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PREVALENCE AND ANTIMICROBIAL RESISTANCE OF EXTENDED-SPECTRUM ß-LACTAMASE-PRODUCING *Klebsiella pneumoniae* IN SMALLHOLDER POULTRY FARMS IN ABEOKUTA, NIGERIA

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ABSTRACT

Food-producing animals like poultry have been identified as potential sources of extended-spectrum ß-lactamase (ESβL)-producing *Klebsiella pneumoniae*. This study aimed to investigate the occurrence and spread of ESβL-producing *Klebsiella pneumoniae* among ten (10) smallholder poultry farms in Abeokuta Metropolis, Ogun State, Nigeria. Antimicrobial-resistant profiles, phenotypic characterizations, and spread of chicken cloacal swab sample isolates were studied. Isolation and characterization of ESβL-producing *Klebsiella pneumoniae* were done using standard microbiological techniques. Standard antimicrobial discs, cefoxitin, vancomycin, tetracycline, ciprofloxacin, amoxiclav, ceftazidime, streptomycin, cefotaxime, trimethoprim, and gentamicin were used for resistant profiling using Kirby Bauer disc diffusion method. In total, one hundred (100) samples were collected, and overall, 22% *Klebsiella pneumoniae* and 45% of ESβL-producing *Klebsiella pneumoniae* isolates were recorded. Among these, ESβL-producing *Klebsiella pneumoniae* was isolated from Adigbe (50%), Onikolobo (33%), Ogun State television area (40%), Ajegunle (67%) and Somorin (25%). ESβL-producing *Klebsiella pneumoniae* was detected using a double disc synergy test, determined between cefotaxime (30 mg) disc alone and combined with clavulanic acid (30:10 mg) at a 15mm distance apart. Antimicrobial susceptibility test showed that among the antibiotics used, cefoxitin and amoxicillin-clavulanic acid (31.8 % resistance) were most effective against the *Klebsiella pneumoniae* isolates, while tetracycline (81.8%) and Cefoxtamine (63.6%) were the least effective antibiotics against the isolates. All ESβL-producing *Klebsiella pneumoniae* isolates recorded a multiple antibiotic-resistant index value of 0.5 to 0.8, thus further demonstrating that they are all multidrug-resistant. Findings have suggested the presence and spread of ESβL-producing *Klebsiella pneumoniae* between smallholder poultry farms and farm handlers in Abeokuta. These findings present a significant public health concern. Coordinated efforts that emphasize antimicrobial stewardship must be implemented to mitigate this problem.

Keywords: Microbial resistance; occurrence; ß-lactamase; *Klebsiella pneumonia;* poultry farming.

INTRODUCTION

Extended-spectrum beta-lactamases (ESβLs) are enzymes that are produced by certain types of bacteria and are capable of breaking down active ingredients in many common antibiotics. These enzymes are plasmid-mediated and hence are transmissible among bacterial species. They are known for their ability to hydrolyze beta-lactam antibiotics such as penicillins, cephalosporins and monobactams, resulting in antimicrobial therapy failure" (Akpaka *et al.*, 2020). *Klebsiella pneumoniae* is an *Enterobacteriaceae* member which often displays resistance towards β-lactam antibiotics, particularly through the β-lactamase expression, of which the most important are cephalosporinases, such as extended-spectrum β-lactamases (ESβLs) and carbapenemases.

Smallholder poultry farming encompasses households engaged in the rearing of poultry, typically including chickens, ducks, or other avian species, for meat and egg production, frequently employing family labour and utilizing locally available resources. Smallholder poultry farming is an integral part of livestock farming in the developing world, contributing multidimensionally to the livelihoods of both rural and urban households. Its contributions span economic, social, cultural, and environmental benefits (Birhanu *et al.*, 2022). It plays a major role in generating additional income for families to pay school fees, cover medical expenses, and buy agricultural inputs such as fertilizer and improved seeds (Birhanu *et al.*, 2021b). Smallholder poultry farming serves as the primary source of high-quality protein for households (Wong *et al.*, 2017). Poultry meat and eggs are crucial sources of protein and other essential nutrients. Poultry meat contains low fats and cholesterol, is rich in n-3 polyunsaturated fatty acids, and provides highly digestible protein, B-group vitamins (mainly thiamin, vitamin B6, and pantothenic acid), and various minerals such as iron, zinc, and copper (Bordoni and Danesi, 2017). Similarly, poultry eggs are rich in protein, lipids, vitamins, choline, minerals, and trace elements (Réhault-Godbert *et al.*, 2019). Accessibility to resource-poor households and multiple nutritional values make poultry products the most preferred animal source foods (ASFs) in the developing world (Birhanu *et al.*, 2023).

The China Bacteria Resistance Surveillance Network, in 2021, ranked the isolation rate of K. pneumoniae to be second after E coli, with a prevalence rate of 13.86%. The same report documented the isolation of K. pneumoniae in livestock and poultry farming (Yang *et al.*, 2021). The bacteria, *K pneumoniae*, cause infection with a high mortality rate and mainly cause mastitis in cows (Yang *et al.*, 2021) and respiratory symptoms in broiler chickens (Hamza *et al.*, 2016). In general, the isolation rate has been reported to be 9 to 35 per cent (Hamza *et al.*, 2016; Zhai *et al.*, 2020; Yang *et al.*, 2021).

The emergence of extended-spectrum beta-lactamase-producing *Enterobacteriaceae* and the increasing resistance of bacteria to broad-spectrum beta-lactam and peptide drugs in both human and veterinary medicine has attracted public attention (Liu *et al.*, 2021; Wang *et al.*, 2021; Li *et al.*, 2022). Even though the Smallholder poultry farming system contributes greatly to the economy of Nigeria, farmers of smallholder poultry farms lack proper guidance on the appropriate use of antibiotics. Yet, the irrational therapeutic and or prophylactic use of antibiotics is still common among smallholder poultry farmers. In Africa, for instance, the inappropriate use of clinical and veterinary antibiotics, which are often readily sold in shops and markets without prescriptions, is considered to be very high and a major contributor to the emergence of antibiotic resistance in this pre-antibiotic era (Mainda *et al*., 2015). Various studies have reported that inappropriate use of antimicrobial agents remains common among smallholder -operated poultry farms due to the lack of awareness and access to quality veterinary services. (Glasgow, 2019).

The bacterium, K. pneumoniae has been reported as among the most prominent zoonotic conditional pathogen in the *Enterobacteriaceae* family (Wang *et al*., 2021). It is also adjudged to be clinically significant in both humans and animals. *Klebsiella spp* are classified as contaminants of surface water, plants, soil, wastewater, and other environments. This, combined with their drug resistance, creates a risk of resistance genes being transferred to other microorganisms (especially genes conferring resistance to carbapenems), as well as a risk of their potentially causing superinfections and exacerbating primary infections in immunocompromised individuals (Rimoldi *et al*., 2017; Effah *et al*., 2020). *Klebsiella spp* often exhibits multiple resistance to chemotherapeutics. Additionally, the presence of genes coding for extended-spectrum β-lactamase (ESβL) in Klebsiella spp. renders ineffective the β-lactam antibiotics widely used to treat bacterial infections in poultry. A review of the available studies on Klebsiella spp. resistance indicates that the problem is global. Its multidrug resistance (MDR) is worrisome, given the risk of food-borne pathogens spreading to humans through the food chain and the emergence of super-resistant bacteria (Hartantyo *et al*., 2020).

Although *K pneumoniae* has been widely reported in hospital-acquired infections, the pathogen is equally reported to spread through foods and food-producing animals, hence often regarded as a foodborne pathogen (Guo *et al*., 2016). For example, K. pneumoniae has been isolated from foodstuffs like seafood, frozen food, and fresh chicken meat (Guo *et al*., 2016). K. pneumoniae is not only a pathogen to humans, but it is also a respiratory disease agent in chickens, which can cause production loss or even death. In research conducted at University of Mosul, Iraq, by Al-AAlim *et al*, 30% (15/50) specimens taken from chickens with respiratory disease contained K. pneumoniae (Al-AAlim *et al*., 2024). However, K. pneumoniae in livestock, especially in poultry, has not been thoroughly investigated in Abeokuta, as in other parts of Nigeria. Identifying and understanding the prevalence, distribution and characterization of ESβL-producing Klebsiella pneumonia in smallholder poultry farms is crucial for assessing the potential risk to human health. ESβL-producing K. pneumoniae were detected rarely in healthy broilers (Hiroi *et al*., 2012b; Yossapol *et al*., 2017; Mahanti *et al*., 2018). Hence, there is a paucity of reports on antibiotic resistance, especially on ESBL-producing K. pneumonia.

This study thus investigated the occurrence and spread of ESβL-producing *Klebsiella pneumoniae* among smallholder poultry farms in Abeokuta South Local Government. From available information, this study is the first to document the prevalence of ESβL-producing *Klebsiella pneumoniae* among smallholder poultry farms in Abeokuta. The data presented as the findings of this study will go a long way in developing the prevalence and characteristics of K. pneumoniae in Abeokuta.

MATERIALS AND METHODS

Sample Collection

A total of 100 Chicken cloacal swab samples were collected from various Smallholder poultry farms in Abeokuta metropolis Ogun, State, Nigeria between April and July 2023 (20 samples from Adigbe area, 16 samples from Onikolobo area, 20 samples from Ogun Television area, 20 samples from Ajegunle area and 24 samples from Somorin area. The samples were collected using a sterile swab stick, after obtaining ethical approval and informed consent from the College of Veterinary Medicine, FUNAAB and the farm owners, respectively. The samples were transported in an ice-pack, immediately to the Department of Microbiology, Federal University of Agriculture, Abeokuta, for isolation, identification, characterization and antimicrobial susceptibility testing of bacterial isolates using standard microbiological techniques.

Isolation and Characterization of Bacterial Isolates

All chicken cloacal swab samples were immediately streaked onto MacConkey agar and blood agar plates (Oxoid, UK) and incubated at 37°c for 24 hours. After 24 h incubation of the test organism, a single colony of bacteria isolate that appeared grey-white in blood agar, mucoid pink colonies on MacConkey agar, were identified to be Klebsiella pneumonia, which was further proven using Gram staining, microscopy and standard biochemical tests (Cheesebrough, 2006). Biochemical tests carried out included: citrate utilization test using Simmons citrate agar, urease test using modified Christensen's urea broths and glucose fermentation tests. The isolated colonies were purified by first streaking on a fresh MacConkey agar plate and incubated at 37°c for 24 hours. The pure isolated colonies were transferred to a 5 ml nutrient agar slant and stored in a refrigerator for further characterization.

Antimicrobial Susceptibility Test

This was done using the Kirby-Bauer disc diffusion technique on Mueller-Hinton agar according to the CLSI procedure (CLSI, 2014). A Mueller-Hinton agar plate was prepared according to the manufacturer's specifications. The Inoculum of the isolate was prepared by suspending 10 µg of pure colonies from a nutrient agar culture plate in a 5 ml normal saline and standardized by comparing with a 0.5 MacFarland turbidity standard. The standardized inoculum was then swabbed on the surface of the agar plate using a sterile swab stick. The plate was allowed 15 min for pre-diffusion to take place. After which, the following antibiotic discs were placed on the surface of the agar plate: Ceftazidime (30 µg/ml), Cefoxitin (30 µg/ml), Ciprofloxacin (25 µg/ml), gentamicin (10 µg/ml), streptomycin (10 µg/ml), Tetracycline (30µg/ml), Trimethoprim/sulfamethoxazole, Cefotaxime, Vancomycin and Amoxicillin-clavulanic acid (25µg/ml). They were incubated at 37°C for 24 h and radial zones of inhibition were taken. Diameters of the zones of inhibition for individual antibacterial agents were translated into susceptible and resistant categories, according to the EUCAST. Multiple drug-resistant (MDR) organisms were identified as resistant to three or more antibacterial classes.

Susceptibility Test with Beta-lactam Antibiotics

Mueller Hinton agar plates for the sensitivity test were prepared following the manufacturer's instructions, and the following antibiotics were placed on their surface: ceftazidime (30 µg), Cefotaxime (30 µg) and cefoxitin (30 µg). These were incubated at 37°C for 24 h, and the radial zones of inhibition were taken. Any organism resistant to 3rd generation cephalosporins (ceftazidime and cefotaxime) and sensitive to the 2nd generation cephalosporins (cefoxitin) used was subject to ESβL studies.

Phenotype Detection of ESβL using Double Disc Synergy Test (DDST)

This was carried out using three antibiotics, namely Amoxicillin-Clavulanic disc (20 µg), Cefotaxime (30 µg) and Ceftazidime (30 µg). A Mueller-Hinton agar plate (Oxoid, UK) was prepared following the manufacturer's specification an Amoxicillin-Clavulanic disc was placed at the centre of the Petri dish, and a cefotaxime and ceftazidime disc was placed 15 mm apart from the centre disc (amoxicillin-clavulanate disc (20 µg). The test organisms were considered ESβL producers (DDS test positive) if the zone size around the test antibiotic disc increases from 5 mm and above towards the Amoxicillin-Clavulanic disc after 24 h incubation at 37°C.

RESULTS

A total of 100 chicken cloacal swab samples were collected and 22 (22%) *Klebsiella pneumoniae* isolates were isolated (Table 1). The isolates were obtained from five different locations: Adigbe (4), Onikolobo (3), Ogun TV (5), Ajegunle (6), and Somorin (4). The DDST showed that out of 22 isolates of K. pneumoniae screened for extended-spectrum beta-lactamase production, 10 (45.5%) were positive. Adigbe had 2 (50%), Onikolobo had 1 (33%), Ogun TV had 2 (40%), Ajegunle had 4 (67%), and Somorin had 1 (25%) - Table 1.

All of the bacterial isolates, except for CSJ3 and CSS1, were found to be susceptible to gentamicin (Table 2). Out of the 22 K. pneumoniae isolates that were tested, 15 of them showed susceptibility to the Amoxicillin Clavulanic Acid (AUG) antibiotic. A test conducted on the 22 K. pneumoniae isolates revealed that Ceftazidime and Ciprofloxacin antibiotics were effective against 14 of the isolates. However, only three (3) and four (4) isolates showed susceptibility to Vancomycin and Tetracycline, respectively. The analysis showed that the isolates have a high resistance level against Vancomycin and Tetracycline antibiotics (Table 2).

The ESβL-producing K. pneumoniae isolates exhibited varying levels of resistance to antibiotics such as Gentamycin (0%), Vancomycin (90%), Tetracycline (80%), Ciprofloxacin (60%), Trimethoprim/Sulfamethoxazole (90%), Cefotaxime (80%), Cefoxitin (50%), Ceftazidime (50%), Streptomycin (60%), and Amoxicillin Clavulanic Acid had 50% (Figure 1).

The non-ESβL-producing K. pneumoniae isolates showed resistance to specific antibiotics such as Gentamycin 17%, Vancomycin 83%, Tetracycline 83%, Ciprofloxacin 25%, Trimethoprim/Sulfamethoxazole 50%, Cefotaxime 50%, Cefoxitin 33%, Ceftazidime 25 %, Streptomycin 58 %, and Amoxicillin Clavulanic Acid 17 % (Figure 2).

The K. pneumoniae isolates were found to be resistant to certain antibiotics. Specifically, Gentamycin showed a resistance rate of 32%, Vancomycin 86%, Tetracycline 82%, Ciprofloxacin 59%, Trimethoprim/Sulfamethoxazole 68%, Cefotaxime 64 %, Cefoxitin 41 %, Ceftazidime 36%, Streptomycin 59% and Amoxicillin Clavulanic Acid, with 32% (Fig. 3).

All of the *Klebsiella pneumoniae* bacteria that produced ESβL were found to be resistant to vancomycin, trimethoprim/ sulfamethoxazole, and tetracycline, except for the CSO6 isolate, which was found to be susceptible to trimethoprim/ sulfamethoxazole and tetracycline, and the CSG1 isolate, which was only susceptible to tetracycline (Table 2). The ESβL-producing *Klebsiella pneumoniae* bacteria also had a high multiple antibiotic resistance (MAR) index value. The highest MAR index value of 0.8 was recorded by the CSA7 isolate, while the CSA3 isolate had a MAR index value of 0.7. Other isolates had MAR index values ranging from 0.5 to 0.6 (Table 2).

Table 1: Distribution of ESβL-producing *Klebsiella pneumoniae* isolated from smallholder poultry farms in Abeokuta metropolis

Location	No. of Samples Collected	No of *K. pneumoniae* Isolated	ESβL Positive (%)	ESβL Negative (%)
Adigbe	20	4 (20)	2 (50)	2 (50)
Onikolobo	16	3 (19)	1 (33)	2 (67)
Ogun TV area	20	5 (25)	2 (40)	3 (60)
Ajegunle	20	6 (30)	4 (67)	2 (33)
Somorin	24	4 (17)	1 (25)	3 (75)
Total	100	22 (22 %)	10 (45 %)	12 (55 n%)

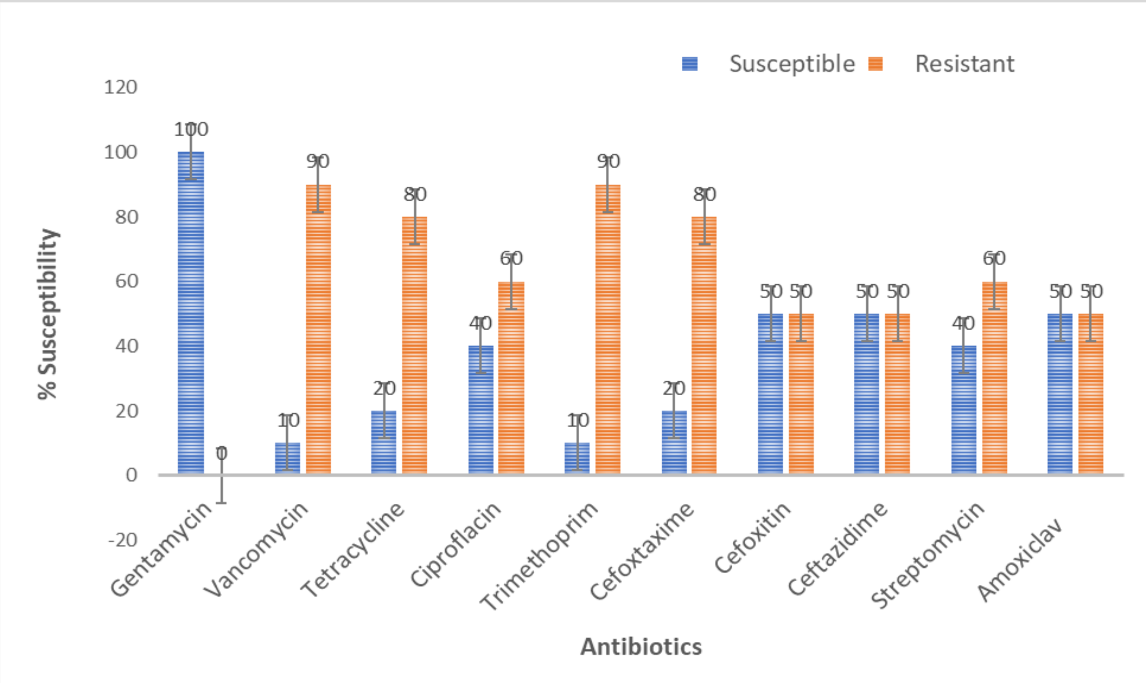

Figure 1: Percentage susceptibility and resistance patterns of ESβL-producing *K. pneumoniae* isolated from Smallholder poultry farms

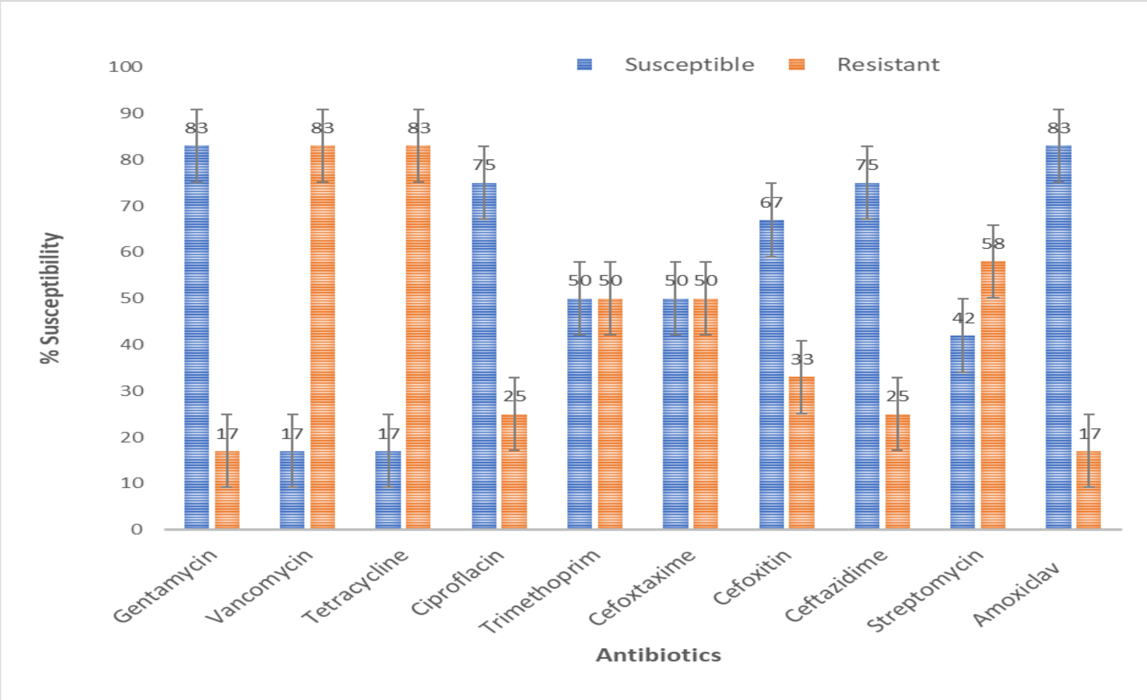

Figure 2: Percentage susceptibility and resistance patterns of non-Esβl-producing *K. pneumoniae* isolated from Smallholder poultry farms

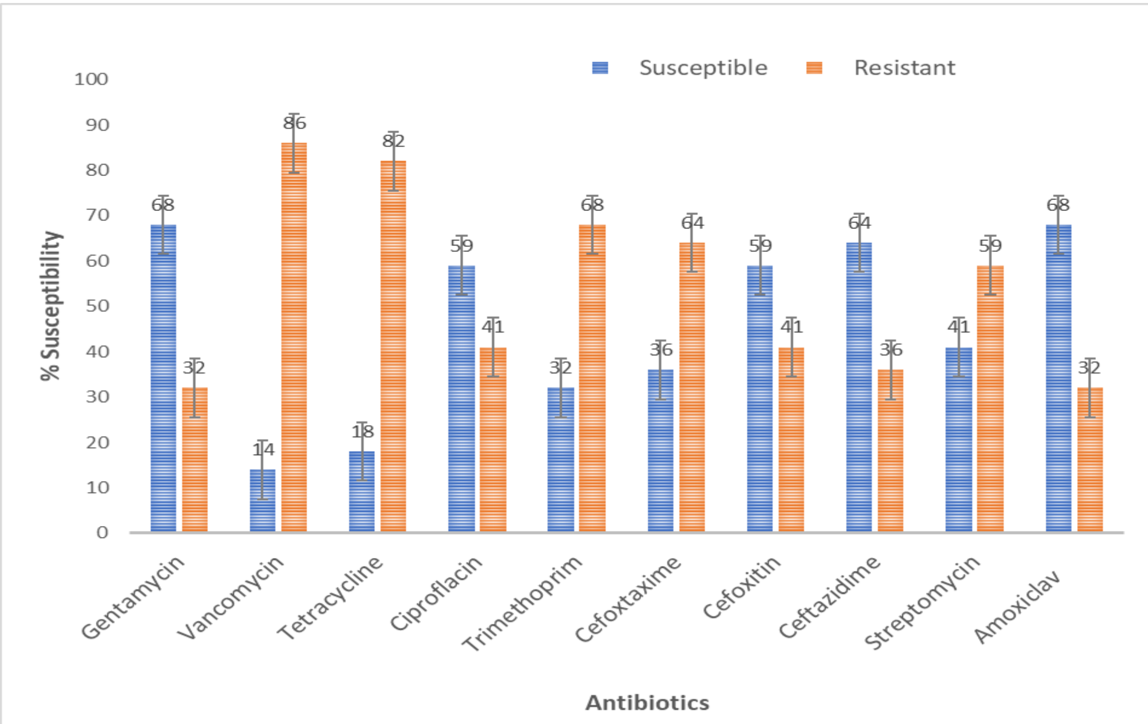

Figure 3: Percentage susceptibility and resistance patterns of *Klebsiella pneumoniae* isolated from smallholder poultry farms

Table 2: Antibiotic resistance profile and MAR index of ESβL-producing *Klebsiella pneumoniae* isolated from smallholder poultry farms

Isolates Code	Antibiotics	MAR Index
CSA 3	VAN, CTX, TET, SXT, FOX, CAZ, STM	0.7
CSA 5	CIP, VAN CTX, TET, SXT, FOX	0,6
CSA 7	VAN, CTX, TET, SXT, FOX, CAZ, STM, AUG	0.8
CSO 3	CIP, VAN, CTX, TET, SXT, STM	0.6
CSJ 6	CIP, VAN, TET, SXT, FOX, STM	0.6
CSJ 7	VAN, TET, SXT, CAZ, STM, AUG	0.6
CSO 6	CIP, VAN, CTX, FOX, CAZ, AUG	0.6
CSS 4	CIP, CTX, TET, SXT, STM, AUG	0.6
CSG 1	VAN, CTX, CIP, SXT, CAZ	0.5
CSG 6	VAN, CTX, TET, SXT, FOX	0.5

Key: CN = Gentamycin, CIP = Ciprofloxacin, VAN = Vancomycin, CTX = Cefotaxime, TET = Tetracycline, SXT = Trimethoprim/Sulfamethoxazole, FOX = Cefoxitin, CAZ = Ceftazidime, STM = Streptomycin, AUG = Amoxicillin Clavulanic Acid, R = Resistant and S = Susceptible.

DISCUSSION

The emergence of bacteria that produce extended-spectrum beta-lactamase (ESβL) is a significant threat because they can break down a wide range of β-lactam antibiotics, making them ineffective. K. pneumoniae is a zoonotic pathogen of great importance, and the high prevalence of ESβL-producing K. pneumoniae makes it one of the multi-drug-resistant (MDR) pathogens that pose a significant public health risk (Li *et al.*, 2022). A study conducted in the Netherlands has affirmed that antibiotic-resistant genes can be passed from poultry through the food chain to humans who are in close contact with them. Similar ESβL-producing isolates have been found in both poultry meat and humans who have close contact with them (Leverstein-van Hall *et al.*, 2011).

The result of the study has revealed that out of 22 (22%) *Klebsiella pneumoniae* isolates identified, 45.5 % were ESβL-positive and were spread across the study area. The total isolation rate of K. pneumoniae recorded in this study was higher than the 7.80 % recorded in a study conducted by Bhardwaj *et al* on a poultry farm in India in 2021 (Bhardwaj *et al.*, 2021). Also, the 45% ESβL prevalence rate recorded in this study is lower than that of a Shandong broiler slaughterhouse, where 96.67 % (87/90) ESβL-producing strains were isolated (Wu *et al.*, 2016), but lower than the 17.50 % ESβL prevalence recorded in Germany's separation rate of chicken meat in a study conducted by Eibach *et al.* in 2018. The reason for this frequency may be due to the widespread use of antibiotics in healthy animals for growth promotion, a practice that is now banned in some countries of Africa but still active in others (Adebowale *et al.*, 2016). The high prevalence of ESβL-producing K. pneumoniae found in poultry could lead to an increase in human carriage if the chicken meat's microbial flora is not properly inactivated.

All bacterial isolates, except for CSJ3 and CSS1, were susceptible to gentamicin, which was the most effective antibiotic. On the other hand, the analysis of our research showed that the isolates have a high resistance level against Vancomycin and Tetracycline antibiotics. The ESβL-producing K. pneumoniae isolates exhibited varying levels of resistance to antibiotics. This observation is not unexpected since Gentamicin is an aminoglycoside group of antibiotics that is important in treating bacterial infections in livestock and pets. Gentamicin is among the Office International des Epizooties (OIE), now World Organization for Animal Health (WOAH)-identified antibiotics to be of utmost importance in veterinary medicine. If those identified antibiotics become ineffective, it would require alternative antibiotics to be used. However, the use of alternatives such as fluoroquinolones and colistin is limited in animals, and they are considered to be extremely important for human medicine, according to the European Medicines Agency in 2016. The high resistance to tetracycline observed in this study may be due to the common use of this antibiotic as a feed additive and for prophylaxis in animal production in Nigeria, as pointed out by Olatoye (2010). Similarly, Usman *et al.* (2016) noted that the affordability and availability of tetracycline have led to resistance being a major problem in veterinary practice in Nigeria. The observed resistance to cefotaxime could be a result of the frequent use of cephalosporins in poultry in the study area, which has led to the indiscriminate use of vancomycin as well.

A high MAR index value that was recorded among ESβL-producing *Klebsiella pneumoniae* Isolates means that for every isolate in this research, the bacterial MAR index was >0.2, which is in agreement with the report of Subramani and Vignesh who revealed that all the isolates they used in their study had a very high MAR index value of >0.2 (Ogefere and Idoko, 2024). The statement suggests that the bacterial isolates might have been exposed to multiple antibiotics. The selective pressure exerted by the antibiotics used in managing bacterial infections could be the primary reason for the development of resistance, besides the bacteria acquiring the resistance gene through mutation or interspecies gene transmission (Poonia *et al.*, 2014; Ariom *et al.*, 2019). The ESβL-producing *Klebsiella pneumoniae* bacteria, in addition to having a high MAR index value, were resistant to more than three (3) different classes of antibiotics used in the study, thus making them multidrug-resistant (MDR). This MDR bacteria has the potential to spread from poultry to humans which is a big concern (Wang *et al.*, 2021). The high MAR index found in this study can be attributed to an excessive and/or improper use of antibiotics in the study area (Wang *et al.*, 2021; Yu *et al.*, 2022). The isolation of these MDR bacteria from both the poultry and handlers is an indication that they can easily spread to humans (Montso *et al.*, 2019).

Conclusion and recommendation

The study recorded a high prevalence of ESβL-producing *Klebsiella pneumoniae*, which spread across the study area with a multiple antibiotic-resistant index value of >0.2.

The prevalence rate of EsβL in smallholder poultry farms poses a new threat to human food safety. The spread of ESβL globally in both humans and animals can serve as a reference in efforts to prevent and treat ESβL bacterial infections. It is crucial to monitor and control this spread, or it will cause a serious problem in poultry farms and communities. Every laboratory should perform routine detection of ESβL-producing microbes using standard detection methods to control the spread of these infections and develop proper therapeutic strategies. Monitoring and judicious usage of extended-spectrum cephalosporins, periodic surveillance of antibiotic resistance patterns, and efforts to reduce empirical antibiotic therapy are recommended to address some of the problems associated with ESβLs in the community.

REFERENCES

Adebowale, O. O., Adeyemo, O. K., Awoyomi, O., Dada, R., Adebowale, O. 2016. Antibiotic use and practices in commercial poultry laying hens in Ogun State Nigeria. *Rev. Elev. Med. Vet. Pays Trop.* 69:41–45. doi: 10.19182/remvt.31170.

Akpaka, P. E., Vaillant, A., Wilson, C., & Jayaratne, P. 2020. Extended Spectrum Beta- Lactamase (ESβL) Produced by Gram-Negative Bacteria in Trinidad and Tobago. *International Journal of Microbiology*, 2021(1), 5582755. https://doi.org/10.1155/2021/5582755.

Al-AAlim, A. M., Al-Chalaby, A. Y., & Al -Abedi, S. F. 2024. Isolation and identification of *Klebsiella pneumoniae* from respiratory disease in chicken. *Iraqi Journal of Veterinary Sciences*, 38(3), 583-588. https://doi.org/10.33899/ijvs.2024.144877.3335

Ariom, T. O., Iroha, I. R., Moses, I. B., Iroha, C. S., Ude, U. I. and Kalu, A. C. 2019. Detection and Phenotypic Characterization of Methicillin-Resistant *Staphylococcus aureus* from Clinical and Community Samples in Abakaliki, Ebonyi State, Nigeria. *African Health Science.* 19(2); 2026-2035. Available online at https*://dx.doi.org/10.4314/ahs. v19i2.26*

Bhardwaj, K., Shenoy, M. S., Baliga, S. B., Baliga, B. S and Shetty, V. K. 2021. Research Note: Characterization of antibiotic-resistant phenotypes and linked genes of *Escherichia coli* and *Klebsiella pneumoniae* from healthy broiler chickens, Karnataka, India. *Poult. Sci.*;100

Birhanu, M. Y., Bruno, J.E., Alemayehu, T., Esatu, W., Geremew, K., Yemane, T., Kebede, F.G., and Dessie, T. 2022. Beyond diffusion to sustained adoption of innovation: a case of smallholder poultry development in sub-Saharan Africa. *International Journal of Agriculture and Sustainability.* In press:1–19.doi: 10.1080/14735903.2022.2041235

Birhanu, M. Y., Geremew, K., Esatu, W., Girma, T., Worku, S.F.G., and Dessie, T. 2021b. Economic and marketing performance of chicken value chain actors in Ethiopia: challenges and business opportunities for sustainable livelihoods. Nairobi, Kenya: *International Livestock Research Institute (ILRI).*

Birhanu, M. Y., Yeboah O. F., and Dessie, T. 2023. Smallholder poultry production in the context of increasing global food prices: Roles in poverty reduction and food security. *Animal Frontiers*, 13(1), 17-25. https://doi.org/10.1093/af/vfac069

Bordoni, A., and Danesi, F. 2017. Poultry meat nutritive value and human health. In: M. Petracci, and C. Berri, editors. *Food science, technology and nutrition, poultry quality evaluation.*

Woodhead Publishing, Sawston, Cambridge.

Cheesbrough, M. 2006. District Laboratory Practice in Tropical Countries, Part Two, 2nd Edition. Cambridge University Press, UK. Pp 134 – 180

Chenouf, N.S., Carvalho, I., Messaï, C.R., Ruiz-Ripa, L., Mama, O.M., Titouche, Y., Zitouni, A., Hakem A. and Torres, C. 2021. Extended-spectrum β-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* from Broiler Liver in the Centre of Algeria, with detection of CTX-M-55 and B2/ST131-CTX-M-15 in *Escherichia coli. Microb. Drug Resist; 27:268*–276.

Clinical and Laboratory Standards Institute (CLSI) 2014. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fourth Informational Supplement. CLSI Document M100-S24, *Wayne*, 34(1).

Effah, C.Y., Sun, T., Liu, S., Wu, Y. 2020. *Klebsiella pneumoniae*: An increasing threat to public health. *Ann. Clin. Microbiol. Antimicrob;* 19:1. Doi: 10.1186/s12941-019-0343-8.

Eibach, D., Dekker, D., Gyau Boahen, K., Wiafe Akenten C., Sarpong, N., Belmar Campos, C., Berneking, L., Aepfelbacher, M., Krumkamp, R., Owusu-Dabo, E., May, J. 2018. Extended-spectrum beta-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* in local and imported poultry meat in Ghana. *Vet Microbiology* 217:7–12.

European Medicines Agency. Reflection Paper on Use of Aminoglycosides in Animals in the European Union: Development of Resistance and Impact on Human and Animal Health. London (UK): *European Medicines Agency*; 2016.

Glasgow, L., Forde, M., Brown, D., Mahoney, C., Fletcher, S. and Rodrigo, S. 2019. Antimicrobial use in poultry production in Grenada *Vet Med Int.* 2:1-7 doi: 10.1155/2019/675519.

Guo, Z. H., Qin, L., Pang, Z., Qin, T., Ren, H. 2016. Frequency, antimicrobial resistance and genetic diversity of *Klebsiella pneumoniae* in food samples. *PLoS One*; 11(4): e0153561.

Hamza, E., and Dorgham, D. A. (2016) Carbapenemase-producing *Klebsiella pneumoniae* in broiler poultry farming in Egypt *J. Glob. Antimicrob.* Res., 7, pp. 8-10

Hartantyo, S.H.P., M.L. Chau, T.H. Koh, M. Yap, T. Yi, D.Y.H. Cao, R.A. Gut iÉrrez, L.C. 2020. Ng Foodborne *Klebsiella pneumoniae*: virulence potential, antibiotic resistance, and risks to food safety *J. Food Prot.*, 83:1096-1103.

Hiroi, M., Yamazaki, F., Harada, T., Takahashi, N., Iida, N., Noda, Y., Yagi, M., Nishio, T., Kanda, T., Kawamori, F., Sugiyama, K., Masuda, T., Hara-Kudo, Y., Ohashi, N. 2012b. Prevalence of extended-spectrum beta-lactamase-producing Escherichia coli and *Klebsiella pneumoniae* in food-producing animals. *Journal of Veterinary Medical Science*, 74.

Leverstein-van Hall, M.A., Dierikx, C. M., Cohen Stuart, J., Voets, G. M., van den Munckhof, M.P., van Essen-Zandbergen, A., Platteel, T., Fluit, A. C., van de Sande-Bruinsma, N., Scharinga, J., Bonten, M. J., Mevius, D.J. 2011. Dutch patients, retail chicken meat and poultry share the same ESβL genes, plasmids and strains. *Clin. Microbiol. Infect;* 17:873–880.

Li, Z., Peng, C., Zhang, G., Shen, Y., Zhang, Y., Liu, C., Liu, M. and Wang. F. 2022. Prevalence and characteristics of multidrug-resistant Proteus mirabilis from broiler farms in Shandong Province, China. *Poult Sci.* 101:101710

Li, Z., Xin, L., Peng, C., Liu, C., Wang, P., Yu, L., Liu, M and Wang, F. 2022. Prevalence and antimicrobial susceptibility profiles of ESβL-producing *Klebsiella pneumoniae* from broiler chicken farms in Shandong Province, China. *Poultry Science* 101:102002 https://doi.org/10.1016/j.psj.2022.102002

Liu, C., Feng, C., Liu, Y., Wang, P., Yu, L., Liu, M., Sun, S. and Wang, F. 2021. Distribution characteristics and potential risks of heavy metals and antimicrobial resistant *Escherichia coli* in dairy farm wastewater in Tai'an, China. Chemosphere 262:127768

Mahanti, A., Ghosh, P., Samanta, I., Joardar, S. N., Bandyopadhyay, S. and Bhattacharyya, D. 2018. Prevalence of CTX-M-Producing *Klebsiella sp*p. in broiler, kuroiler, and indigenous poultry in West Bengal State, *India. Microb. Drug Resist.* 24, 299–306. Doi: 10.1089/mdr.0096

Mainda, G., Bessell, P. R., Muma, J. B., McAteer, S. P., Chase-Topping, M. E., Gibbons, J. 2015. Prevalence and patterns of antimicrobial resistance among Escherichia coli isolated from Zambian dairy cattle across different production systems. *Sci. Rep.* 5:12439. doi: 10.1038/srep 12439

Montso, K. P., Dlamini, S. B., Kumar, A. and Ateba, C. N. 2019. Antimicrobial resistance factors of extended-spectrum beta-lactamases producing Escherichia coli and *Klebsiella pneumoniae* isolated from cattle farms and raw beef in north-west province, South Africa. *Hindawi Biomed Research International* 1:13

Ogefere, H. O and Idoko, M. O. 2024. Multiple Antibiotic Resistance Index of *Klebsiella pneumoniae* Isolated from Clinical Specimens in a Tertiary Hospital in Benin City, Nigeria. *Journal of Medicine and Biomedical Research*. 23(1) 5-11. https://dx.doi.org/10.4314/jmbr.v23i1.3

Olatoye, I. O. 2010. The incidence and antibiotics susceptibility of *Escherichia coli* O157:H7 from beef in Ibadan Municipal, Nigeria. *Afric. J. Biotechnol.*, 9 (8) 1196-1199.

Poonia, S., Singh, T. S., Tsering, D. C. 2014. Antibiotic susceptibility profile of bacteria isolated from natural sources of water from rural areas of East Sikkim. *Indian Journal of Community Medicine Official Publication of Indian Association of Preventive and Social Medicine.* 39(3):156–160.

Réhault-Godbert, S., Guyot, N, and Nys, Y. 2019. The golden egg: nutritional value, bioactivities, and emerging benefits for human health. *Nutrients.* 11:684. doi:10.3390/nu11030684

Rimoldi, S.G., Gentile, B., Pagani, C., Digregorio, A., Anselmo, A., Palozzi, A.M., Fortunato, A., Pittigliio, V., Ridolfo, A.L. and Gismondo, M. R. 2017. Whole genome sequencing for the molecular characterization of carbapenem resistant *Klebsiella pneumoniae* strains isolated at the Italian ASST Fatebenefratelli Sacco Hospital, 2012–2014. *BMC Infect. Dis*; 17:666.

Doi: 10.1186/s12879-017-2760-7

Samandoulougou, S., Ilboudo, A, J., Bagre, T. S., Tapsoba, F. W., Savadogo, A., Scippo, M. and Traore, A. S. 2015. Screening of antibiotics residues in beef consumed in Ouagadougou, Burkina Faso. *African Journal of Food Science* 9(6):367-371.

Smet, A., Martel, A., Persoons, D., Dew ulf, J., Heyndrickx, M., Catry, B., Herm an, L., Haesebrouck, F. and Butaye, P. 2008. Diversity of extended -spectrum beta-lactamases and class C beta-lactamases among Cloacal *Escherichia coli* Isolates in Belgian broiler farms. *Antimicrobial Agents and Chemotherapy* 52: 1238–1243.

Usman, R. Z., Mustapha, B. M. and Mohammed, F. I., 2016. Isolation and identification of Methicillin Resistant *Staphylococcus aureus* (MRSA) from traditionally fermented milk "nono" and yoghurt in Kaduna Metropolis, Nigeria. *Food Sci. Qual. Manag.* 55: 42-50.

Wang, W., Yu, L., Hao, W., Zhang, F., Jiang, M., Zhao, S. and Wang, F. 2021. Multi-locus Sequence Typing and Drug Resistance Analysis of Swine Origin Escherichia coli in Shandong of China and Its Potential Risk on Public Health. *Front Public Health.* 9:780700.

Wang, W., Yu, W., Hao, F., Zhang, M., Jiang, S. and Zhao, F. 2021. Wang Multi-Locus Sequence Typing and Drug Resistance Analysis of Swine Origin Escherichia coli in Shandong of China and Its Potential Risk on Public Health Front Public Health., 9, p. 780700

Wu, H., Wang, M., Liu, Y., Wang, X., Wang, Y., Lu, J. and Xu, H. 2016. Characterization of antimicrobial resistance in *Klebsiella* species isolated from chicken broilers. *Int. J. Food Microbiol*: 232:95–102.

Yang, Y., Peng, J., Jiang, Z., Gong, H., Z hu, K., Wang, Q., Zhou, Y., Tian, A., Qin, Z., Yang, S., Hang, S. 2021. Isolation and characterization of multidrug-resistant *Klebsiella pneumoniae* from raw cow milk in Jiangsu and Shandong provinces China Transbound. Emerge. Dis., 68:1033-1039.

Yossapol, M., Sugiyama, M. and Asai, T. 2017. The occurrence of CTX-M-25- producing *Enterobacteriaceae* in day-old broiler chicks in Japan. *J. Veter. Med. Sci.* 79, 1644–1647. Doi: 10.1292/jvms.17-0294.

Yu, L., Liu, Y., Liu, M., Li, Z., Li, L., Wang, F. 2022. Molecular characterization of antimicrobial resistance and virulence gene analysis of *Enterococcus faecalis* in poultry in Tai'an, China. *Poult Sci*; 101:101763.

Zhai, R., Fu, B., Shi, X., Sun, C., Liu, Z., Wang, S., Shen, Z., Walsh, T.R., Cai, C., Wang, Y. and Wu, C. 2020. Contaminated in-house environment contributes to the persistence and transmission of *NDM*-producing bacteria in a Chinese poultry farm *Environ. Int.*, 139, Article 105715

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