Common Pathogens Associated with Poultry Production in Awka South Local Government Area of Anambra State

Okoye MC[1]; Okonkwo JC[2]; Mba Anthonia Nkiruka[3]; Ogu CT[4*]; Okonkwo IF[5]

[1,2]Department of Animal Science and Technology, Faculty of Agriculture, Nnamdi Azikiwe University, Awka, Nigeria
[3]David Umahi Federal University of Health Sciences, Uburu, Ebonyi State, Nigeria
[4,5]Department of Applied Microbiology and Brewery, Nnamdi Azikiwe University, Awka, Nigeria
*Corresponding Author

Received:- 01 March 2025/ Revised:- 14 March 2025/ Accepted:- 20 March 2025/ Published: 31-03-2025

Abstract— *The worldwide poultry production is continually intensifying with progressively more challenges due to pathogens; hence it is crucial to ensure the bio-safety of the poultry farms and poultry products. This research was therefore designed to isolate and characterize the common pathogens associated with poultry production in Awka South Local Government Area of Anambra State. In addition, sensitivity tests were carried out to proffer solution to farmers in this area. Thirty fecal samples were collected from poultry farms in three towns in the study area using systematic random sampling. Standard microbiological techniques were employed to cultivate, isolate and characterize pathogens from each town. The study revealed the presence of various pathogenic bacteria including Salmonella spp, Escherichia coli, Shigella spp, Streptococcal spp and Staphylococcus aureus. The antimicrobial susceptibility testing of the isolated pathogens was conducted to determine their sensitivity to commonly used antibiotics. Some of these pathogens were resistant to common antibiotics often used by farmers indicating subnormal applications. The study therefore underscores the urgent need for the government to detail Extension Agents to Awka South Local Government Area of Anambra State in order to educate the poultry farmers on proper use of antibiotics. Again, since most of these isolates are zoonotic, caution should be applied in consumption of animal products from this area. The use of alternative bio security measures such as herbs are recommended in order to mitigate the imminent danger of using antibiotics.*

Keywords— Antibiotic Resistant, Bacterial Isolates, Pathogens, Zoonotic Disease.

I. INTRODUCTION

1.1 Background of the study:

Poultry production is a significant contributor to global food security, providing a vital source of protein; eggs and income for millions of people worldwide (Farrell, 2013). However, this industry faces challenges due to presence of various pathogens that can cause diseases in poultry (Sierżant *et al*., 2021). These diseases not only impact animal health and welfare but also lead to significant economic losses for farmers due to decreased productivity and mortality (Grzinic *et al.*, 2023). Pathogens can be introduced to a poultry flock via air, pests, people, water, feed, to mention but a few. The prevalence and impact of these pathogens is largely dependent upon the quality of environment and the health and welfare of the birds.

Pathogens such as *Salmonella spp.*, *Staphylococcus aureus*, *Escherichia coli,* and *Enterococcus spp* and *Campylobacter spp.* present a major concern for the poultry industry on a yearly basis due to their association with poultry-related foodborne illnesses. Crates use in transportation, poor environmental conditions, poor worker hygiene, and bird-to-bird pathogen transfer have all been identified as major preharvest contamination risk factors (Heyndrickx *et al.,* 2002; Bull *et al.,* 2006). During processing, poultry carcasses are primarily contaminated with pathogenic bacteria due to the leakage of fecal matter during major processing steps (Berrang *et al.,* 2001). Cross-contamination has also been identified as a major risk factor during processing (Rasschaert *et al.,* 2008). Intervention strategies are implemented at the pre harvest and postharvest levels to mitigate the risk of contamination of the poultry product by these pathogenic bacteria.

Globally, there is increased demand for antibiotic-free animal products, causing consumers to move towards the organic food market (Dimitri and Oberholtzer, 2009; Reisch *et al.,* 2013). This has impacted the poultry industry, where broiler meat harvested from alternative poultry farming production facilities, such as organic and free-range, have increased in demand (Van Loo *et al.,* 2011; Rothrock Jr. *et al.,* 2016). These types of operations are characterized by the lack of antibiotic use and the allowance of birds to access the outside environment. As such, birds are exposed to a less controlled environment, indicating an increased risk of microbial contamination of the birds.

Pathogens continue as a considerable threat to public health. Intensification of livestock production, especially poultry, facilitates diseases transmission by increasing population size and density (Feßler *et al*., 2011; Dhama *et al.,* 2014)

Studies by Timbermont *et al.* (2009) and Dolka *et al.* (2020) emphasized that poultry immunity, health and production are some of the factors that will challenge the future growth of the industry. Many foodborne and zoonotic diseases, emerging and re-emerging worldwide, are strictly linked with poultry farming. A foodborne disease outbreak is an incident during which at least two people contract the same illness from the same contaminated food or drink. Developing strategies to eliminate and control foodborne pathogens, while tackling the public health hazards linked to consuming foods with high antibiotic residues and the threat of antimicrobial resistance, remains as critical challenges for the industry.

Colibacillosis is the most common bacterial disease in poultry and it is caused by Avian Pathogenic *Escherichia coli* (APEC). It can be present among poultry of all ages. APEC is opportunistic in nature and it can grow rapidly in stress conditions. Initial exposure to APEC might occur in the hatchery from infected or contaminated eggs; however infections are commonly triggered by immunosuppressive diseases such as Infectious Bursal Disease, Mareks Disease, or Chicken Anemia. Colibacillosis is a major cause of morbidity, mortality, and economic loss for all types of poultry worldwide (Ramazani *et al.*, 2021).

Another common bacterium on poultry farms is *Salmonella*, which is pathogenic to both poultry and humans. Although the target habitat of Salmonella is the gastrointestinal tract, it is widely present in nature and makes a major microbial hazard in animal feed, as it can persist for long periods of time. Bacteria belonging to the *Salmonella* genus are responsible for a variety of acute and chronic diseases in poultry. Moreover, poultry flocks infected with this pathogen are the main zoonotic reservoir which can transmit infection through the food chain to humans, thus posing a serious health problem as well. Potential symptoms of *Salmonella* presence in birds include drowsiness, huddling together, poor growth, chalky white diarrhea, dehydration, reduced egg production, and increased mortality, among others. Salmonella symptoms, especially at subclinical level, can go unnoticed and not recognized as caused by *Salmonella* bacteria, while the birds' body is using its vital resources to fight off the bacteria instead of utilizing them for growth and productivity. This bacterium causes significant disturbances in chicken gut and it weakens overall bird health. *Salmonella* is the most reported cause of foodborne outbreaks in the European Union nearly one in three foodborne outbreaks in the EU in 2018 were caused by this bacterium. This is one of the main findings of the annual report on trends and sources of zoonoses published by the European Food Safety Authority (EFSA) and the European Centre for Disease Prevention and Control (ECDC). In 2020, EU Member States reported 3 166 foodborne outbreaks affecting 22,010 people. Over 56% of the outbreaks were linked to *Salmonella* (Ricke, 2021; Timbermont *et al.,* 2009).

In the United States, The Centre for Disease Control and Prevention (CDC) has cited about 1.35 million *Salmonella* infection cases and approximately 420 deaths in the United States every year.

Understanding the prevalence, distribution, and characteristics of these pathogens is essential for implementing effective disease control and prevention measures in poultry production systems. Therefore, studies focusing on the isolation and characterization of common pathogens associated with poultry production are crucial for enhancing biosecurity practices, improving animal welfare, and safeguarding public health.

1.2 Statement of Problem:

Limited research reports exist on how different pathogens affect the poultry production. However, poultry production faces numerous challenges, with disease outbreaks being a primary concern. Diseases not only lead to significant economic losses for poultry farmers but also pose potential threats to public health through the transmission of zoonotic pathogens.

The poultry industry is susceptible to various diseases that affect both layers and broilers, resulting in reduced production, increased mortality rates, and decreased profitability for farmers. However, there is a lack of comprehensive data on the prevalence and economic impact of these diseases in the local poultry population. Diseases affecting poultry can also have implications for human health, particularly if they are zoonotic in nature. The transmission of pathogens from infected birds to

humans through direct contact, consumption of contaminated poultry products, or environmental exposure represents a significant public health risk. However, the specific pathogens responsible for such risks and their prevalence in the local poultry population are not well-documented.

Effective disease management strategies rely on a thorough understanding of the pathogens present in the poultry population, their modes of transmission, and their susceptibility to available treatments. However, the lack of data on the specific pathogens affecting poultry hinders the development and implementation of targeted disease control measures.

Disease outbreaks in poultry farms can often be attributed to lapses in biosecurity practices and inadequate disease control measures. Without proper identification of the pathogens involved, it is challenging for poultry farmers to implement appropriate biosecurity protocols and preventive measures to mitigate disease spread effectively.

Sustainability and Food Sustainable poultry production is essential for ensuring food security and meeting the nutritional needs of the population. However, disease outbreaks can undermine the sustainability of poultry farming enterprises, leading to food shortages, increased prices, and reduced access to affordable protein sources for consumers.

This research is essential for informing targeted intervention strategies, improving biosecurity measures, safeguarding public health, and ensuring the sustainability and economic viability of the poultry industry in the region.

1.3 Objective of the Study:

The general objective of this study is to identify and characterize common pathogens associated with poultry production in Awka South Local Government Area of Anambra State with a view of drafting bio-security measures appropriate to farmers in this area.

1.4 Justification of the Study:

The study on isolating and characterizing common pathogens associated with poultry production is significant for several reasons. Understanding the prevalence and identity of pathogens affecting poultry is crucial for safeguarding public health. Many poultry pathogens have zoonotic potential, meaning they can be transmitted from animals to humans. By identifying these pathogens, we can implement measures to prevent human exposure and reduce the risk of disease outbreaks in both animal and human populations.

Knowledge of prevalent pathogens enables the development of effective disease management and control strategies. With accurate identification, targeted treatment protocols can be implemented to mitigate disease spread and minimize economic losses for poultry farmers. Additionally, understanding the antimicrobial susceptibility patterns of isolated pathogens can guide prudent antimicrobial use practices, thereby reducing the risk of antimicrobial resistance.

Disease outbreaks in poultry farms result in significant economic losses due to decreased productivity, increased mortality rates, and veterinary expenses. By identifying common pathogens and understanding their impact on poultry health, farmers can implement proactive measures to prevent disease transmission, reduce production losses, and enhance profitability. The study findings can inform the development and implementation of robust bio security measures tailored to the specific pathogens identified in the local poultry population. Improved bio security practices reduce the risk of disease introduction and transmission within and between poultry farms, thereby enhancing overall farm productivity and sustainability.

Sustainable poultry production is essential for ensuring long-term food security and environmental sustainability. By identifying and managing common pathogens, farmers can reduce reliance on prophylactic antimicrobials and promote environmentally friendly farming practices. Additionally, sustainable disease management strategies contribute to the resilience of the poultry industry in the face of emerging pathogens and changing environmental conditions.

Research findings on prevalent poultry pathogens can inform policy formulation and regulatory frameworks aimed at protecting public health, promoting animal welfare, and ensuring the sustainability of the poultry industry. Evidence-based policies can facilitate the adoption of best practices in disease management, bio security, and antimicrobial stewardship. The outcome of this study contributes to the advancement of disease management strategies, enhancement of bio security measures, promotion of sustainable farming practices, and formulation of evidence-based policies to support the poultry industry and protect public health.

II. MATERIALS AND METHODS

2.1 Study Area:

The study was carried out in Awka South Local Government Area Anambra State. Awka South Local Government Area lies in latitude 6°10'N and 6°15' N of the Equator and longitude 7°02'E and 7°07'E of the Greenwich Meridian. It has an average temperature of approximately 27°C, and an average humidity level of about 70 percent. The rainfall here is 2950mm per year. The local government comprised of 9 towns which are Amawbia, Awka, Ezinato, Isiagu, Mbaukwu, Nibo, Nise, Okpuno and Umuawulu with a population of about 270,300 and a density of 63.4sqm (Nigeria Media, 2023)

2.2 Sample Collection and Sampling Techniques:

Three towns were selected for this experiment using simple random sampling technique. One biggest farm was selected from each of the three towns (systematic random sampling) for this study. In each farm, 10 birds were sampled making it a total of 30 samples. A total number of thirty (30) freshly excreted feacal samples of chicken droppings were collected from the three towns in Awka South Local Government Area namely, Ify poultry farm Ifite Awka, KennyPassy Integrated farms Ltd Amawbia and Divine Favour poutlry farm Okpuno. They were properly labeled and transported with plastic test tubes to Amazing Grace Research laboratory, Nkwelle Izunaka in Oyi local government area of Anambra State, Nigeria for analysis. Each sample was prepared by making dilutions in distilled water.

2.3 Culture Media Preparation:

The media prepared were Salmonella Shigellar Agar, Macconkey Agar and Nutrient Agar for the isolation of bacteria respectively. They were prepared according to the manufacturer's instructions.

2.3.1 Salmonella-Shigellar (SS) Agar 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 microorganisms was achieved by the 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 or pink colonies. Lactose non-fermenters form colourless colonies.

2.3.2 MacConkey Agar Test:

MacConkey agar was the first solid differential media to be formulated which was developed in the 20th century by Alfred Theodore. MacConkey Agar is the earliest selective and differential medium for the cultivation of coliform organisms. It is used for the isolation and differentiation of non-fastidous gram-negative rods, particularly members of the family Enterobacteriaceae and the genius *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 15 minutes. It was then cooled to 40 mins to 50mins. The isolates were mixed well before pouring into sterile plates.

2.3.3 Nutrient Agar Test:

Nutrient Agar is a basic culture medium commonly used for the culture of non-fastidous microorganisms, 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. 28g of nutrient agar powder was suspended in 1L of distilled water; mixed and dissolved completely. It was sterilized by autoclaving at 121°C for 15 minutes. The liquid was poured into the Petri dish and wait for the medium to solidify. The agar was prepared in a clean environment to prevent any contamination. Once the agar solidifies, the agar is ready for use. The media forms light yellow colored clear to slightly opalescent gel on Petri plates after cooling.

2.4 Inoculation of the Media for Bacteria Isolation:

The test tubes containing the test samples were inoculated by streaking using a wire loop into both Macconkey and Salmonella Shigellar agar. 1ml of the test samples was transferred into a prepared Salmonella Shigellar Agar and Macconkey agar in culture plates and inoculated at 37°C for 24 hours (overnight). After 24 hours, the growth of organisms was checked. Fresh media was prepared, and then poured into the Petri dishes. Then, it was allowed to cool, it was subcultured with the aid of the wire loop. Then, it was incubated at 37°C for 24 hours (overnight) to get pure colonies.

2.5 Sensitivity Test:

Sensitivity test (the drug that can kill the organisms) was used to determine the actual active ingredient that can eradicate the organism. The various pure isolates were subjected to sensitivity test with commercial antibiotics discs using disc diffusion method. They were incubated at 37°C for 24 hours after which the zones of inhibitions were measured using a meter rule.

2.6 Characterization and Identification of the Isolates:

Different morphological and biochemical characteristics accompanied with colony characteristics on different selective medium were observed for the identification of isolates.

2.7 Biochemical Test:

The pure colonies were subjected to various biochemical tests for proper identification using methods as described by Chessburugh (2010). The biochemical tests carried out include Gram staining, Catalase test, Coagulase test, Oxidase test, Citrate utilization test, Indole test, Ureese test, Methyl red test, Voges proskauer test, Hydrogen sulphide test and Motility test.

2.7.1 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 Greece 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 strains for 60 seconds, rinse with clean water and drained quickly before it was flooded with Lugols iodine for 60 seconds and was then washed off with distilled water. The slide was flooded with 95% ethanol which is a decolourizer for 5 secs. After that, the slide was washed using distilled water and then flooded with Safranin (counter stain) for 30 seconds and then washed off. The back of the slide was then cleaned and placed in a draining rack for the stained smear to air dry. The standard smear was then allowed to air dry. Then, a drop of oil immersion was added on the smear to prior to viewing under the microscope for examination.

2.7.2 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 indicator for the presence of catalase was looked out for. This is done to differentiate between *Staphylococci* and *Streptococci*. Effervescence of gas indicates the presence of gram-positive organisms.

2.7.3 Coagulase Test:

For the analysis, a drop of sterile distilled water was added to both ends of the slide. A colony of the test organism was emulsified in each drop of water and a look full of plasma added to one of the suspension with thorough mixing. Change is observed within 10 seconds to determine the identity of the organism.

2.7.4 Oxidase Test:

Over-well isolated (pure culture) colonies of test bacteria from fresh culture; three drops of Kovac's oxidase reagent were added. Tilt the plate and shake it gently so that the colonies get exposed to oxygen. Observe for the formation of purple (deep blue) colour over the reagent moistened colonies and note the time requires for change in color for up to 60 secs. When the test is positive (+), there's development of purple to deep blue colour within 10-30 secs indicating a positive (+) oxidase test and development of purple to deep blue colour within 30-60 secs indicating a weak oxidase positive (+) reaction or delayed oxidase positive. When the test is negative (-), there is no development of purple to deep blue colour within 60 seconds and development of purple to deep blue colour after 60 seconds.

2.7.5 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 30°C for 24 hours. A colour change in the agar from green to blue indicates a positive (+) reaction.

2.7.6 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 24 hours. After incubation, few drops of Kovac's reagents were added to the tube and were shaken gently and allowed to stand. The pinkish and ring like color and the upper layer indicates Indole production in the tubes and if otherwise, no Indole production.

2.7.7 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 colour while no colour change is a negative (-) result.

2.7.8 Methyl Red Test:

Wire loops full of isolates under investigation were inoculated and incubated for 24 hours, three drops of methyl red solution was added and colour change observed. A colour change from light yellow to pink indicates a methyl red positive reaction, meaning that acid is produced, while no change in colour (colour remains yellow) indicated a methyl red negative (-) reaction.

2.7.9 Voges Proskauer Test (Vp):

Inoculate and voges proskauer broth tube with a pure culture of the test organism, then incubate for 24 hours at 35°C. at the end of this time measure 1ml of broth into a clean test tube. Add 0.6ml of 5% of naptol followed by 0.2 ml of 40% KOH. Shake the tube gently to expose the medium to atmospheric oxygen and allow the tube to remain undisturbed for 10-15 minutes. A positive VP test is development of a pink red colour at the surface within 15 minutes or more after the addition of the reagents. The rest should not be read after standing for over one hour because negative Voges-Proskauer cultures may produce a colourless colour, resulting in a false positive interpretation.

2.7.10 Hydrogen Sulphide:

Using a sterile rod, innoculate 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 37°C for about 24 hours. Observe the colour of the developed colonies. Black colonies or colourless or coloured colonies with a black center will appear.

2.7.11 Motility Test:

With a sterile straight needle, touch a colony of a young (18-24 hours) culture growing up on an agar medium, then single stab down the center of the tube to about half the development of the medium, then incubate at 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's non motile, the organism remains in the site of inoculation.

2.8 Experimental Design:

This study was conducted using CRD (Complete Randomized Design), where the towns are the treatment and the poultry units within the towns are the replicates.

Given the formula,

$$Yij = \mu + Ti + Eij \quad (1)$$

where;

Yij= single observation made on jth observation and ith treatment

μ = Overall mean

Ti = Effect of treatment (i=1,2,.....n)

Eij= Random error.

2.9 Statistical Analysis:

Analysis of Variance (ANOVA) was carried out using SPSS version 17, and differences between the treatment means were separated using Duncans New Multiple Range Test.

III. RESULTS AND DISCUSSIONS

3.1 Characterization of pathogens:

Biochemical reaction patterns used include: colony morphology, gram reactions, catalase, oxidase, coagulase, citrate, indole, urease, methyl red, voges Proskauer, hydrogen sulphide and motility tests.

3.1.1 Isolated pathogens from Ifite town:

A total of four dominant isolates were obtained from 10 faecal samples collected from Ifite village in Awka South Local Government Area.

The biochemical tests carried out using isolates from Ifite town revealed the organism to be *E-coli*, Streptococcal *spp*, *Salmonella spp* and *Shigella spp.* (Table 1).

TABLE 1
BIOCHEMICAL REACTION OF THE ISOLATES FROM IFITE

Colony Morphology	Probable Organisms	Gram Reaction	Catalase	Coaguse	Oxidase	Citrate	Indole	Urease	Methl Red	VP	H2s	Motility
Pink Smooth	*E-coli*	-	+	-	-	-	+	-	+	-	-	+
White colonies in chains	*Streptococcal spp*	+	-	-	-	-	-	-	-	-	-	-
Dark coloured rods	*Salmonella*	-	+	-	+	-	-	-	+	+	+	+
Pink rod like colours	*Shigella*	-	+	-	-	-	+	-	+	-	-	-

3.1.2 Isolated pathogens in Okpuno town:

A total of five dominant isolates were obtained from 30 faecal samples collected from Okpuno in Awka South Local Government Area. The biochemical tests carried out using isolates from Okpuno town revealed the organism to be *E-coli*, Streptococcal *spp, Salmonella spp*, *Enterococcus spp* and *Shigella spp.* as presented in Table 2.

TABLE 2
BIOCHEMICAL REACTION OF THE ISOLATES FROM OKPUNO

Colony Morphology	Probable Organisms	Gram Reaction	Catalase	Coaguse	Oxidase	Citrate	Indole	Urease	Methl Red	VP	H2s	Motility
Dark coloured rods	Salmonella spp	-	+	-	+	-	-	-	+	-	+	+
Pink rods	*Shigella spp*	-	+	-	-	-	-	-	+	-	-	-
Pink smooth colonies	*E-coli*	-	+	-	-	-	+	-	+	-	-	+
Small white colonies in chain	*Streptococcal spp*	+	-	-	-	-	-	-	-	-	-	-
Colourless small colonies	*Enterococcus spp*	+	-	-	-	-	-	-	-	+	-	-

3.1.3 Isolated pathogens in Amawbia town:

A total of six dominant isolates were obtained from 30 faecal samples collected from Amawbia in Awka South Local Government Area.

Table 3 presents the biochemical tests carried out using isolates from Amawbia town. The organisms identified include *E-coli,* Streptococcal *spp, Salmonella spp, Staphylococcal spp, Enterococcus spp* an *Shigella spp.*

TABLE 3
BIOCHEMICAL REACTION PATTERN OF ISOLATES FROM AMAWBIA

Colony morphology	Probable Organisms	Gram rxn	Catalase	Coagulase	Oxidase	Citrate	Indole	Urease	Methyl red	Vp	H2s	Motility
Pink smooth colonies	*E-coli*	-	+	-	-	-	+	-	+	-	-	+
Dark colored rods	*Salmonella spp*	-	+	-	+	-	-	-	+	-	+	+
Pink rods	*Shigella spp*	-	+	-	-	-	+	-	+	-	-	-
Small white colonies in chain	*Streptococcal spp*	+	-	-	-	-	-	-	-	_	-	-
Clustered cocci	*Staphylococcal spp*	+	+	-	-	+	-	+	+	+	-	-
Colourless small colonies	*Enterococcus spp*	-	+	-	-	+	-	+	-	+	-	+

3.2 Common pathogens identified in poultry industries in Awka south local government area of Anambra state, Nigeria

The summary of the bacterial isolates identified in Ifite (T1), Okpuno (T2) and Amawbia (T3) are presented in Table 4.

TABLE 4
THE SUMMARY OF THE BACTERIAL ISOLATES IDENTIFIED IN IFITE (T1), OKPUNO (T2) AND AMAWBIA (T3)

Isolates	Ifite (T1)	Okpuno (T2)	Amawbia (T3)
E-Coli	+	+	+
Salmonella spp	+	+	+
Shigella spp	+	+	+
Streptococcal spp	+	+	+
Staphylococcal spp	_	_	+

In Amawbia, virtually all the bacterial isolates studied were present while *Staphloccoccal spp* was absent in Okpuno. So, *E.coli, Salmonella spp, Shigella spp and Streptococcal spp* are more endemic in Awka South Local Government Area.

3.3 Sensitivity test of the identified pathogens:

These isolates were subjected to sensitivity tests with commercially prepared antibiotics discs using disc diffusion methods.

3.3.1 Sensitivity test of the identified pathogens from ifite town:

These isolates were subjected to sensitivity tests with commercially prepared antibiotics discs using disc diffusion methods and the results showed that only *salmonella spp* were sensitive to Peflacine with inhibitor zone diameter of 17mm, Gentamicin with zone diameter diameter of 17mm and Nalidixic acid with zone diameter of 12mm.

3.3.2 Sensitivity test of the identified pathogens in Okpuno town:

These isolates were subjected to sensitivity tests with commercially prepared antibiotics discs using disc diffusion methods and the results showed that *Salmonella spp* were sensitive to Ofloxacin with zone diameter of 20mm, Ceporex (16mm), Nalidix acid (15mm), Pelacine (12mm), Ciprpflaxin (13mm) and Streptomycin (10mm).

Also, *shigella spp* were sensitive to Oflaxacin with zone of inhibitor of 17mm, Peflacine (12mm) and Ciproflaxin (10mm). Both *E-coli spp, strept spp and entercoccus spp* were all resistant to the antibiotics.

3.3.3 Sensitivity test of the identified pathogens in Amawbia town:

These isolates were subjected to sensitivity tests with commercially prepared antibiotics discs using disc diffusion methods and the results showed that only *streptococcal spp* were sensitive to Rifampin with inhibitor zone diameter of 20mm, Gentamicin (17mm), Streptomycin (15mm), Amoxicillin (20mm) and Ampiclox (20mm). Table 5 presents the summary of the sensitivity pattern of the isolates from the three towns studied

TABLE 5
SUMMARY OF THE SENSITIVITY PATTERN OF THE ISOLATES FROM THE THREE TOWNS STUDIED

Isolates	Ifite (T1)	Okpuno (T2)	Amawbia (T3)
E-Coli	Resistant	Resistant	Resistant
Salmonella spp	Resistant	Sensitive	Resistant
Shigella spp	Sensitive	Sensitive	Resistant
Streptococcal spp	Resistant	Resistant	Resistant
Staphylococcal spp	-	-	Sensitive
Enterococcus spp	-	Resistant	Resistant

3.4 Location effect on the number of bacteria isolates:

The effect of location on the number of the bacteria isolates obtained at poultry industries in Awka South Local Government Area of Anambra State, Nigeria is presented in Table 6.

TABLE 6
EFFECT OF LOCATION ON THE NUMBER OF BACTERIA ISOLATES

Isolates	Ifite (T1)	Okpuno (T2)	Amawbia (T3)
E-Coli	10	10	10
Salmonella Spp	10	10	10
Shigella Spp	10	10	10
Streptococcal Spp	10	10	10
Staphylococcal Spp	0.00b	0.00b	10.00a
Enterococcus Spp	0.00b	10.00a	10.00a
Key = + Indicates Presence of A Given Isolate While – Indicates Its Absence.			
Means Bearing Different Letter along with the Same Rows are Significantly Different (P<0.05)			

Amawbia poultry farms recorded the highest number of isolates followed by Okpuno and Ifite being the least.

IV. DISCUSSION

4.1 Escherichia Coli (*E. Coli*):

*Escherichia coli*is 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.

E. coli was identified in all three towns: Ifite, Okpuno, and Amawbia. Similar to findings by Akinpelu *et al.* (2023), who reported a high prevalence of *E. coli* in poultry farms in Southwestern Nigeria, our results confirm its ubiquitous presence in poultry environments. The researchers also highlighted that *E. coli* thrives in unhygienic conditions, which may explain its widespread occurrence in Awka South Local Government Area. *E. coli* infections can cause colibacillosis in poultry, leading to respiratory infections, reduced productivity, and increased mortality rates. Its presence suggests poor biosecurity measures. *E. coli* isolates from all towns showed resistance to antibiotics tested. Resistance could be due to the overuse of antibiotics in feed and treatment, as observed by Roth *et al. (*2019). Antibiotic-resistant *E. coli* strains pose challenges in managing infections in poultry. Antibiotic-resistant *E. coli* in poultry may transfer to humans via contaminated meat or direct contact, potentially causing foodborne illnesses or urinary tract infections (UTIs). This is consistent with findings by Kumar *et al.* (2019).

4.2 *Salmonella Spp.:*

Salmonella was present in all the towns studied, but exhibited varying antibiotic sensitivity. 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. The prevalence aligns with findings by Ugbo *et al.* (2018), who reported high *Salmonella* presence in poultry farms in Eastern Nigeria. In contrast, our results showed variable sensitivity to antibiotics. *Salmonella* causes salmonellosis in poultry, leading to economic losses through poor growth and egg production. It also increases the cost of treatment when resistant strains emerge. Resistance patterns varied, with isolates from Okpuno being the most sensitive. 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. Misuse of antibiotics in poultry farming, as noted by Olovo *et al.* (2019), may contribute to this resistance. 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. Drug-resistant *Salmonella* strains can cause severe foodborne outbreaks in humans. Contaminated poultry products are a major source of infection.

4.3 *Shigella Spp:*

The genus *Shigella* was first identified by Japanese microbiologist Kiyoshi Shiga in 1897 during an outbreak of dysentery. *Shigella* was found in Ifite and Okpuno but absent in Amawbia. *Shigella's* occurrence is less common in poultry compared to *Salmonella*, but our findings are consistent with studies like Odo *et al.* (2021), who identified *Shigella* in poultry litter in Nigeria. While *Shigella* primarily infects humans, its presence in poultry indicates possible contamination through water or feed, posing risks of zoonotic transmission. *Shigella* spp from Okpuno showed sensitivity to multiple antibiotics, suggesting recent introduction into the ecosystem. However, Ifite isolates showed limited sensitivity, likely due to previous exposure to antimicrobial agents. 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. *Shigella* causes dysentery in humans, with resistant strains exacerbating outbreaks. Zoonotic transfer is possible, especially in unsanitary conditions, as noted by Jenkins *et al.*, (2023).

4.4 *Streptococcal Spp.:*

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 identified in all towns, with the highest sensitivity recorded in Amawbia. Our findings align with that of Odeyemi *et al.* (2019) who noted *Streptococcal spp* in poultry environments as opportunistic pathogens. These bacteria can cause infections such as septicemia in poultry, leading to reduced productivity. Resistant strains found in Ifite and Okpuno indicates prolonged antibiotic misuse in these towns. However, Amawbia isolates showed sensitivity to Rifampin and Amoxicillin, indicating less exposure to these drugs. 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. *Streptococcal* infections in humans, like pharyngitis or invasive diseases, could arise through zoonotic transmission, especially when handling infected birds without proper hygiene.

4.5 *Staphylococcal Spp.:*

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. *Staphylococcus* was identified only in Amawbia. This is consistent with the findings of Ezeh *et al*. (2023) and Islam *et al*. (2023), who found *Staphylococcus* to be less prevalent in rural poultry farms compared to urban areas. *Staphylococcus* causes conditions like bumblefoot and septicemia, affecting bird welfare and economic outputs. Sensitive to antibiotics like Ampiclox and Amoxicillin is indicating the possibility of effective treatment of the ailment caused by the pathogen in Amawbia. The occurrence of antibiotic-resistant *Staphylococcal spp*. in poultry farms could present significant challenges. Infected birds may exhibit prolonged illness, reduced growth rates, and increased mortality, leading to economic losses. *Staphylococcus aureus*, particularly drug-resistant strains (e.g., MRSA), poses significant risks to humans, especially farm workers. *Staphylococcus* infections in humans can range from mild skin infections to severe, life-threatening conditions, such as sepsis.

4.6 *Enterococcus Spp.:*

Enterococcus species are part of the normal gut flora in animals, including poultry, and can be found in various environmental sources. Although many *Enterococcus* strains are commensal, some can cause infections under certain conditions, particularly in immune compromised hosts. It was detected only in Okpuno and Amawbia. *Enterococcus spp* have been reported by Chukwu *et al*, (2022) in poultry farms with high antimicrobial use. *Enterococcus* infections in poultry, though rare, can complicate other diseases. Their presence suggests environmental contamination. Both towns showed high resistance, which is troubling since *Enterococcus* can act as a reservoir for resistance genes, as highlighted by Morgan *et al*.(2023). 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. This can make bacterial infections harder to treat, leading to increased disease incidence and economic losses.

Enterococcus spp, particularly vancomycin-resistant strains (VRE), pose serious health risks in clinical settings. 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.

V. CONCLUSIONS

This study underscores the prevalence of bacterial pathogens in poultry industries in Awka South Loal Government of Anambra State. The major pathogens include *E-coli spp., Salmonella spp., Shigella spp., Streptococcal spp., Staphylococcal spp.* and *Enterococcus spp.* These pathogens pose significant economic losses to the poultry industry and public health risks due to their zoonotic potential.

Again, poultry farmers in this area are not lettered, and as such are not practicing proper management and bio-security measures. Equally, they use sub-lethal levels/ doses of antibiotics when confronting these pathogens. Hence, most of these antibiotics are resistant to commonly used antibiotics. The most resistant isolates identified were *E. coli spp., Salmonella spp., Shigella spp. and Streptococcal spp.* which are very common in the area.

The study therefore calls for close monitoring of antibiotic resistance in our environment and controlled use of antibiotics in poultry industries since these pathogens are zoonotic, and birds serve as the major source of protein in many homes. The use of the bird droppings for manure should be checked, because of the health hazard to the general populace when crops are consumed.

RECOMMENDATIONS

A multi-faceted approach is essential in combating the menace of bacterial pathogens in Awka south local government of Anambra state. Rigorous bio security measures, such as strict hygiene protocols, controlled access, and vaccination programs are pivotal in preventing disease outbreaks. Additionally, judicious antibiotic use and antimicrobial stewardship are crucial to combat antimicrobial resistance and maintain the efficacy of treatments.

REFERENCES

[1] Akinpelu, D. A., Adekoya, O. A., Oladoye, P. O., Ogbaga, C. C., & Okolie, J. A. (2023). Machine learning applications in biomass pyrolysis: from biorefinery to end-of-life product management. *Digital Chemical Engineering*, *8*, 100103.

[2] Akinpelu *et al.* (2023)

[3] Berrang, M. E., Buhr, R. J., Cason, J. A., & Dickens, J. A. (2001). Broiler carcass contamination with Campylobacter from feces during defeathering. *Journal of food protection*, 64(12), 2063-2066.

[4] Bull, S. A., Allen, V. M., Domingue, G., Jørgensen, F., Frost, J. A., Ure, R., ... & Humphrey, T. J. (2006). Sources of Campylobacter spp. colonizing housed broiler flocks during rearing. *Applied and environmental microbiology*, 72(1), 645-652.

[5] Chukwu, C. A., Mahmood, K., Elmakki, S., Gorton, J., Kalra, P. A., Poulikakos, D., & Middleton, R. (2022). Evaluating the antibody response to SARS-COV-2 vaccination amongst kidney transplant recipients at a single nephrology centre. *PLoS One*, *17*(3), e0265130.

[6] Dhama, K., Tiwari, R., Chakraborty, S., Saminathan, M., Kumar, A., Karthik, K., ... & Rahal, A. (2014). Evidence based antibacterial potentials of medicinal plants and herbs countering bacterial pathogens especially in the era of emerging drug resistance: an integrated update. *International Journal of Pharmacology*, 10(1), 1-43.

[7] Dimitri, C., & Oberholtzer, L. (2009). Meeting market demand in the organic sector: Handler–supplier relationships in the face of tight supply. *Renewable Agriculture and Food Systems*, 24(2), 137-145.

[8] Dolka, B., Czopowicz, M., Chrobak-Chmiel, D., Ledwoń, A., & Szeleszczuk, P. (2020). Prevalence, antibiotic susceptibility and virulence factors of Enterococcus species in racing pigeons (Columba livia f. domestica*). BMC veterinary research,* 16, 1-14.

[9] European, T.; One, U.; Report, Z. The European Union One Health 2020 Zoonoses Report. EFSA J. 2021,19, e06971

[10] Ezeh, C. K., Eze, C. N., Dibua, M. E. U., & Emencheta, S. C. (2023). A meta-analysis on the prevalence of resistance of Staphylococcus aureus to different antibiotics in Nigeria. *Antimicrobial Resistance & Infection Control*, *12*(1), 40.

[11] Farrell, T. S. (2013). Reflecting on ESL teacher expertise: A case study. *System*, *41*(4), 1070-1082.

[12] Feßler, A. T., Kadlec, K., Hassel, M., Hauschild, T., Eidam, C., Ehricht, R., ... & Schwarz, S. (2011). Characterization of methicillin-resistant Staphylococcus aureus isolates from food and food products of poultry origin in Germany, *Applied and environmental microbiology*, 77(20), 7151-7157.

[13] Gržinić, G., Piotrowicz-Cieślak, A., Klimkowicz-Pawlas, A., Górny, R. L., Ławniczek-Wałczyk, A., Piechowicz, L., ... & Wolska, L. (2023). Intensive poultry farming: A review of the impact on the environment and human health. *Science of the Total Environment*, 858, 160014.

[14] Heyndrickx, M., Vandekerchove, D., Herman, L., Rollier, I., Grijspeerdt, K., & De Zutter, L. (2002). Routes for Salmonella contamination of poultry meat: epidemiological study from hatchery to slaughterhouse. *Epidemiology & Infection*, 129(2): 253-265.

[15] Islam, M. S., Nayeem, M. M. H., Sobur, M. A., Ievy, S., Islam, M. A., Rahman, S., ... & Rahman, M. T. (2021). Virulence determinants and multidrug resistance of Escherichia coli isolated from migratory birds. *Antibiotics*, 10(2): 190.

[16] Jenkin, D., Wright, D., Folegatti, P. M., Platt, A., Poulton, I., Lawrie, A., ... & Warimwe, G. M. (2023). Safety and immunogenicity of a ChAdOx1 vaccine against Rift Valley fever in UK adults: an open-label, non-randomised, first-in-human phase 1 clinical trial. *The Lancet Infectious Diseases*, *23*(8): 956-964.

[17] Kumar, D., Pornsukarom, S., & Thakur, S. (2019). Antibiotic usage in poultry production and antimicrobial-resistant Salmonella in poultry. Food safety in poultry meat production, 47-66.

[18] Morgan, A. L., Woolhouse, M. E., Wagenaar, J. A., & van Bunnik, B. A. (2023). Modelling the effects of antibiotic usage in livestock on human salmonellosis. *One Health*, 17, 100639.

[19] Olovo, C. V., Reward, E. E., Obi, S. N., & Ike, A. C. (2019). Isolation, identification and antibiogram of salmonella from cloacal swabs of free range poultry in Nsukka, Nigeria. J. Adv. Microbiol, 1, 1-9.

[20] Odo, D. B., Yang, I. A., & Knibbs, L. D. (2021). A systematic review and appraisal of epidemiological studies on household fuel use and its health effects using demographic and health surveys. *International journal of environmental research and public health*, *18*(4), 1411.

[21] Odeyemi, O. A., Alegbeleye, O. O., Strateva, M., & Stratev, D. (2019). Understanding spoilage microbial community and spoilage mechanisms in foods of animal origin. *Comprehensive reviews in food science and food safety*, *19*(2), 311-331.

[22] Ramazani, E., Emami, S. A., Tayarani-Najaran, N., Sahebkar, A., & Tayarani-Najaran, Z. (2021). Antiviral plants in view of avicenna's the canon of medicine and modern medicine against common cold. *Natural Products and Human Diseases: Pharmacology, Molecular Targets, and Therapeutic Benefits*, 99-121.

[23] Rasschaert, G., Houf, K., Godard, C., Wildemauwe, C., Pastuszczak-Frak, M., & De Zutter, L. (2008). Contamination of carcasses with Salmonella during poultry slaughter. *Journal of Food Protection*, 71(1), 146-152.

[24] Reisch, L., Eberle, U., & Lorek, S. (2013). Sustainable food consumption: an overview of contemporary issues and policies. *Sustainability: Science, Practice and Policy*, 9(2), 7-25.

[25] Ricke, S. C. (2003). Perspectives on the use of organic acids and short chain fatty acids as antimicrobials. *Poultry science*, 82(4), 632-639.

[26] Ricke, S. C. (2021). Strategies to improve poultry food safety, a landscape review. *Annual review of animal biosciences*, 9(1), 379-400.

[27] Rothrock Jr, M. J., Keen, P. L., Cook, K. L., Durso, L. M., Franklin, A. M., & Dungan, R. S. (2016). How should we be determining background and baseline antibiotic resistance levels in agroecosystem research?. *Journal of environmental quality*, 45(2), 420-431.

[28] Sierżant, K., Korzeniowska, M., Orda, J., Wojdyło, A., Gondret, F., & Półbrat, T. (2021). The effect of rosemary (Rosmarinus officinalis) and blackcurrant extracts (Ribes nigrum) supplementation on performance indices and oxidative stability of chicken broiler meat. *Animals*, 11(4), 1155

[29] Timbermont, L., Lanckriet, A., Gholamiandehkordi, A. R., Pasmans, F., Martel, A., Haesebrouck, F., ... & Van Immerseel, F. (2009). Origin of Clostridium perfringens isolates determines the ability to induce necrotic enteritis in broilers. *Comparative immunology, microbiology and infectious diseases*, 32(6), 503-512.

[30] Ugbo, E. N., Moses, I. B., Orji, J. O., Ukpai, E. G., Eluu, S. C., Egbule, C. U., ... & Okata-Nwali, D. O. (2018). Antimicrobial susceptibility patterns of uropathogenic microorganisms associated with vesico-vaginal fistula (VVF) patients in Abakaliki, South eastern Nigeria. *African Journal of Microbiology Research*, *12*(46), 1039-1043.

[31] Van Loo, E. J., Caputo, V., Nayga Jr, R. M., Meullenet, J. F., & Ricke, S. C. (2011). Consumers' willingness to pay for organic chicken breast: Evidence from choice experiment. *Food quality and preference*, 22(7), 603-613.