance to antibiotics may change overtime to other conventional veterinary drugs used in the treatment of infections.

The high level of antibiotic resistance in poultry bird droppings could occur from the uncontrolled use of antibiotics as antibiotic growth promotors (AGP). Poultry feed that contains antibiotics, used in poultry farming is one of the main components that can contribute to antimicrobial resistance.[26] Thus, resistant strains in poultry birds could occur when the host is exposed to resistant strains in the environment.[27] Antibiotic resistance causes *K. pneumoniae* to be identified as an important disease in public health because of the difficulty in selecting therapeutic agents.[28] The potential risk of spreading bacteria from poultry farms birds, equipment and their products either through contact or via consumption of their products to humans and the emergence of multidrug resistance bacteria drew the world's attention to the health risks.[29]

Multidrug resistance *Klebsiella pnuemoniae* isolates were detected and the isolates had multidrug resistance (MDR) index below 0.2, while one-fourth had a MDR index of 0.47. According to previous studies [30, 31] organisms with MDR index above 0.2 potentially possess antibiotic resistance genes.

In conclusion, multidrug-resistant *K. pneumoniae isolates were reported from poultry farms in Abakaliki with the prevalence of 17.8% and the isolates were resistant to more than three different classes of antibiotics. Notably,* ciprofloxacin and gentamicin were reported to show great inhibitory effect on the multidrug resistance *Klebsiella pnuemoniae* isolates. This research has reported the current resistance profile of *Klebsiella pnuemoniae* isolates to the antibiotics commonly used in treatment of infection and growth promotion in poultry farms in Abakaliki. Therefore, the use of antibiotics as growth promoters and prophylaxis in poultry farms should be discouraged and blind treatment of diseases in birds with antibiotics without the recommendation of a veterinarian should be stopped.

References

1. Wang Y, Zhang R, Li J, Wu Z, Yin W, Schwarz, S. Comprehensive resistance analysis reveals the prevalence of NDM and MCR-1 in Chinese poultry production. *Nat Microbiol.* 2017; 2:16260-16263.
2. Octavia S, Sara J, Lan R. Characterization of a large novel phage-like plasmid in Salmonella enterica serovar Typhimurium. *FEMS Microbiol Lett.* 2015; 362 (8): fnv044 doi:10.1093/femsle/fnv044/2467663
3. Gutierrez A, De J, Schneider KR. Prevalence, Concentration, and Antimicrobial Resistance Profiles of *Salmonella* Isolated from Florida Poultry Litter. *Journal of Food Protection*. 2020; 83 (12): 2179–2186. doi: 10.4315/JFP-20-215

4. Agyare C, Boamah VE, Zumbi CN, Osei FB. Antibiotic use in poultry production and its effects on bacterial resistance. *Antimicrobial resistance A global threat*. 2018: 33-51.
5. Hartantyo SH, Chau ML, Koh TH, Yap M, Yi T, Cao DY et al Foodborne *Klebsiella pneumoniae*: virulence potential, antibiotic resistance, and risks to food safety. *J Food Prot*. 2020; 83(7):1096–103.
6. Behnke RH, Muthami D. The contribution of livestock to the Kenyan economy. IGAD LPI Working Paper. 2011; 03:11-16.
7. Hundie D, Goshu G, Tamir B, Duguma G. Assessment on rural poultry production and marketing system of Horro chicken ecotypes in Western Ethiopia. *J Agricultural Ext Rural Dev.* 2019; 11(12):248–259
8. Adegbeye MJ, Salem AZ, Reddy PR, Elghandour MM, Oyebamiji KJ. Waste recycling for the eco-friendly input use efficiency in agriculture and livestock feeding. *Resour Use Effi Agric.* 2020: 1–45.
9. Manyi-Loh C, Mamphweli S, Meyer E, Okoh A. Antibiotic use in agriculture and its consequential resistance in environmental sources: potential public health implications. *Molecules.* 2018; 23(4):795.
10. Hayati M, Indrawati A, Mayasari NLOI, Istiyaningsih I, Atikah N. Molecular detection of extended-spectrum β-lactamase-producing *Klebsiella pneumoniae* isolates of chicken origin from East Java, Indonesia. *Vet World.* 2019; 12 (4): 578.
11. Davis GS, Waits K, Nordstrom L, Grande H, Weaver B, Papp K et al. Antibiotic-resistant *Escherichia coli* from retail poultry meat with different antibiotic use claims. *BMC Microbiol.* 2018; 18:1–7.
12. Hedman HD, Vasco KA, Zhang L. A review of antimicrobial resistance in poultry farming within low-resource settings. *Animals.* 2020; 10(8):1264.
13. Navon-Venezia S, Kondratyeva K, Carattoli. K. *Klebsiella pneumoniae*: a major worldwide source and shuttle for antibiotic resistance. *FEMS Microbiol Rev,* 2017; 41 (3): 252-275.
14. Wu F, Ying Y, Yin M, Jiang Y, Wu C, Qian C et al Molecular characterization of a multidrug-resistant *Klebsiella pneumoniae* strain R46 isolated from a rabbit. *Intl J Genom.* 2019: 5459190. DOI: 10.1155/2019/5459190
15. Riwu KHP, Effendi MH, Rantam FA**.** A review of extended-spectrum β-Lactamase (ESBL) producing *Klebsiella pneumoniae* and Multidrug-Resistant (MDR) on companion animals. *Syst Rev Pharm.* 2020; 11 (7): 270-277.
16. **Darniati D, Kadir MDA, Rezfha D. Detection of multidrug-resistance *Klebsiella pneumoniae* isolated from oropharyngeal swab in broiler chickens.** *IOP Conf. Series: Earth and Environmental Science.* **2024;** 1297:012-050.
17. Ugbo EN, Effendi MH, Witaningrum AM, Tyasningsih W, Agumah BN, Ugbo AI et al Antimicrobial resistance pattern of *Salmonella* spp. isolated from poultry farms in Abakaliki, Nigeria. *Biodiversitas*. 2023; 24(9): 5207-5214.
18. Clinical Laboratory Standard Institute. Performance standard for antibiotics susceptibility testing. 11th information supplement NCCLS Documents M100-991 (ISBN 1-56238426-0) 2020; NCCL, Pennsyvania, USA.
19. Boamah, V, Agyare, C, Odoi, H, Dalsgaard, A. Practices and factors influencing the use of antibiotics in selected poultry farms in Ghana. *Journal of antimicrobial Agents.* 2016; 2(2):2-8.
20. Bushen A, Tekalign E, Abayneh M. Drug and Multidrug Resistance Pattern of Enterobacteriaceae Isolated from Droppings of Healthy Chickens on a Poultry Farm in Southwest Ethiopia. Infect. *Drug Resist.* 2021 14: 2051–2058.
21. Bamidele O, Yakubu A, Joseph EB, Amole TA, Antibiotic Resistance of Bacterial

Isolates from Smallholder Poultry Droppings in the Guinea Savanna Zone of Nigeria. *Antibiotic.* 2022; 11: 973. https://doi.org/10.3390/antibiotics11070973

22. Kahin MA, Mohamed AH, Mohomed AA, Mubarik AH, Haben G, Kebede AI. Occurrence, antibiotic resistance profiles and associated risk factors of *Klebsiella pneumoniae* in poultry farms in selected districts of Somalia Reginal State, Ethiopia. *BMC Microbiol.* 2024; 24:137.
23. Food and Agricultural Organization. Country STAT. Available online: http://nigeria.countrystat.org/search-and-visualize-data/en/ (accessed on 8 December 2021).
24. Ajayi KO, Omoya FO. Antibiotic Usage Pattern in Poultry and Resistance Pattern of Human Pathogenic Bacteria Isolated from Poultry Droppings in Akure, Nigeria. *Int. J. Biomed. Sci. Eng.* 2017; 5: 35–40.
25. Kakooza S, Muwonge A, Nabatta E, Eneku W, Ndoboli D, Wampande E et al Retrospective analysis of antimicrobial resistance in pathogenic *Escherichia coli* and *Salmonella* spp. isolates from poultry in Uganda. *Int. J. Vet. Sci. Med.* 2021; 9: 11–21.
26. Untari T, Herawati O, Anggita M, Asmara W, Wahyuni AE, Wibowo MH. The effect of Antibiotic Growth Promoters (AGP) on antibiotic resistance and the digestive system of Broiler Chicken in Sleman, Yogyakarta. *J InBIO Web of Confer.* 2021; 33: 40-50.
27. Kunhikannan S, Thomas CJ, Franks AE, Mahadevaiah S, Kumar S, Petrovski S. Environmental hotspots for antibiotic resistance genes. *J Microbiology Open.* 2021; 10: e1197.
28. Opoku-Temeng C, Kobayashi SD, DeLeo FR. *Klebsiella pneumoniae* capsule polysaccharide as a target for therapeutics and vaccines. *J Computational and Structural Biotech.* 2019; 17:1360–1366.
29. Pokharel S, Shrestha P, Adhikari B. Antimicrobial use in food animals and human health: time to implement One Health approach. *J Antimicrobial Resistance & Infection Contr.* 2020; 9: 1-5.