CHARACTERISING ANTIBIOTIC RESISTANCE OF BACTERIAL ISOLATES FROM SMALLHOLDER POULTRY DROPPINGS IN SELECTED LOCATIONS IN NASARAWA STATE, NIGERIA

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ABSTRACT

The misuse of antibiotics within poultry settings, which is globally recognized as a major health and food security burden is less emphasized in the smallholder poultry production systems (SPPS). The present study, therefore, aimed to investigate the antibiotic resistance patterns of bacterial species isolated from chickens raised under SPPS in the Guinea Savanna agro-ecological zone of Nigeria. A total of one hundred and twenty (120) fresh poultry droppings were aseptically collected randomly from two tropically adapted [Noiler (40) and FUNAAB

Alpha (40)] and Local chickens (40) of equal sexes in Gitta Mbasha and Karshi, Nasarawa State. The birds were subjected to two management conditions (use of antibiotics and ethnoveterinary medicine practice). Bacteria were isolated, identified, characterized and analyzed using standard protocols and appropriate statistical tools. Salmonella species (57.5%) and Escherichia coli (34.2%) were the most prevalent ($\chi^2 = 96.67$; $P<0.001$) in the study area. Prevalence of bacterial species was only significantly ($P <0.05$) influenced by antibiotics usage in poultry farms. All the four bacterial species isolated were multidrug resistant. FUNAAB alpha (58.0%) and Noiler (44.0%) were highly resistant to Quinolones, while their local counterparts (22.6%) exhibited more resistance to Aminoglycosides class of antibiotics. The clustering of bacterial species isolated with similar antibiogram revealed an evidence of inter–species dependence with possible animal and human health including environment risk implications. However, the present findings should be consolidated with further studies on genomics to better comprehend and exploit the genetic nature of antimicrobial resistance. Farmers should also exercise caution in the use of antibiotic drugs while adopting appropriate biosecurity measures and good hygiene.

Keywords: Bacteria, Antibiotics, Resistance, Poultry, Smallholder

INTRODUCTION

Smallholder farmers contribute significantly to the entire poultry value chain as over 80% of rural households, practise smallholder poultry ventures. This has a significant implication on household food patterns, consumption of animal-sourced foods, food chain, and food safety. Poultry production has been identified as a hot-spot for the development of antimicrobial resistance (AMR), and transfer of drug-resistant micro-organisms between food-producing animals and humans (Zalewska et al., 2021). The indiscriminate use of antibiotics, both for therapeutic and non-therapeutic purposes presents a public health threat to humans (Hedman et al., 2020). Village chickens are produced under scavenging, and semi-scavenging smallholder poultry production systems (SPPS) which have a lower risk of AMR. The lower risk of AMR can be attributed to the unique characteristics of the SPPS which relies on ethnoveterinary practices for therapeutic and non-therapeutic animal care purposes. However, the introduction of improved chicken genetics (FUNAAB Alpha, Noiler, Shika Brown, Sasso, Kuroiler) in Nigeria, as a developmental programme for improving SPPS (Bamidele et al*.,* 2020), has contributed to a decline in farmers' use of ethnoveterinary medicine and an increase in indiscriminate use of antibiotics. This intervention, though with positive developmental outcomes (food security, livelihoods) may have unintended public health-related consequences for the environment, animals and humans. Therefore, the objective of this study was to characterise the bacteriological profiles of poultry droppings in two farmer-preferred, chicken breeds (FUNAAB Alpha and Noiler), and identify their antibiotic resistance patterns relative to the local chickens raised under SPPS in selected locations in Nasarawa State, Nigeria.

MATERIALS AND METHODS

The samples were collected from smallholder poultry households located in two villages (Gitta Mbasha and Karshi) of Nasarawa State, Nigeria. The geographical location and agro-ecological features of Nasarawa State has been described by Bamidele and Amole (2021). The two villages were selected based on the practice of ethnoveterinary medicine (Gitta Mbasha) and administration of antibiotics (Karshi) to the flocks. At 21 weeks of age, a total of one hundred and twenty (120) fresh poultry droppings were aseptically collected using sterile spatulas from randomly selected apparently healthy chickens [10 cocks and 10 hens per breed (Noiler, FUNAAB Alpha and Locals) and per village (Gitta Mbasha and Karshi. The samples, which were placed into sterile universal sampling bottles, were kept in a mobile box containing ice packs and immediately transported to the Bafawat Biomedical Diagnostic Laboratory, Lafia, Nasarawa State for microbiological analyses. Bacteriological examinations were carried out in the Laboratory using standard procedures for aerobic bacteria while antibiotic susceptibility test of bacteria isolates was determined by the disk diffusion method. The prevalence of the bacterial species isolated was determined using Chi-square. A binomial logistic regression model was applied to determine the effects of independent factors on the odds of occurrence of bacterial species. Kruskal–Wallis H test was used to compare the antibiotic resistance rates of the different bacterial species isolated. IBM-SPSS and

R statistical packages were employed in the analysis. A dendrogram was drawn using PAST software to identify clusters of bacterial species isolated with similar antibiogram.

RESULTS

The proportion of the bacterial species in the study area indicated that *Salmonella* species was statistically ($\chi^2 = 96.67$; $P<0.001$) the most prevalent (57.5%), followed by *Escherichia coli* (34.2%), *Klebsiella* species (5.8%) and *Pseudomonas* species (2.5%). In the binomial logistic regression model, the risk of contracting bacterial species (*Salmonella* species and *Escherichia coli* only*)* through antibiotics usage was high (P = 0.025; Odds Ratio = 2.529; CI= 1.125-5.684). Interestingly, this effect was noticeable in *Escherichia coli* where its proportion in poultry farms where antibiotics were used was higher than in those that did not use antibiotics (65.9% vs. 23.3%). The Hosmer-Lemeshow test statistic indicated that the model fitted well ($\chi^2 = 7.328$; P = 0.502). All the four bacteria species showed medium resistance to Quinolones (Table 1). *Klebsiella* species was more resistant to Penicillins while *Pseudomonas* species was more resistant to Sulfonamides. While FUNAAB alpha were highly (P=0.003) resistant to Quinolones, their local counterparts exhibited more resistance to Aminoglycosides ((Table 2). There were no significant (P>0.05) sex and antibiotics usage effects (Tables 3 and 4).

The dendrogram obtained showed five clusters (Figure not shown). The pattern of the distribution revealed that there was an evidence of inter–species dependence concerning the resistance/susceptibility of the bacterial speciesto the different antibiotics.

DISCUSSION

The higher prevalence of *Salmonella* species and *Escherichia coli* could be an indication of their better adaptability and fitness in the adverse environments of the low-input poultry farmers of the present study area. Their endemic nature may equally be attributed to lack of or ineffective measures on the prevention and control of infections, poor husbandry, hygienic and biosecurity practices on the part of the farmers. Our present prevalent rates are comparable to the values reported for *Escherichia coli* (39.0%) in Ethiopia (Bushen et al., 2021) and *Salmonella* species (59.5%) in Malaysia (Osman et al., 2021). In the binary regression, *Escherichia coli* was more pronounced in the farms where antibiotics were used contrary to expectation. The possible reasons that could be adduced for the higher number of *Escherichia coli* could be its potential antibiotic resistance capability, and other possible management and environmental sources of bacterial contamination (WHO, 2021). Medium to low antibiotic resistance could be due to the classes and types of the drugs tested in this study. There is the tendency that the pattern of *Salmonella* species, *Escherichia coli, Klebsiella* species and *Pseudomonas* species resistance to antibiotics may change if other conventional veterinary drugs are tested. FUNAAB alpha and Noiler chickens were more highly resistant to Quinolones compared to their local counterparts. Although the use of antibiotics is being globally discouraged (Bean-Hodgins and Kiarie, 2021), where its moderate use becomes necessary either for prevention or treatment, Quinolones appear not to be the best for FUNAAB alpha and Noiler chickens. The therapeutic efficacy of such drugs might be compromised in the birds as a result of their antimicrobial resistance level. On the other hands, it is most likely that drugs of the Aminoglycosides class might not be suitable for the local chicken in the study area. The pattern of clustering indicates co-existence of the bacteria under similar environmental pressures within the poultry production system which could lead to inter-species genomic exchange that can constitute environmental hazards, thereby posing greater risk to animal and human health (Aworh et al., 2021).

Table 1. Antibiotic resistance (number, %) pattern of the bacterial species

Parameters	Bacterial Species				Kruskal-Wallis test	P-value
	Salmonella N= 91	*Pseudomonas* N= 2	*Klebsiella* N= 9	*Escherichia coli* N= 51		
Antibiotic Class						
Quinolones	37 (40.7)	1 (50.0)	3 (33.3)	24 (47.1)	0.886	0.829
β-lactams	8 (8.8)	0 (0.0)	1 (11.1)	7 (13.7)		
Penicillins	8 (8.8)	0 (0.0)	3 (33.3)	4 (7.8)		
Aminoglycosides	18 (19.8)	0 (0.0)	0 (0.0)	5 (9.8)		

Sulfonamides	11 (12.1)	1 (50.0)	1 (11.1)	7 (13.7)
Cephalosporins	9 (9.9)	0 (0.0)	1(11.1)	4 (7.8)

Table 2. Antibiotic resistance (number, %) pattern based on chicken genotype

	Chicken genotype			Kruskal-Wallis test	P-value
	Local N= 53	FUNAAB alpha N= 50	Noiler N= 50		
Antibiotic Class					
Quinolones	14 (26.4)	29 (58.0)	22 (44.0)	11.817	0.003
β-lactams	5 (9.4)	7 (14.0)	4 (8.0)		
Penicillins	6 (11.3)	3 (6.0)	6 (12.0)		
Aminoglycosides	12 (22.6)	3 (6.0)	8 (16.0)		
Sulfonamides	8 (15.1)	4 (8.0)	8 (16.0)		
Cephalosporins	8 (15.1)	4 (8.0)	2 (4.0)		

Table 3. Antibiotic resistance (number, %) pattern based on sex of chicken

	Sex		Kruskal-Wallis test	P-value
	Male N= 73	Female N= 80		
Antibiotic Class				
Quinolones	28 (38.4)	37 (46.3)	0.566	0.452
β-lactams	7 (9.6)	9 (11.3)		
Penicillins	11 (15.1)	4 (5.0)		
Aminoglycosides	10 (13.7)	13 (16.3)		
Sulfonamides	10 (13.7)	10 (12.5)		
Cephalosporins	7 (9.6)	7 (8.8)		

Table 4. Antibiotic resistance (number, %) pattern based on antibiotics usage by farmers

	Antibiotics usage		Kruskal-Wallis test	P-value
	No Antibiotics N= 73	Antibiotics N= 80		
Antibiotic Class				
Quinolones	28 (38.4)	37 (46.3)	0.668	0.414
β-lactams	7 (9.6)	9 (11.3)		
Penicillins	11 (15.1)	4 (5.0)		
Aminoglycosides	10 (13.7)	13 (16.3)		
Sulfonamides	9 (12.3)	11 (13.8)		
Cephalosporins	8 (11.0)	6 (7.5)		

CONCLUSION

Salmonella species and *Escherichia coli* were the most prevalent bacteria in the study area. Prevalence was influenced by the use of antibiotics in the poultry farms. Multidrug resistance of the bacteria was observed. While FUNAAB alpha and Noiler chickens were highly resistant to Quinolones, their local counterparts exhibited more resistance to Aminoglycosides. The pattern of clustering to a variety of antibiotics revealed the co-existence of the four bacterial species isolated with possible animal and human health implications as well as environmental risks. However, further molecular research is advocated to track the role of inter-species gene transfer in antibiotic resistance. Farmers should also adopt the best practices to stem the tide of contributing factors to antimicrobial resistance in the ecosystem.

ACKNOWLEDGMENTS: This research received funding from CGIAR Research Program for Agriculture for Nutrition and Health (A4NH) as part of the CGIAR COVID-19 Hub project in Nigeria.

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POST-EXPOSURE *PESTE DES PETITS RUMINANTS* VACCINATION WITH CONCURRENT ANTIBACTERIAL THERAPY INFLUENCES THE DISEASE OUTCOMES FOLLOWING NATURAL EXPOSURE TO *PESTE DES PETITS RUMINANTS* VIRUS IN GOATS.

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ABSTRACT