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EVALUATION AND PREVALENCE OF SOME BACTERIA ISOLATED FROM POULTRY ENVIRONMENT IN ABRAKA, NIGERIA

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ABSTRACT

Poultry are birds domesticated by man such as domestic fowl, duck, geese, turkey and guinea fowl. They are among the most intensively reared of all livestock species worldwide, serving as an important source of animal protein globally. The study aimed to investigate the prevalence of bacterial isolates present in poultry environments in Abraka, Nigeria. A total of 102 samples were obtained (in triplicates) from the air, feeds, litters, floors, clothing and hands of farm workers. Two poultry farms A (Center for Entrepreneurship Studies Poultry Farm, behind digital examination center, Site 3, DELSU, Abraka) and B (University Farm, Site 1, Delta State University, Abraka) were used to carry out investigations for this study. Samples were examined using standard bacteriological culture techniques. The settle plate technique was employed for collection of the air sample. The overall prevalence of the bacterial spectrum recovered from this study was 99% and included *Escherichia coli* (21%), *Staphylococcus aureus* (18%), *Klebsiella pneumoniae* (16%), *Proteus mirabilis* (14%), *Proteus terrae* (12%), *Streptococcus pnuemoniae* (7%), *Klebsiella oxytoca* (5%), Campylobacter spp. (3%), *Enterobacter roggenkampii* (2%), and *Enterobacter hormaechei* (1%) in decreasing order. Invariably, both poultry farms contributed to the bacterial diversity encountered in this study. Frequencies of isolation from sample sources were: Farm A: Litter (25), Clothing (24), Hands (22), Floor (18), Air (15), Feed (5); Farm B: Air (8), Litter (7), Clothing (6), Hands (5), Floor (5), Feed (5). The presence of these organisms in the poultry environment is indicative of possible health risks to poultry and poultry workers.

Key words: Poultry, Bacterial Isolates, Prevalence, Sample sources.

Introduction

Poultry are birds domesticated by man, such as domestic fowl, duck, geese, turkey, guinea fowl, pigeons and ostriches (Barakat, 2004). It is among the most intensively reared (especially in developing countries) of all livestock species and is a significant contributor to global food supply. It is versatile and rich in protein which makes them the most consumed meats. Poultry is an important aspect of the global economy yet poses a significant threat to both animals and humans, due to risk of bacterial contamination/transmission, being a major vector (Okogun *et al.*, 2016). Previous studies have shown Salmonella species to be one of the most important foodborne pathogens associated with poultry, spreading mostly through the usage/consumption of their infected products such as chicken and eggs (Schjørring and Krogfelt, 2011). It presents a clinically identifiable syndrome in humans such as acute fever, diarrhea, and sometimes vomiting and nausea.

Poultry remains one of the most efficient forms of livestock management with regards to natural resource use and protein gain, yet poses a significant threat to human health and the environment. De Vries and De Boer (2010); Gržinić *et al*. (2022). Thus, diseases of poultry are a major concern as they put the food and health sector on the edge. Being aware that poultry meat and eggs represents one of the most important food items globally especially in Nigeria which cuts across all barriers of religions, race and age class all over the world (United Nations, 2019). This study is therefore hinged on determining the bacteriological assessment and the prevalence of antimicrobial resistance genes in poultry farms in Abraka environment, Nigeria.

Materials and Methods

Area of Study

This study was carried out on two poultry farms, Center for Entrepreneurship Studies Poultry Farm, behind digital examination center, Site 3, DELSU, Abraka (A) and University Farm, Site 1, Delta State University, Abraka (B). Abraka lies on a longitude 5.79170N and latitude 6.09870E, located in the South-South geopolitical zone of Nigeria (Figure 1).

Figure 1: Map of Delta State Nigeria showing location of study area
Source: Irwin and Oghenevwede (2014).

Collection of Samples

A total of 102 samples were randomly collected from the air, floors, litters, feeds, hands and clothing of the farm workers. All samples were collected in triplicates. Samples were collected from the floors and hands of the workers using sterile swabs, after being dipped in sterile peptone water. Sterile universal containers were used to collect samples from the feeds and litters while the sweep plate technique was used to obtain samples from the workers' clothing. For sampling the air, the "Settle Plate Technique" was used where, the lids of sterile Blood Agar (BA) and MacConkey Agar (MCA) plates (properly placed on bench tops), were removed and exposed to air within the poultry environment for 30 minutes (Napoli *et al*., 2012). Thereafter, the lids were replaced. All samples were accurately labelled and sent to Microbiology Laboratory of the Department of Microbiology, DELSU, Abraka for analysis. Samples not analysed within 4-6 hours after collection were discarded.

Sample Analysis

i. Microbiological analysis

The following media were prepared and used:- Blood agar, MacConkey agar, Nutrient agar, Chocolate agar, lauryl Britany broth, Christensen Urea broth, Citrate medium and peptone water. All media was prepared according to the manufacturer's guide.

ii. Culture method

All samples were inoculated on blood agar, chocolate agar and MacConkey agar. The inoculated plates were incubated at 37ºC for 24 hours. Plates with mixed cultures were sub-cultured to obtain pure colonies of bacteria. Morphological and biochemical characteristics of the discreet colonies were determined in accordance with standard laboratory practices. The tests performed include Gram staining, motility, catalase, oxidase, indole, methyl-red, Voges Proskauer, citrate utilization, urease, coagulase, hemolysis and sugar fermentation (glucose, lactose and mannitol).

Biochemical Identification of Bacterial Isolates

The isolates were characterized and identified based on colony morphology, cultural appearance and biochemical tests (Cowan and Steel, 1974; Cruishank *et al*., 1975; Sanders, 1994).

STATISTICAL ANALYSIS

Data collected were entered in Microsoft Excel, presented in tables, percentages, bar charts and proportions (in pie charts). Data were imported for analysis in IBM Statistical Package for the Social Sciences, SPSS version 25. The Mann-Whitney U test was performed and the median values were determined to compare the bacterial prevalence between farms A and B. For all tests, a P-value of ≤ 0.05 was considered significant.

RESULTS

Two (2) poultry farms A and B were evaluated and a total of 102 samples were collected from sample source and examined (Table 1). Organisms were isolated and identified based on their morphological, microbiological/biochemical characteristics and a total of 145 isolates were recorded (Table 2). Of the 145 bacterial isolates encountered, 109 was from poultry A and 36 from poultry B. The identified isolates from the sampled sources in poultry A, were Escherichia coli and *Proteus mirabilis* (19.30%), followed by *Klebsiella pneumoniae* (17.40%), *Staphylococcus aureus* (14.70%), *Proteus terrae* (11%), *Streptococcus pnuemoniae* (9.20%), Campylobacter species (4.60%), *Klebsiella oxytoca* (3.70%) and *Enterobacter hormaechei* (0.90%) by decreasing order of prevalence (Table 3). From poultry B, the identified bacterial isolates from the sampled sources in decreasing order of prevalence (Table 4) were *Escherichia coli* and *Staphylococcus aureus* (27.80%), *Proteus terrae* (16.70%), *Klebsiella pneumoniae* (11.10%), followed by *Klebsiella oxytoca* and *Enterobacter roggenkampii* (8.30%).

Table 1: Total number of samples collected from Poultries A and B

POULTRY/ LOCATION	AIR	FLOOR	LITTER	FEED	WORKER'S HAND	WORKER'S CLOTHING	TOTAL SAMPLE COLLECTED
A	9	9	9	9	9	9	**54**
B	9	9	9	9	6	6	**48**
SUM TOTAL	**18**	**18**	**18**	**18**	**15**	**15**	**102**

Table 2: ***Morphological and biochemical characteristics of bacteria isolated from Poultries A & B***

S/N	Cellular Morphology	Gram Reaction	Catalase	Motility	Oxidase	Indole	Methyl-Red	Voges Proskauer	Citrate	Urease	Coagulase	Hemolysis	Glucose	Lactose	Mannitol	Number of Isolates Showing	Most Probable Organism
1	Spiral rod	−	+	+	+	−	−	NA	+	+	−	+	−	−	−	5	*Campylobacter* species
2	Rod	−	+	+	−	−	v	+	+	+	−	−	+	−	+	1	*Enterobacter hormaechei*
3	Rod	−	+	+	−	−	−	+	+	+	−	+	+	+	+	3	*Enterobacter roggenkampii*
4	Rod	−	+	+	−	+	+	−	−	−	−	v	+	+	+	31	*Escherichia coli*
5	Rod	−	+	−	−	+	−	+	+	+	−	−	+	+	+	7	*Klebsiella oxytoca*
6	Rod	−	+	−	−	−	−	+	+	+	−	−	+	+	−	23	*Klebsiella pneumoniae*
7	Rod	−	+	+	−	−	+	−	+	+	−	−	+	−	−	21	*Proteus mirabilis*
8	Rod	−	+	+	−	−	+	−	+	+	−	−	+	−	−	18	*Proteus terrae*
9	Cocci	+	+	−	−	−	+	+	−	+	+	+ (β)	+	+	+	26	*Staphylococcus aureus*
10	Diplococci	+	−	−	−	−	−	−	−	−	−	+ (α)	+	+	−	10	*Streptococcus pnuemoniae*
	Total Number of Gram +ve Isolates															**36**	
	Total Number of Gram -ve Isolates															**109***	
		Total Number of Isolates														**145**	

Key Notations: NA =Not Available, + = Positive, - = Negative, v = Variable, β = Beta Hemolysis, α = Alpha Hemolysis.

TABLE 3: PREVALENCE OF BACTERIAL ISOLATES FROM SAMPLE SOURCE IN POULTRY A

Source	**No of Samples**	***Campylobacter species***	***Enterobacter hormaechei***	***Escherichia coli***	***Klebsiella oxytoca***	***Klebsiella pneumoniae***	***Proteus mirabilis***	***Proteus terrae***	***Staphylococcus aureus***	***Streptococcus pneumoniae***	**% Cumulative of Isolates**
Air	9	−	−	−	−	2(13.3%)	5(33.3%)	3(20.0%)	3(20.0%)	2(13.3%)	**13.80%**
Clothing	9	−	−	7(29.2%)	3(12.5%)	3(12.5%)	3(12.5%)	1(4.2%)	5(20.8%)	2(8.3%)	**22.00%**
Feed	9	−	−	−	−	1(20.0%)	2(40.0%)	−	2(40.0%)	−	**4.60%**
Floor	9	2(11.1%)	−	5(27.8%)	1(5.6%)	3(16.7%)	3(16.7%)	3(16.7%)	−	1(5.6%)	**16.50%**
Hand	9	−	1(4.5%)	3(13.6%)	−	4(18.2%)	3(13.6%)	−	6(27.3%)	5(22.7%)	**20.20%**
Litter	9	3(12.0%)	−	6(24.0%)	−	6(24.0%)	5(20.0%)	5(20.0%)	−	−	**22.90%**
Total	**54**	5(4.6%)	1(0.9%)	21(19.3%)	4(3.7%)	19(17.4%)	21(19.3%)	12(11.0%)	16(14.7%)	10(9.2%)	

TABLE 4: PREVALENCE OF BACTERIAL ISOLATES FROM SAMPLE SOURCE IN POULTRY

Source	**No of Samples**	***Enterobacter roggenkampii***	***Escherichia coli***	***Klebsiella oxytoca***	***Klebsiella pneumoniae***	***Proteus terrae***	***Staphylococcus aureus***
Air	9	3(37.5%)	–	–	–	3(37.5%)	2(25.0%)
Clothing	6	–	3(50.0%)	2(33.3%)	–	–	1(16.7%)
Feed	9	–	–	1(20.0%)	–	1(20.0%)	3(60.0%)
Floor	9	–	3(60.0%)	–	–	–	2(40.0%)
Hand	6	–	1(20.0%)	–	1(20.0%)	1(20.0%)	2(40.0%)
Litter	9	–	3(42.9%)	–	3(42.9%)	1(14.3%)	–
Total	**48**	3(8.3%)	10(27.8%)	3(8.3%)	4(11.1%)	6(16.7%)	10(27.8%)

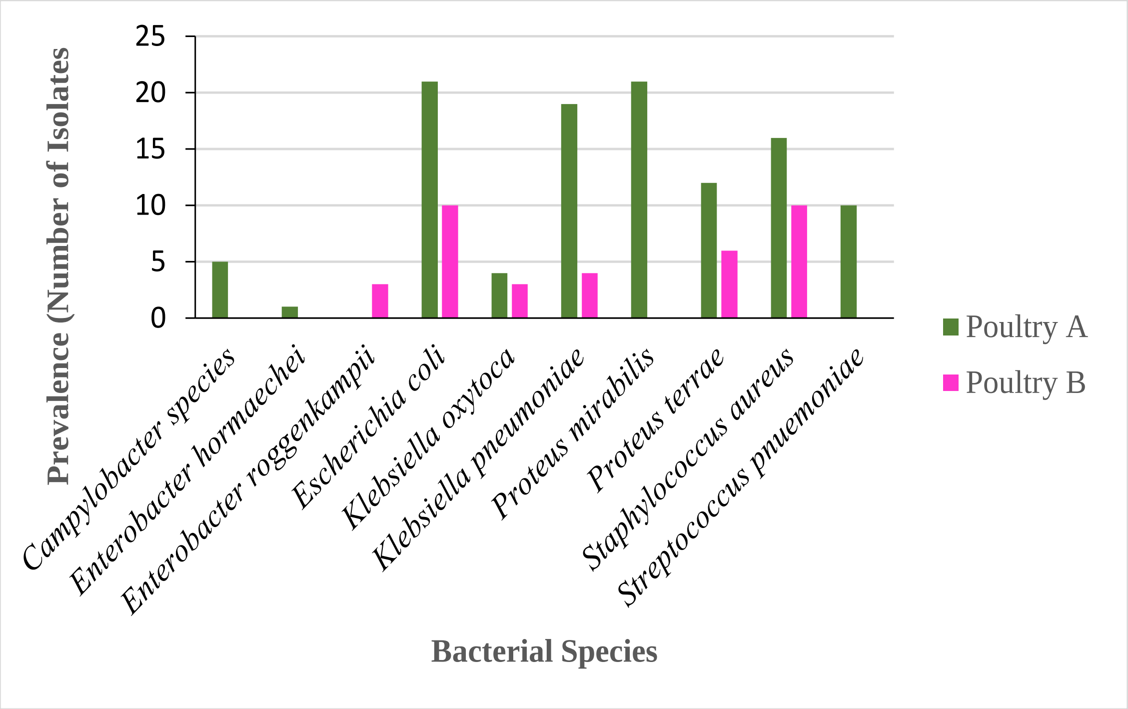

FIGURE 2: Prevalence of bacterial Species between Poultry A and Poultry B

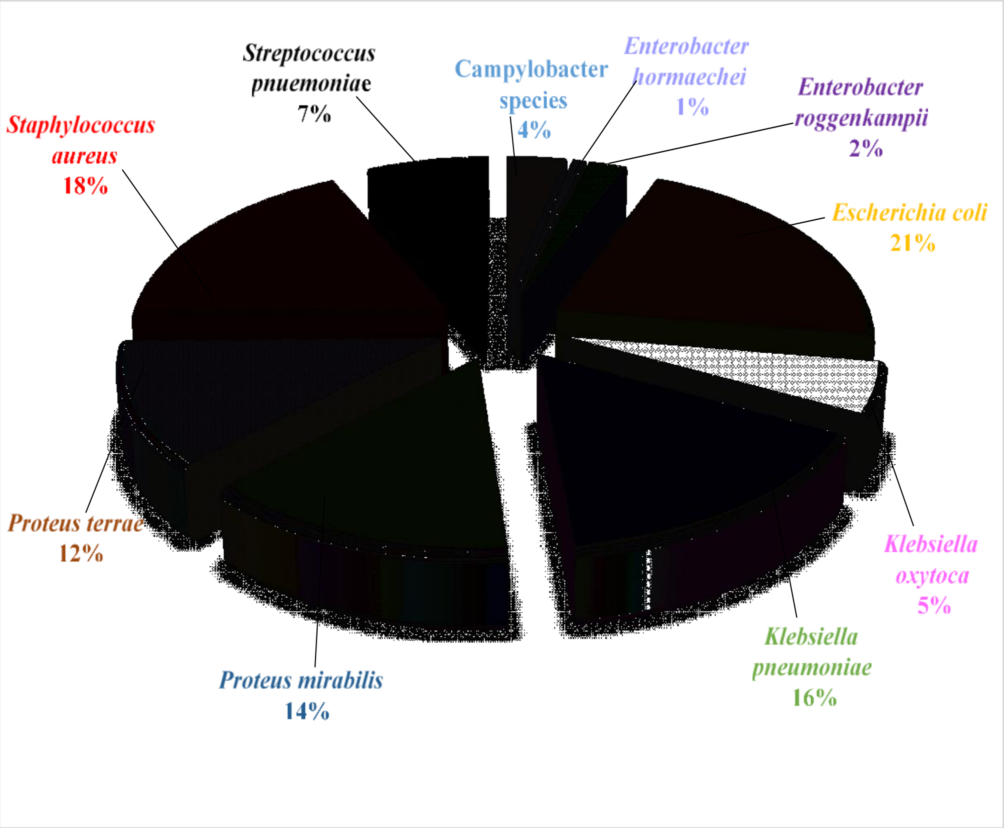

Figure 3: Overall prevalence of bacterial Isolates in Poultries A and B

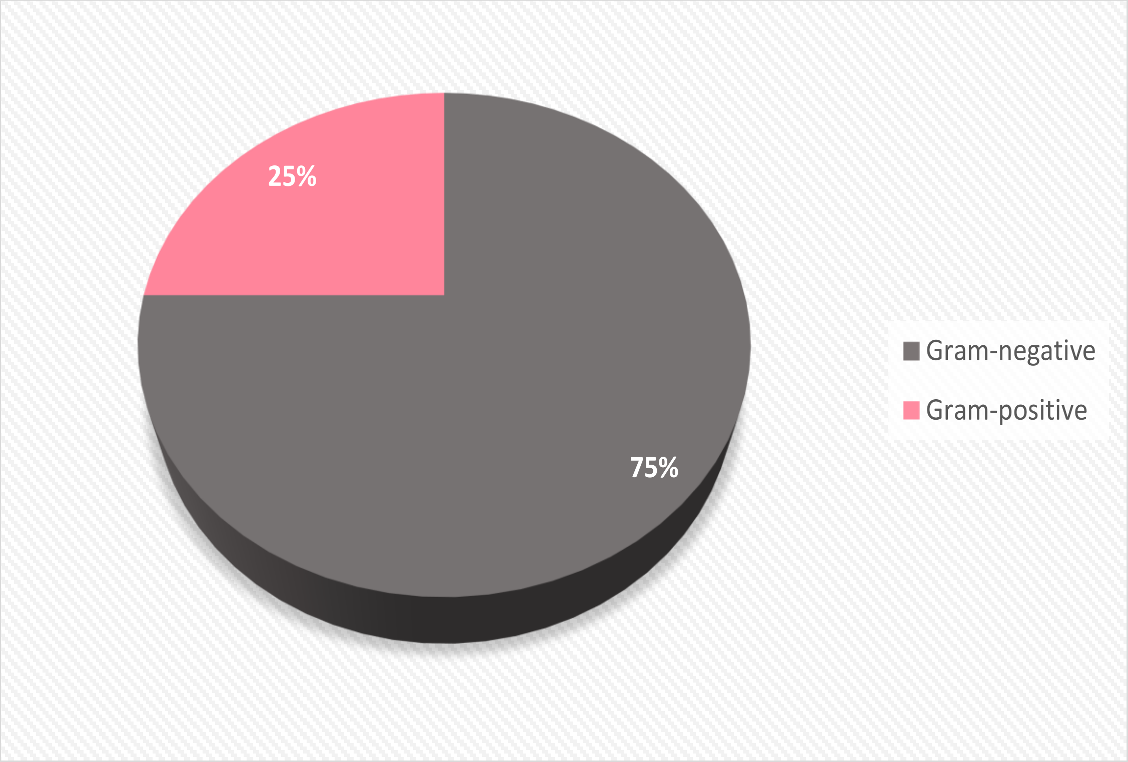

Figure 4: Overall gram-negative and gram-postive bacterial Isolate Occurrence (%) from Poultries A and B

Discussion

Intensive conventional poultry farm practices have resulted in accumulation of large amount of wastes especially manure and litter, antibiotic resistance, excessive resource consumption, water pollution, and ethical concerns Grzinic *et al*. (2023). The prevalence of pathogenic microorganisms in poultry environment is a serious threat to public health and poultry management system and thus, should not be underrated. In the present study, *Escherichia coli* and *Klebsiella pneumoniae* were the most prevalent gram-negative bacteria isolated (21% and 16% respectively) while *Staphylococcus aureus* was the second (18% prevalence) yet most prevalent gram-positive bacteria isolated. This findings aligned with Egbule *et al*., 2024 which recorded both bacteria as amongst the most prevalent isolated bacterial species from their study. It is worthy of note that *Escherichia coli* and *Proteus mirabilis* were the most prevalent isolates encountered from poultry A in the present study. Same findings were reported in recent studies carried out by Atnafu-Bushen and Abayneh (2021) from Ethopia; Assoumy *et al*. (2021) from Coˆte d'Ivoire; Ibrahim *et al*. (2021) from Malaysia; Langata *et al*. (2019) from Kenya; Omoya and Ajayi (2016) in Nigeria and Tigabie *et al*. (2023) in Ethopia. The present study (data from poultry A) presents same findings with these previous studies except that there were no record of *Salmonella* spp. in this study. The predominance of *E. coli* in this study as well as many other studies may be because *E. coli* is a ubiquitous commensal bacterium that is predominantly found in the gastrointestinal tracts of animals and humans, as a normal flora (Torres *et al*. (2010). All bacterial isolates recovered from this study included *Campylobacter species, Enterobacter hormaechei, Enterobacter roggenkampii, Escherichia coli, Klebsiella oxytoca, Klebsiella pneumoniae, Proteus mirabilis, Proteus terrae, Staphylococcus aureus,* and *Streptococcus pnuemoniae*. All 10 bacterial isolates were present in poultry A except for *E. roggenkampii*, while Campylobacter species, *Enterobacter hormaechei*, *Proteus mirabilis* and *Streptococcus pnuemoniae* were absent from poultry B.

The highest cumulative percentages of bacterial isolates in poultry A were found in litter (22.90%), clothing of farm workers (22.00%), and hands of farm workers (20.20%). This is indicative that these sample sources are significant contributors to the bacterial load and may be critical points for intervention to reducing this load and mitigate the risk of antimicrobial resistance gene transmission in this Poultry. Transmission through poor hygienic handling practices by farm workers is also an important route which may be a threat to public health since transmission of these pathogens can occur from human-to-human, human-to-birds and vice versa and to the surrounding environment (Wang *et al*., 2022; Adeyemi *et al*., 2022). For poultry B, the highest cumulative percentage of bacterial isolates was found in air (22.20%), followed by litter (19.40%). This could indicate potential airborne transmission of bacteria or contamination in the poultry environment. The relatively high cumulative percentage of bacterial isolates in air samples from this poultry (B) farm highlighted the potential importance of airborne transmission and the need for adequate ventilation and air quality management in poultry environments. The differences in cumulative percentages between Poultry A and Poultry B may indicate that farm-specific factors, such as management practices or environmental conditions play a significant role in shaping the bacterial ecology that could impact the development and spread of antimicrobial resistance in poultry environments.

The Mann Whitney-U test gave a p-value ($p = .000$) which is less than 0.05 (in this case, lesser than 0.001) is suggestive that there is a statistically significant difference in the bacterial prevalence between Farm A and Farm B; indicating that certain farm-specific factors may be contributing to this disparity. The median value for Farm A was 2.0000, higher than the median value for Farm B, 1.0000 which showed that Farm A had a higher bacterial prevalence than Farm B. In comparing the Chi-square tests performed on the resistant bacteria as well as the two most prevalent bacterial species encountered in this study, significant associations between farm type and the presence of *Enterobacter roggenkampii* (p-value ≈ 0.0238) and *Klebsiella pneumoniae* (p-value ≈ 0.0044) have been observed. These findings are suggestive that certain farm-specific factors might play a role in the presence or prevalence of these bacteria. On the other hand, there were no statistically significant associations found between farm type and the presence of *Enterobacter hormaechei* (p-value ≈ 0.4915), *Staphylococcus aureus* (p-value ≈ 0.4329), *Proteus terrae* (p-value ≈ 0.2851) and *Escherichia coli* (p-value ≈ 0.0897). These results have indicated that these bacteria might be more widespread or resilient across the different farm types, or other factors may be driving their presence. Some of the key factors that might contribute to the difference in bacterial prevalence between the two farms may include farm management practice, the hygiene practices such as the differences in cleaning and disinfection protocols which may contribute to varying bacterial loads, animal health involving vaccination programs, and disease management which may have an impact on the bacterial prevalence. The quality, source, storage/handling of feed and water could also influence bacterial contamination. Environmental factors such as temperature, humidity, ventilation, farm management practices and sanitation might influence bacterial growth and transmission especially through the air. Also, the difference in biosecurity measures/protocols, such as access control, quarantine procedures, and staff training, could impact the transmission and spread of bacterial contamination (Gržinić *et al.* (2022)).

Conclusion

The present study have revealed the prevalence of certain bacterial species in poultries in Abraka, Delta State, Nigeria. With the current rise in antimicrobial resistance in poultry farms globally, multidrug resistant bacterial strains have emerged, making its spread via horizontal gene transfer and the high risk contamination of poultry products and processing plants inevitable. The presence of these multidrug-resistant bacteria in poultry poses a serious risk to human health, particularly if these bacteria enter the food chain. This study has therefore highlighted the need for proper handling and cooking practices to minimize the risk of transmission. The mode of these transmissions are due to poor hygiene, animal contact and environmental exposure. These are expressed through foodborne illnesses, treatment decline/ineffectiveness, prolonged/expensive treatment options leading to prolonged hospital stay and even death (Semedo-Lemsaddek *et al.,* 2015). Since poultry represents an important sector of the agricultural economy, providing one of the most important world leading sources of animal protein, there is a need for enhanced surveillance and education among poultry farmers, veterinarians, and consumers to promote awareness and understanding of the presence of these organisms as well as the implications. Awareness on the importance of responsible poultry farming/animal husbandry practices such as improved hygiene practices, proper use of personal protective equipment, and enhanced biosecurity measures are relevant to mitigate the detrimental effects to poultry and humans.

Recommendation

The findings from this study could help inform strategies for creating and promoting outreaches on the awareness of the impacts of these organisms as it relates to overall poultry farm practices. Further studies however, is recommended to be carried out on a broader scope to ascertain the level of bacterial contamination and prevalence of antimicrobial resistance in these poultry environments.

Declaration of Competing Interest

Authors declare no conflict of competing interests.

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