RESEARCH ARTICLE AGROBIOLOGICAL RECORDS
ISSN: 2708-7182 (Print); ISSN: 2708-7190 (Online)
Open Access Journal

PATHOGEN PROFILE OF POULTRY INDUSTRIES IN OYI LOCAL GOVERNMENT AREA OF ANAMBRA STATE, NIGERIA

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ABSTRACT

Common pathogens in poultry industries in Oyi Local Government Area of Anambra State were isolated and characterized using standard methods. Sensitivity test was employed with a view to ascertain the proper control and treatment measures cum drugs for the isolates. A total of 30 fecal samples were collected from three different towns in the area namely Awkwuzu, Nkwelle, and Umunya. Standard microbiological techniques were used for the cultivation of the pathogens, followed by biochemical characterization to ascertain the identity of the pathogens. The identified pathogens include *Salmonella spp*., *Shigella* spp., *E. coli*, *Enterococcus spp.*, *Streptococcus spp*., and *Staphylococcus spp*.. The sensitivity of these isolates to various antibiotics was evaluated using the diffusion method, and the results revealed disparity in resistance patterns. *Salmonella spp.* and *E. coli* demonstrated significant resistance to several antibiotics including cephalosporins and fluoroquinolones, raising concerns about antimicrobial resistance (AMR) in poultry farming. Sensitivity to alternative drugs like ofloxacin and ciprofloxacin was observed for some pathogens. It could be deduced that the poultry farmers in Oyi Local Government Area of Anambra State lack knowledge on the proper use of antibiotics. The findings further highlight the importance of correct use of antibiotics in poultry farming as substandard application or overuse could lead to bacteria becoming resistant. The study therefore recommends detailing of livestock extension worker to Oyi local government area in order to teach the farmers on the proper ways of application as antibiotics. In addition, herbal alternatives like *Moring oleifera*, could be used to reduce reliance on antibiotics.

***Keywords*:** Antibiotics, Isolates, Pathogen, Sensitivity Disc, Poultry, Resistance

Article History (ABR-24-288) || Received: 08 Nov 2024 || Revised: 22 Jan 2025 || Accepted: 25 Jan-2025 || Published Online: 27 Jan 2025

1. INTRODUCTION

Poultry production is an integral part of the global food industry. However, it faces a lot of challenges ranging from poor nutrition to havoc caused by pathogens. Poultry are domesticated birds that are reared for their meat, egg and feather. Poultry meat products are popular globally because of their rich nutritional value, cost-effective production, and quick cooking time. Chicken raised for meat provides a nutrient-rich, low-acid food source containing phosphorus, various minerals, and B-complex vitamins (Gamble et al. 2015). Chicken is an excellent animal protein source, offering low levels of fat and high biological value. It provides all essential amino acids and unsaturated fatty acids necessary for a balanced human diet (Takma and Korel 2019).

Poultry production is a vital sector of agriculture, contributing significantly to food security and economic development in many regions, including Oyi Local Government local government area of Anambra state. However, industry faces numerous challenges, including the threat of diseases caused by various pathogens. Several pathogenic microorganisms such as *Salmonella spp., Campylobacter spp., Staphylococcus aureus, Escherichia coli,* and *Enterococcus spp*., have been found to cause serious threats to poultry industries. Illnesses originating from animals can transmit to humans through indirect exposure to the environment, direct interaction, or consumption of contaminated food (Chlebicz and Śliżewska 2018).

Situated in the heart of Nigeria, Oyi Local Government serves as a vital hub for poultry farming, contributing significantly to the agricultural landscape and the local economy. However, despite its importance, poultry production in Oyi is not without its challenges, chief among them the presence of various pathogens that can lead to disease outbreaks and subsequent economic losses for farmers.

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Open Access Journal

In Oyi Local Government, like in many other poultry-producing regions, several pathogens pose significant risks to the health of poultry flocks. These pathogens can cause a range of diseases, including respiratory infections, gastrointestinal disorders, and systemic illnesses, leading to reduced production efficiency, increased mortality rates, and economic losses for farmers.

A member of the Enterobacteriaceae family such as Salmonella is a gram-negative rod genus. They are straight-rod, motile, gram-negative bacteria that do not sporulate, infecting the gastrointestinal tract and causing cramps, nausea and diarrhea in humans, it is one of the most common foodborne illnesses in the world (Jung et al. 2022). Campylobacter species are gram negative, motile, non-spore-forming bacteria with a characteristic helical structure that change into filamentous or coccid forms in response to environmental stimuli. Campylobacter can change into a filamentous or coccid form to adapt to severe environments (Tresse et al. 2017). *Escherichia coli* is a facultative anaerobic, gram-negative bacterium with a rod-like form that ranges in length from 2 to 6µm and width from 1.1 to 1.5µm. Its peritrichous flagella allow it to move and is not spore-forming (Percival 2014; Islam et al. 2023). It is thought to be one of the most common bacterial infections globally and is common in domesticated poultry species (Nolan et al. 2020).

Globally, food borne illnesses stem from microbial pathogens found in food, particularly animal products, posing a threat to food safety. Consuming contaminated food, either with live pathogens or their toxins, presents a significant risk. Many of these microorganisms are zoonotic, meaning they can have substantial effects on both public health and economic sectors (Abebe et al. 2020). Understanding and effectively managing these pathogens are crucial for maintaining the health and productivity of poultry populations. Isolation, identification and characterization of common pathogens associated with poultry production play pivotal role in disease surveillance, control, and prevention strategies. By accurately identifying these pathogens, stakeholders in the poultry industry can implement targeted measures to mitigate their impact, such as vaccination programs, bio-security protocols, and treatment strategies.

Isolation and identification of these pathogens involve a combination of laboratory techniques, including microbiological culture, biochemical tests, Sensitivity Tests and serological assays. These methods allow for the precise identification of the causative agents responsible for poultry diseases, enabling targeted interventions to control their spread and minimize their impact on flock health.

Hence, the general objective of the study is to isolate, identify and characterize the common pathogens often found in poultry industries in Oyi local government area of Anambra state, Nigeria.

2. MATERIALS AND METHODS

2.1. Study Area

Oyi is a local Government Area that is found in Anambra state, Nigeria with the coordinate's 6°10'36''N 6°51'43''E.It is located on the map along the longitude 6.45°E and the latitude 6.14°N (Google Map, 2024). Oyi comprises of six (6) towns namely: Nkwelle-Ezunaka, Awkuzu, Ogbunike, Umunede, Umunya and Nteje. The land is fairly low about 500feet (150m) above sea level.

2.2. Sample Collection and Analysis

A total of thirty (30) freshly excreted chicken fecal samples were collected from three (3) towns in Oyi Local Government, Anambra State using random sampling. The towns selected include Umunya (10 samples), Awkuzu (10 samples) and Nkwelle-Ezunaka (10 samples). The samples were properly labeled and transported with plastic test tubes to the laboratory for analysis. Each sample was prepared by making dilutions in distilled water. The laboratory analysis was conducted at Amazing Grace Research Laboratory situated in Nkwelle, Oyi LGA, Anambra State, Nigeria.

2.3. Culture Media Preparation

Nutrient Agar, Salmonella Shigellar Agar and MacConkey Agar were prepared for the study in accordance with the manufacturer's instructions.

***2.3.1. Salmonella-Shigella Agar (SSA) Test*:** SSA is a selective and differential medium. It was used for the isolation, cultivation and differentiation of gram-negative enteric microorganisms. Differentiation of enteric micro-organisms was achieved by incorporation of lactose in the medium. Isolates were inoculated by streaking and incubated at 37°C for 24 hours. Organisms which ferment lactose produce acid which, in the presence of the neutral red indicator, results in the formation of red/pink colonies. Lactose non-fermenters form colorless colonies.

***2.3.2. MacConkey Agar Test*:** MacConkey Agar was used as selective and differential medium for the cultivation of coliform organisms. It was used for the isolation and differentiation of non-fastidious gram-negative rods,

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Open Access Journal

particularly members of the family Enterobacteriaceae and the genus Pseudomonas. Isolates were in a dehydrated medium in 1000mL of distilled water. The isolated were heated to boiling to dissolve the medium completely. Then it was sterilized by autoclaving at 121°C for 15min. The isolates were well mixed well before pouring into sterile Petri plates.

***2.3.3. Nutrient Agar Test*:** Nutrient Agar was used for culturing of non-fastidious microorganisms, and for quality control and checking purity prior to biochemical testing. It is one of the most important and commonly used non-selective media for the routine cultivation of microorganisms. Then, 28g of nutrient agar powder was suspended. (28g of nutrient agar powder was suspended in 1 L of distilled water. It was mixed and dissolved completely. Then, it was sterilized using autoclave at 121°C for 15min. The liquid was poured into the petri dish and waited for the medium to solidify. Clean environment was maintained to prevent contamination. After solidification, the agar was used. The media forms light yellow colored clear to slightly opalescent gel on Petri plates after cooling.

2.4. Inoculation of the Media for Bacterial Isolation

The test tubes containing the test samples were inoculated by streaking using a wire loop into both MacConkey and Salmonella Shigella agar. 1ml of the test samples was transferred into a prepared Salmonella Shigella agar and MacConkey agar in culture plates and incubated at 37℃ for 24 hours (overnight). After 24 hours, It was checked for the growth of organisms. Fresh media was prepared and poured into the petri dishes. Then it was allowed to gel, and subsequently sub-cultured with the aid of the wire loop. Then it was for 24 hours (overnight). After 24 hours, it was checked for the growth of organisms. Fresh media was prepared and poured into the petri dishes. Then it was allowed to gel, and subsequently sub-cultured with the aid of the wire loop. It was incubated at 37℃ for 24 hours (overnight) to get pure colonies.

2.5. Sensitivity Test

The various pure isolates were subjected to sensitivity test with commercial antibiotic discs using disc diffusion methods. They were incubated at 37℃ for 24 hours after which the various zones of inhibition were measured with a meter rule.

2.6. Characterization and Identification of the Isolates

Different morphological and biochemical characteristics accompanied with colony characteristics on different selective mediums were observed for the identification of isolates. The pure colonies were subjected to various biochemical tests for proper identification using methods as described by Chessburugh (2010). These tests carried out include Gram staining, Catalase Test, Coagulase Test, Oxidase Test, Citrate Utilization Test, Indole Test, Urease Test, Methyl Red Test, Voges Proskauer (VP) Test, Hydrogen Sulfide Test and Motility Test.

***2.6.1. Gram Staining*:** This reaction differentiates the Gram positive from the gram-negative bacteria due to differences in their cell wall structure. A drop of normal saline was placed on a clean grease free slide, using a sterile wire loop, a smear of the culture was made on the slide and heat fixed. The fixed smear was flooded with crystal violet stains for 60s, rinsed with clean water and drained quickly before it was flooded with Lugol's iodine for 60s and then washed off with distilled water. The slide was flooded with 95% ethanol which is a decolorizer for 5s. After which the slide was washed using distilled water and then flooded with safranin (counterstain) for 30s and then washed off. The back of the slide was then cleaned and placed in a draining rack for the stained smear to dry. The standard smear was then allowed to dry air. Then a drop of oil immersion was added on the smear to view the smear under the microscope for examination.

***2.6.2. Catalase Test*:** Using a sterile dropping pipette, a drop of 3% hydrogen peroxide solution was placed on a slide and a colony of the test organism was added to the drop of hydrogen peroxide solution. Fermentation of oxygen bubbles which is an indicative of the presence of catalase was looked out for. This was done to differentiate between staphylococci and streptococci. Effervescence of gas indicates the presence of gram-positive organisms.

***2.6.3. Coagulase Test*:** For the analysis, a drop of sterile distilled water was added to both ends of a slide. A colony of the test organism was emulsified in each drop of water and a loop full of plasma added to one of the suspensions with thorough mixing. Change is observed within 10s to determine the identity of the organism.

***2.6.4. Oxidase Test*:** Over well-isolated (pure culture) colonies of test bacteria from fresh culture, three drops of Kovacs' oxidase reagent were added. The plate was tilted and shaked gently so that the colonies got exposed to oxygen. Observe for the formation of purple (deep blue) color over the reagent-moistened colonies and note the time required for change in color for up to 60s. When the test is Positive**,** there's development of purple to deep

ISSN: 2708-7182 (Print); ISSN: 2708-7190 (Online)
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blue color within 10 to 30s indicating a positive oxidase test and development of purple to deep blue color within 30 to 60s indicating a weak oxidase positive reaction or delayed oxidase positive. When the test is Negative, there is no development of purple to deep blue color within 60s and development of purple to deep blue color after 60s.

***2.6.5. Citrate Utilization Test*:** The test differentiates among bacteria by determining their ability to utilize citrate as their only source of carbon. Test organisms were inoculated on Simmons citrate agar slants streaking gently and incubated at 37°C for 24 hours. A color change in the agar from green to blue indicates a positive reaction.

***2.6.6. Indole Test*:** This test is used to distinguish among members of the families of Enterobacteriaceae by testing their ability to degrade an essential alpha amino acid, tryptophan, to produce Indole. The isolates were inoculated in nutrient broth and incubated at 37°C for 16-24 hours. After incubation, a few drops of Kovac's reagents were added and the tube shaken gently and allowed it to stand. The pinkish and ring-like color at the upper layer indicates Indole production in the tubes, and if otherwise, no Indole production.

2.6.7. Urease Test: Urease test is a biochemical test that detects the alkaline fermentation of urine (urea) with the resultant production of ammonia by microorganisms. It is performed by growing the test organisms on the agar medium, containing the pH indicator phenol red. Positive results show deep pink color while no color change is a negative result.

***2.6.8. Methyl Red Test*:** Wire loops full of isolates under investigation were inoculated and incubated for 24 hours, three drops of methyl red solution were added and color change observed. The color change from light yellow to pink indicates a methyl red positive reaction, meaning that acid produced; while no change in color (color remains yellow) indicates a methyl red negative reaction.

***2.6.9. Voges Proskauer (Vp) Test*:** MR/VP broth with a pure culture of the test organism was incubated 24 hours at 35°C. At the end of this time, 1mL of aliquot broth was poured into a clean test tube. 0.6mL of 5% α-naphthol was added, followed by 0.2mL of 40%KOH. The tube was shaken gently to expose the medium to atmospheric oxygen and allow the tube to remain undisturbed for 10 to 15min. A positive Vp test is a development of a pink-red color at the surface within 15min or more after the addition of the reagents.

***2.6.10. Hydrogen Sulfide Test*:** Using a sterile inoculation to touch a well-isolated colony from a fresh culture of the test bacterium, streak culture over the agar plate to get well-isolated colonies and then incubate at 35±2°C for 24 hours. Then, the color of the developed colonies was observed. Black colonies or colorless or colored colonies with a black center will appear.

***2.6.11. Motility Test*:** With a sterile straight needle, touch a colony of a young (18-24 hours) culture growing on an agar medium, then single stab down the center of the tube to about half the depth of the medium, then incubate at 35-37°C and examine daily for up to 7 days. If it is motile, the organisms will spread out into the medium from the site of inoculation and if it is non-motile, the organisms remain at the site of inoculation.

2.7. Sample Design

This project was conducted using CRD, where the towns are the treatments and the poultry units within the towns are the replicates. Given the formula:

$Y=\mu+Ti+£ij$

Where Yij= Single observation or j^{th} observation in the i^{th} treatment

μ = Overall mean

Ti=Effect of treatment (i= 1, 2…., n)

Eij = Random error.

2.8. Statistical Analysis

Analysis of Variance (ANOVA) was carried out using SPSS version 17. The treatment means were separated using Duncan's New Multiple Range Test (Duncan 1995).

3. RESULTS

3.1. Characterization of Bacteria Isolates

The characteristics of the isolates from fecal samples collected from the three towns namely Awkuzu (T1), Nkwelle (T2) and Umunya (T3) in Oyi local government area of Anambra state were presented in Table 1, 2 and 3.

ISSN: 2708-7182 (Print); ISSN: 2708-7190 (Online)
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Table 1: Biochemical characteristics of Bacteria Isolates from Chicken feces in Awkuzu

Colony morphology	Organisms	Gram rxn	Catalase	Coagulase	Oxidase	Citrate utilization	Indole	Urease	Methyl red	Vp	H_2S	Motility
Pink smooth colonies	E-coli	-	+	-	-	-	+	-	+	-	-	+
Colorless small colonies	Enterococcus spp	+	-	-	-	-	-	-	-	+	-	-
Dark coloured rods	Salmonella spp	-	+	-	+	-	-	-	+	-	+	+
colonies small white color in chains	Streptococcal spp	+	-	-	-	-	-	-	-	-	-	-
Pink rods	Shigella spp	-	+	-	-	-	+/-	-	+	-	-	-
Clustered cocci	Staphylococcal spp	+	+	+	-	+	-	+	+	+	-	-

Table 2: Biochemical characteristics of Bacteria Isolates from Chicken feces in Nkwelle

Colony morphology	Organisms	Gram rxn	Catalase	coagulase	Oxidase	Citrate	indole	Urease	Methyl red	vp	H2s	motility
Small white colonies in chains	Streptococcal spp	+	-	-	-	-	-	-	-	-	-	-
Clustered cocci	Staphylococcal spp	+	+	+	-	+	-	+	+	+	-	-
Colorless small colonies	Enterococcus spp	+	-	-	-	-	-	-	-	+	-	-
Pink smooth colonies	E-coli	-	+	-	-	-	+	-	+	-	-	+
Pink rods	Shigella spp	-	+	-	-	-	+	-	+	-	-	-

Table 3: Biochemical characteristics of Bacteria Isolates from Chicken feces in Umunya

Colony morphology	Probable organisms	Gram rxn	Catalase	Coagulase	Oxidase	Citrate	Indole	Urese	Methyl red	vp	H2s	mot
Dark colored rods	Salmonella	-	+	-	-	+	-	-	+	-	+	+
Pinkish rods	Shigella spp	-	+	-	-	-	+	-	+	-	-	-
Pink smooth colonies	E-coli	-	+	-	-	-	+	-	+	-	-	+
Small white colonies in chains	Streptococcal spp	+	-	-	-	-	-	-	-	-	-	-

3.1.1. Characterization of Awkuzu Pathogens: A total of six (6) dormant isolates were obtained from 30 fecal samples collected from Awkuzu. The biochemical characteristics of these isolates are shown in Table 1. Biochemical tests revealed the organisms to be *Salmonella spp, Shigella spp, E. coli spp, Enterococcus spp, Streptococcus spp and Staphylococcus spp.* These isolates were subjected to antibiotic sensitivity tests with commercial antibiotic discs using disc diffusion methods and the results showed that only *Salmonella spp*, were sensitive to ofloxacin with zone diameter of 12mm and pefloxacin with zone diameter of 10mm. Other isolates showed resistance to the tested antibiotics.

***3.1.2. Characterization of Nkwelle Pathogens*:** A total of five (5) dormant isolates were obtained from 30 fecal samples collected from Nkwelle town in Oyi Local Government (Table 2). Biochemical tests revealed the organisms to be *Streptococcal spp, Staphylococcal spp, Enterococcal spp, E. coli spp,* and *Shigella spp*. No *Salmonella spp* was isolated. These isolates were subjected to sensitivity tests with commercially prepared antibiotics discs using disc diffusion method. The results showed that all the isolates were resistant to all the antibiotics tested except *Shigella spp.*, which were sensitive to Ofloxacin with zone diameter of 15mm and Levofloxacin with zone of inhibition of 12mm.

***3.1.3. Characterization of Umunya Pathogens*:** A total of four (4) dormant isolates were recovered from 30 fecal samples obtained from Umunya town in Oyi Local Government. The biochemical tests revealed the organisms to be *Salmonella spp, Shigella spp, E. coli spp,* and *Streptococcal spp.* (Table 3). These isolates were subjected to sensitivity tests with commercially prepared antibiotic discs using disc diffusion methods. The results showed that all the *E-coli* isolates were resistant to the entire antibiotic and *Salmonella spp* were sensitive to levofloxacin (15mm), ciprofloxacin (10mm), and erythromycin (12mm) respectively, while *Shigella spp* were sensitive to ciprofloxacin (17mm), erythromycin (12mm) and levofloxacin (15mm). Also *streptococcal spp* were sensitive to levofloxacin (12mm), ciprofloxacin (10mm) and resistant to other antibiotics.

3.2. Major Isolates According to Location

The summary of the bacterial isolates identified in Awkuzu (T1), Nkwelle (T2) and Umunya (T3) are presented in Table 4. In Okwuzu, virtually all the bacterial isolates studied were present while *Salmonella spp*. was absent in Nkwelle. Similarly, *Shigella spp., Staphylococcal spp.* and *Enterococcus spp.* were absent in Umunya. So, *Enterococcus spp* and *Streptococcus spp.* are more endemic in Oyi local government area of Anambra state,

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ISSN: 2708-7182 (Print); ISSN: 2708-7190 (Online)
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Nigeria. The effect of location on the number of the bacteria isolates obtained at poultry industries in Oyi Local Government Area of Anambra State, Nigeria is presented in Table 5. Awkuzu poultry farms recorded the highest number of isolates followed by Nkwelle, and Umunya being the least. Typical example of the isolates and sensitivity test are shown in Fig. 1.

Table 4: The bacterial isolates identified in Awkuzu (T1), Nkwelle (T2) and Umunya (T3)

Isolates	T1	T2	T3
Salmonella spp	+	-	+
Shigella spp	+	+	-
E-coli spp.	+	+	+
Enterococcus spp	+	+	-
Streptococcal spp.	+	+	+
Staphylococcal spp.	+	+	-

Key = + indicates presence of a given isolate while – indicates its absence

Table 5: Effect of Location on the Number of Bacteria Isolates obtained at Poultry Industries in Oyi Local Government Area of Anambra State, Nigeria

Isolates	T1 (Awkuzu)	T2 (Nkwelle)	T3 (Umunya)
Salmonella spp	9.00b	0.10a	10.00b
Shigella spp	10.00b	9.00b	0.20a
E-coli spp.	10.00b	10.00b	10.00b
Enterococcus spp	10.00b	10.00b	0.00a
Streptococcal spp.	9.00	10.00	10.00
Staphylococcal spp.	10.00b	10.00b	0.00a

Means bearing different alphabets in the same row are significantly ($P<0.05$) different

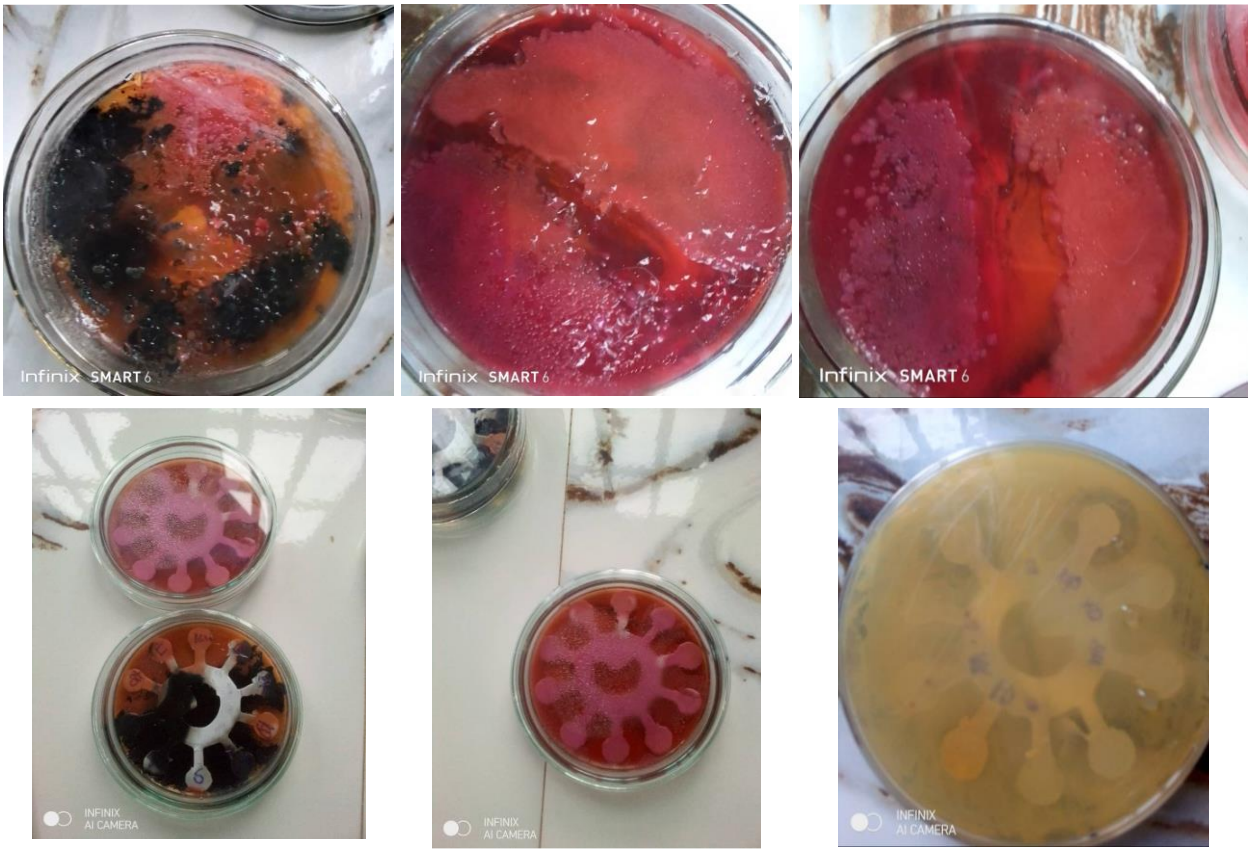

Fig. 1: Samples of the isolates and Sensitivity Tests.

4. DISCUSSION

Generally, four to six different bacterial isolates were obtained from the study area. These include *Salmonella spp*, *Shigella spp*, *E-coli spp.*, *Enterococcus spp.*, *Streptococcal spp.* and *Staphylococcal spp. Salmonella spp*. was identified as one of the bacterial isolates in this study, particularly in samples from Awkuzu and Umunya. The frequency of its occurrence suggests that these locations might present favorable conditions for the survival and transmission of Salmonella, a common food borne pathogen in poultry environments. No *Salmonella* was found in samples collected from Nkwelle, suggesting that environmental factors such as farm management practices, or biosecurity measures might be stricter in Nkwelle. In this study, antibiotic sensitivity testing revealed that *Salmonella spp*. from Awkuzu were sensitive only to ofloxacin and pefloxacin, while those from Umunya showed sensitivity to levofloxacin, ciprofloxacin, and erythromycin. The limited sensitivity observed suggests a growing resistance trend among *Salmonella* isolates, possibly due to the excessive use of antibiotics in poultry farming.

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Resistance in pathogenic bacteria can lead to recurrent disease outbreaks, increased mortality rates, and higher production costs for poultry farmers who need to invest in alternative treatments or adopt stricter management protocols. For instance, the findings are consistent with Adeyanju and Ishola (2014), who reported that the overuse of common antibiotics in poultry farms has led to increased antibiotic resistance in pathogenic bacteria. Additionally, resistant strains may persist in the environment and infect humans through direct contact or consumption of contaminated poultry products, thus posing a risk to public health. Again, the antibiotic sensitivity results for *Salmonella spp.* isolated in this study showed variability across different locations. At Awkuzu, *Salmonella* demonstrated sensitivity to ofloxacin (zone of inhibition: 12mm) and pefloxacin (zone of inhibition: 10mm), indicating that these antibiotics could potentially be effective in managing *Salmonella* infections in poultry from this location. Conversely, in Umunya, the *Salmonella* isolates were sensitive to levofloxacin (15mm), ciprofloxacin (10mm), and erythromycin (12mm), suggesting some degree of variation in antibiotic efficacy, which may be influenced by local factors such as antibiotic usage patterns or the introduction of new resistant strains.

The presence of antibiotic-resistant *Salmonella* in poultry poses a significant public health risk, especially in communities where poultry products form a major part of the diet. Human infections with *Salmonella* are commonly linked to the consumption of contaminated meat or eggs, which can lead to food borne illnesses characterized by symptoms such as diarrhea, fever, and abdominal cramps. According to the Centers for Disease Control and Prevention (CDC), antibiotic-resistant *Salmonella* infections are more challenging to treat, resulting in prolonged illnesses and increased risk of complications, particularly in vulnerable populations such as children, the elderly, and immune compromised individuals (Keely Boyle et al. 2018).

The genus Shigella was first identified by Japanese microbiologist Kiyoshi Shiga in 1897 by Okui (2024) during an outbreak of dysentery. It is a significant cause of bacterial dysentery in both humans and animals. In this study, *Shigella spp*. were isolated from poultry farms in Awkuzu and Nkwelle but were absent in Umunya. The presence of Shigella in two out of the three locations suggests it may be a common pathogen in poultry farms within Oyi Local Government, potentially introduced through contaminated water, feed, or poor sanitary conditions.

The detection of *Shigella spp*. in Awkuzu and Nkwelle aligns with other studies in Nigeria and similar regions, where Shigella has been reported in poultry farms. For instance, Kura et al. (2022) and Islam et al. (2021) reported high prevalence of Shigella spp. in broiler farms in Northern Nigeria, linked to poor farm hygiene and water quality. The prevalence in Awkuzu (10 out of 30 samples) and Nkwelle (9 out of 30 samples) supports these findings, indicating a potential for widespread contamination within the local poultry environment.

Antibiotic resistance in Shigella poses a significant threat to poultry farming. In this study, antibiotic sensitivity testing showed that Shigella spp. was sensitive to ofloxacin and levofloxacin in Nkwelle, with zone diameters of 15mm and 12mm, respectively. In Umunya, where no Shigella was isolated, no sensitivity results were available for comparison. The sensitivity of Shigella to these fluoroquinolones suggests that they could be effective in managing infections; however, resistance to other antibiotics remains a concern.

Antibiotic-resistant Shigella can lead to higher morbidity and mortality rates in poultry, necessitating frequent use of alternative treatments or combinations of antibiotics, which can increase production costs. This is consistent with findings from Olumide et al. (2015), who reported that antibiotic-resistant strains of Shigella are associated with increased treatment failures and recurrent infections in poultry farms.

The antibiotic sensitivity tests conducted in this study revealed that *Shigella spp*. from Nkwelle showed sensitivity to ofloxacin (15mm) and levofloxacin (12mm), while demonstrating resistance to other antibiotics tested. In Awkuzu, Shigella isolates were not subjected to the same sensitivity tests due to study limitations, but the pattern observed in Nkwelle suggests that fluoroquinolones may still be effective against this pathogen.

Escherichia coli are a common bacterium found in the intestines of warm-blooded animals, including poultry. While many E. coli strains are harmless, some can cause serious infections in animals and humans. The presence of pathogenic E. coli in poultry is associated with various diseases, such as colibacillosis, which can lead to economic losses in poultry farming due to reduced productivity and increased mortality.

In this study, *E. coli* was isolated from poultry fecal samples in all three locations—Awkuzu, Nkwelle, and Umunya. This bacterium's presence across these towns indicates that it is widespread in poultry farms in Oyi Local Government Area. This has significant health implications for both poultry and human safety.

The findings of this study are consistent with those of earlier research, such as Anyanwu et al. (2022) and Karim which reported high prevalence rates of *E. coli* in poultry farms across southeastern Nigeria. Their studies highlighted factors like poor hygiene, substandard farming practices, and the absence of proper waste management as key contributors to the widespread presence of *E. coli* in poultry environments.

Again, *E. coli* isolates from the three locations showed resistance to all tested antibiotics, indicating the presence of multi-drug resistant strains. It could be that the farmers have been using sub-lethal level of antibiotics. This resistance poses a serious threat to poultry health, as it limits the treatment options available for controlling infections. The presence of multi-drug-resistant *E. coli* in poultry poses a significant risk to public health. Humans

ISSN: 2708-7182 (Print); ISSN: 2708-7190 (Online)
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can be exposed to resistant *E. coli* through the consumption of contaminated poultry products or through direct contact with infected birds. Infections caused by resistant *E. coli* can be challenging to treat, leading to prolonged illness, increased healthcare costs, and, in some cases, severe complications such as septicemia or kidney damage (hemolytic uremic syndrome).

Enterococcus spp are part of the normal gut flora in animals, including poultry, and can be found in various environmental sources (Sanz et al. 2021; Rajendiran et al. 2022; Ribeiro et al. 2023; Rebelo et al. 2023). Although many Enterococcus strains are commensal, some can cause infections under certain conditions, particularly in immune compromised hosts. *Enterococcus* was isolated from poultry fecal samples collected in Awkuzu and Nkwelle, but not from Umunya. The presence of *Enterococcus* in two out of the three towns indicates that the bacterium is common in some areas of Oyi Local Government. The findings align with previous studies conducted in southeastern Nigeria, where *Enterococcus* has been reported frequently in poultry farms. Research by Mola et al. (2021) documented the widespread occurrence of *Enterococcus* in poultry, attributing its prevalence to factors such as poor farm hygiene and the use of untreated poultry waste as fertilizer.

The resistance of *Enterococcus* to antibiotics can have significant implications for poultry farming, as these bacteria can act as reservoirs for antibiotic resistance genes that may transfer to other pathogenic organisms (Ribeiro et al. 2023; Mwikuma et al. 2023; Martinez-Laorden et al. 2023; Rebelo et al. 2023). This can make bacterial infections harder to treat, leading to increased disease incidence and economic losses. The results showed that *Enterococcus* isolates from both Awkuzu and Nkwelle were resistant to all the antibiotics tested, indicating the presence of multi-drug-resistant strains. The presence of multi-drug-resistant Enterococcus in poultry poses a risk to human health, especially when considering the potential for zoonotic transmission. Humans can be exposed to these resistant strains through direct contact with poultry, handling contaminated poultry products, or consuming undercooked meat. Infections caused by resistant *Enterococcus* in humans can lead to conditions such as urinary tract infections, bacteremia, or endocarditis, which can be challenging to treat due to limited therapeutic options.

Streptococcal species are a group of Gram-positive bacteria commonly found in the environment, including in the intestines of poultry. While many Streptococcus strains are non-pathogenic, certain species can cause diseases in poultry, such as septicemia, respiratory infections, and arthritis. *Streptococcal spp*. were detected in samples from all the three towns: Awkuzu, Nkwelle, and Umunya. This widespread presence indicates that Streptococcal spp. is a common bacterial isolate in poultry farms within Oyi Local Government Area. The results are consistent with previous findings by Odo et al. (2021) and Ezemba et al. (2022), who reported the prevalence of Streptococcal spp. in poultry farms in southeastern Nigeria. The studies attributed the widespread occurrence to environmental contamination and inadequate farm hygiene practices.

Compared to the previous studies, the detection rate of *Streptococcal spp*. in this research is high, reflecting ongoing issues with bacterial contamination in the region. If Streptococcal spp. is resistant to antibiotics, it could pose significant challenges in managing bacterial infections in poultry. Resistance can lead to prolonged illness, increased mortality rates, and economic losses due to reduced productivity. The potential for resistant strains to act as reservoirs for resistance genes also raises concerns about the spread of antibiotic resistance to other bacterial species within the farm environment.

In this study, Streptococcal spp. isolates from the three towns showed varying degrees of antibiotic sensitivity. While isolates from Umunya were sensitive to levofloxacin (12mm) and ciprofloxacin (10mm), resistance to other tested antibiotics was noted. This pattern suggests that certain Streptococcal strains may have acquired resistance mechanisms; likely due to factors such as antibiotic overuse or environmental exposure to resistant bacteria. The presence of antibiotic-resistant Streptococcal spp. in poultry farms poses a risk to human health, as these bacteria can cause infections that range from mild throat infections to severe conditions like meningitis and bacteremia. Humans may become exposed to these resistant strains through direct contact with poultry or contaminated environments, or by consuming undercooked poultry products.

Staphylococcal spp. is Gram-positive cocci known for their capacity to colonize various environments, including poultry. They are commonly associated with skin infections, respiratory diseases, and septicemia in poultry. *Staphylococcal spp.* were isolated from Awkuzu and Nkwelle but were absent in Umunya. This indicates that Staphylococcal spp. is not uniformly present across all sampled locations in Oyi Local Government Area. The absence in Umunya could be due to environmental factors, hygiene practices, or farm management differences that may influence bacterial colonization and persistence. Studies by Nwosu et al. (2023) and Okafor et al. (2023), have reported the prevalence of Staphyloc*occal spp.* in poultry farms across Nigeria. These studies found that *Staphylococcus aureus*, a common pathogenic species, was frequently associated with poultry environments, particularly in areas with intensive farming practices. The findings from this study align with their reports, confirming the widespread nature of *Staphylococcal spp*. in poultry farms.

The occurrence of antibiotic-resistant *Staphylococcal spp*. in poultry farms could present significant challenges.

ISSN: 2708-7182 (Print); ISSN: 2708-7190 (Online)
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Infected birds may exhibit prolonged illness, reduced growth rates, and increased mortality, leading to economic losses. The antibiotic sensitivity results from this study indicated that *Staphylococcal spp*. isolated from Awkuzu and Nkwelle were resistant to most antibiotics tested. However, in Awkuzu, they showed sensitivity to ofloxacin and ciprofloxacin, while those from Nkwelle did not exhibit any sensitivity to the tested antibiotics.

Antibiotic-resistant *Staphylococcus spp*., such as Methicillin-resistant *Staphylococcus aureus* (MRSA), poses serious health risks to humans (Silva et al. 2023; Lade and Kim, 2023; Marciniak et al. 2024; Zhao et al. 2024). Humans can be exposed to resistant Staphylococcus strains through direct contact with infected poultry, handling contaminated poultry products, or through environmental exposure in farms. If transmitted to humans, these resistant bacteria can cause difficult-to-treat infections such as skin infections, respiratory infections, or even systemic infections in vulnerable individuals (Zhao et al. 2024; Verma et al. 2024).

There was little disparity in the number of isolates obtained from Awkuzu, Nkwelle and Umunya.The test results indicated little disparity in the number of bacterial isolates obtained from the three towns—Awkuzu, Nkwelle, and Umunya—suggesting a relatively similar prevalence of pathogenic bacteria across the locations. However, certain factors may still account for differences in the specific bacterial profiles and resistance patterns observed. These include bio security Measures, Management Practices, Antibiotic Applications, and other environmental factors such as weather, water quality, and surrounding vegetation, among other (Barrow and Methner, 2013). Similarly, nearby wild birds or rodents may introduce bacteria into poultry farms, affecting the number of isolates in different towns. Other possible Causes of infection in Poultry are contaminated Feed and Water [Poor storage conditions or the use of untreated water can introduce bacteria such as *E. coli, Salmonella spp*., and *Clostridium spp*., resulting in gastrointestinal infections and systemic diseases (Gyles 2008; Amir et al. 2022); inadequate housing conditions [sub-optimal standards for temperature, ventilation, and space can predispose birds to infections, overcrowding, damp litter, and poor airflow can increase stress and facilitate the growth of bacteria in the environment, leading to respiratory and enteric diseases; direct and indirect contact with Infected Birds facilitate the spread of pathogens like *Staphylococcal spp*. and *Shigellaspp*. across farms (Kabir 2009); vertical transmission from parent birds to offspring via eggs and rodents cum wild birds (Benskin et al. 2009; Mola et al. 2021).

5. CONCLUSION

Six dormant bacterial pathogens namely *E. coli, Salmonella spp, Shigella spp, Streptococcal spp, Enterococcus spp,* and *Staphylococcal spp* were identified in poultry farms in Oyi local government area of Anambra State. Virtually all these isolates were resistant to antibiotics in the three towns studied, and they are all zoonotic. The isolation of these microorganisms is an indication that poultry farmers in this area are not properly guided in the rudimentary hygiene and bio security measures in poultry farms. They have misused antibiotics in poultry farming; hence, most of these isolates have developed resistance to antibiotics.

The antibiotic resistance patterns observed, particularly in *E. coli*, raise concerns about the overuse of antibiotics in poultry farming and the risk of spreading resistant strains to humans. Improved farm management practices, the use of natural herbs that possess antimicrobial, antiviral and immune boosting properties are recommended for farmers in these area as these herbs can offer a viable option for managing and preventing diseases in poultry without the adverse effects associated with synthetic antibiotics which is necessary to protect both poultry and public health.

Recommendation

The government should engage extension workers in Oyi local government of Anambra state to educate the farmers on basic management practices and proper use of antibiotics in poultry farms. In addition, the study recommends the use of herbs as an alternative to antibiotics due their proven antimicrobial properties already reported by researchers (Savoia 2012). Herbs such as *Moringa oleifera*, Garlic (*Allium sativum*), Turmeric (*Curcuma longa*), and Aloe vera have been reported to exhibit significant antimicrobial effects without deleterious effects. These herbs can be incorporated into poultry feed or used as natural treatments to enhance their immune system, reduce pathogen load and prevent disease outbreaks. Further research may be encouraged to establish optimal dosages and methods of incorporating these herbs into poultry management systems.

Author's Contribution

Ezejesi HC, Okafor EC and Nwankwo CA designed the project and analyzed the data statistically. Okpalaji Nkeoma Carol, Okonkwo AP and JC Okonkwo are the people that carried out the field work. Ezenyilimba BN and Ejivade OM were involved in supervising and proofreading the work.

Citation: Okpalaji NC, Okonkwo AP, Okonkwo JC, Ezenyilimba BN, Ejivade OM, Okafor EC, Ezejesi HC and Nwankwo CA, 2025. Pathogen profile of poultry industries in Oyi local government area of Anambra State, Nigeria. Agrobiological Records 19: 24-34. https://doi.org/10.47278/journal.abr/2025.004

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