Tropical Environment, Biology, and Technology

Research Article
Volume 1(2), 2023, 67–75
https://doi.org/10.53623/tbhr.v1i2.283

Evaluation of the Antibiotic Susceptibility Patterns of Isolates from Feed and Water of Selected Poultry Farms in Awka Anambra State, Nigeria

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SUBMITTED: 26 July 2023; REVISED: 11 September 2023; ACCEPTED: 18 September 2023

ABSTRACT: The susceptibility of microorganisms isolated from poultry feeds and poultry water samples to selected antibiotics was assessed. Standard methods were used to analyze selected poultry feeds and poultry water samples. The antibiotic susceptibility patterns of the bacterial isolates were determined against the following antibacterial agents: erythromycin (10 µg), ciprofloxacin (10 µg), ampiclox (20 µg), rifampicin (20 µg), amoxil (20 µg), septrin (30 µg), ampicillin (30 µg), ceporex (10 µg), levofloxacin (20 µg), gentamicin (10 µg), streptomycin (30 µg), norfloxacin (10 µg), chloramphenicol (30 µg), ofloxacin (10 µg), nalidixic acid (30 µg), reflecine (10 µg), and augmentin (30 µg).The highest viable counts of bacteria isolated from poultry feed and water samples were $2.7x10^6$ cfu/g and $1.69x10^3$ cfu/ml, respectively. The highest fungal counts in the poultry feed and water samples were $1.60x10^5$ cfu/g and $2x10^5$ cfu/ml, respectively. Bacterial isolates from poultry feed and water samples included *Escherichia coli*, *Klebsiella pneumoniae*, Salmonella species, and *Staphylococcus aureus*. Fungal isolates included Aspergillus species, Penicillium species, Mucor species, and Candida species.*Staphylococcus aureus* exhibited the highest susceptibility to most of the antibiotics, while *Klebsiella pneumoniae* showed the highest resistance, as it was resistant to five out of the ten antibiotics tested in this study. The research has demonstrated that poultry feed and poultry water showed varying levels of contamination, which may pose serious health risks to poultry. Amoxil, levofloxacin, ciprofloxacin, reflecine, and ofloxacin are recommended for use as antibiotics to treat diseases that may be caused by some of these pathogens.

KEYWORDS: Antibiotics; microorganisms; poultry feed; poultry water; susceptibility

1. Introduction

Poultry are domestic birds raised by humans for their meat, eggs, or feathers. A poultry farm refers to a place where domesticated birds are raised to produce meat or eggs for food [1].

Approximately 89% of the world's poultry meat production is contributed by domestic chickens, making them the most important poultry species globally [2]. Poultry farming involves raising domesticated birds to produce eggs or meat for consumption. Annually, humans consume approximately 60 billion chickens. Chickens raised for meat are known as broilers, while those raised for eggs are called layers [3]. Poultry feed consists of a mixture of different ingredients designed to provide the essential nutrients required for the growth and development of poultry birds. The Modern poultry feeds consist largely of grain, protein supplements such as soybean oil meal, mineral supplements, and vitamin supplements. [4]. Poultry water refers to water made available to poultry birds for drinking and other purposes. Water is essential for poultry birds and plays a vital role in various physiological and metabolic processes, including digestion and egg production [5].

Antibiotics are drugs that either kill or inhibit the growth of bacterial pathogens. They are classified as bactericidal if they kill bacteria or bacteriostatic if they slow bacterial growth [6]. Microorganisms in poultry farms play both beneficial and harmful roles. Beneficial microorganisms like probiotics improve poultry health and vitality by enhancing immunity, among other functions. Microorganisms implicated in poultry farm contamination include Escherichia coli, Campylobacter spp., Lactobacillus spp., as well as various yeasts and molds [7]. Feed materials of animal origin are often contaminated with Salmonella. To prevent Salmonella contamination, it is essential to source and use Salmonella-negative feedstuffs in feed-diet formulation. Heat treatments are commonly employed to ensure the microbial quality and safety of animal feeds [8]. The growth of the poultry industry is frequently limited by various diseases. Poultry infections can lead to diseases with varying symptoms, depending on the type of infection [1]. Some fungal diseases of poultry include aspergillosis, candidiasis, dactylariosis, cryptococcosis, favus, rhodotorulic infections, torulopsis, mucormycosis, histoplasmosis, and cryptococcosis. Additionally, some bacterial diseases of poultry include peritonitis, polyserositis, colibacillosis, mycoplasmosis (chronic respiratory disease), and salmonellosis [1, 9]. Antibiotic susceptibility testing (AST) is a laboratory procedure used to identify which antibiotic regimen is effective in treating specific patients. The ability of bacteria to be killed or inhibited by a particular antibiotic is referred to as antibiotic susceptibility. Antibiotic susceptibility is crucial in selecting the appropriate antibiotic for treating specific bacterial infections [9, 10]. The aim of this research was to determine the antibiotic susceptibility pattern of bacterial isolates from feed and water samples collected from selected poultry farms in Awka, Anambra State, Nigeria

2. Materials and Methods

2.1. Study area.

The research was conducted in Awka, the capital of Anambra State, situated in the southeastern region of Nigeria, with geographical coordinates of 6.2222° N and 7.0821° E. Samples were collected from five (5) distinct poultry farms within the study area.

2.2. Sample collection.

Composite samples of feed and water were systematically collected from five different commercial poultry farms in Awka, Anambra State, utilizing the simple random sampling method. Sterile containers and spatulas were used to collect feed samples, while sterile

bottles were employed for poultry water samples. Without delay, all collected samples were transported to the microbiology laboratory at Nnamdi Azikwe University for subsequent analysis.The antibiotics used in this study were procured from a pharmacy store located within the study area. A total of seventeen (17) antibiotics were employed for susceptibility testing against the bacterial isolates and they include erythromycin erythromycin (10 µg), ciprofloxacin (10 µg), ampiclox (20 µg), rifampicin (20 µg), amoxil (20 µg), septrin (30 µg), ampicillin (30 µg), ceporex (10 µg), levofloxacin (20 µg), gentamicin (10 µg), streptomycin (30 µg), norfloxacin (10 µg), chloramphenicol (30 µg), ofloxacin (10 µg), nalidixic acid (30 µg), reflecine (10 µg), and augmentin (30 µg).

2.3 Isolation, characterization and identification of bacterial and fungal isolates.

Enumeration and isolation of microorganismswas done using previously described methods by [11]. Biochemical tests including Gram staining, motility, catalase, coagulase, oxidase, urease, voges-Proskauer, indole, methyl red, citrate utilization, sugar fermentation as well as Lactophenol Cotton Blue staining tests were carried out to identify the isolated organisms using previous standard methods as described by [12].

2.4 Antibiotic susceptibility test.

Antibiotic susceptibility testing was conducted on all bacterial isolates using the disc diffusion method. Pure bacterial isolates were individually streaked evenly on appropriately labeled agar plates. The inoculum from the bacterial pure cultures obtained from poultry feed and water samples of selected poultry farms was prepared by suspending bacterial cells in a sterile broth solution. The turbidity of the suspension was adjusted to match the McFarland standard.Subsequently, agar plates were inoculated with the broth culture containing the bacterial suspension using a sterile wire loop. Following this step, the inoculums were allowed to dry at room temperature for 30 minutes. After drying, antibiotic-impregnated discs were applied to the surface of the inoculated plates using sterile forceps. These antibiotic disks were carefully placed onto the agar surface and incubated. The plates were then observed for bacterial growth, and the zone of inhibition around each antibiotic disk was measured using a meter rule.To interpret the results and determine the susceptibility of the bacterial strain to each antibiotic, the zone diameters of each drug were compared with the standards provided by the Clinical and Laboratory Standards Institute [13]. After 24 hours of incubation, the antibiotic susceptibility results for each bacterial isolate against each antibiotic were determined by measuring the zones of inhibition using a ruler. The interpretation of the zone of inhibition results was based on established standards for each microorganism and antimicrobial agent, typically provided by the Clinical and Laboratory Standards Institute [13].

3. Results and Discussion

3.1. Plate counts of microorganisms from poultry feed and poultry water samples.

Table 1 shows the total viable count of bacteria isolated from the poultry feed and poultry water samples. Sample B had the highest viable count of 2.7×10^6 cfu/g, while Sample A had the lowest viable count of 1.47×10^6 cfu/g for poultry feed. In the case of poultry water

samples, Sample F had the highest viable count of 1.69 x 10^3 cfu/ml, while Sample J had the lowest viable count of 1.20 x 10^3 cfu/ml.

Table 1. Total heterotrophic bacterial count of the poultry feed and water samples.

Poultry Feed Samples	**(10^6 cfu/g)**
A	1.47
B	2.70
C	1.80
D	2.00
E	2.10
Poultry Water Samples	**(10^3 cfu/ml)**
F	1.69
G	1.60
H	1.52
I	1.40
J	1.20

Tables 2 shows the total Fungal count of the poultry feed and water samples. Sample A had the highest count of 1.60 x10^5 cfu/g and sample C and D had the least count of 1.00 x 10^5 cfu/g for poultry feed. Sample H had the highest count of 2.00 x10^5 cfu/ml and sample I had the least count of 1.20 x 10^5 cfu/ml for poultry water. Figure 1 shows the prevalence of microorganisms in poultry feed and poultry water.

Table 2. Total fungal count of the poultry feed and watersamples.

Poultry FeedSamples	**(10^5 cfu/g)**
A	1.60
B	1.10
C	1.00
D	1.00
E	1.30
Poultry WaterSamples	**(10^5 cfu/ml)**
F	1.50
G	1.30
H	2.00
I	1.20
J	1.40

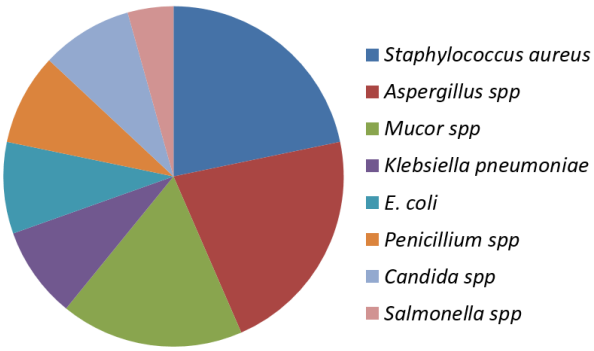

Figure 1. Prevalence of microorganisms in poultry feed and poultry water.

3.2. Morphological and biochemical properties of microorganisms from poultry feed and water samples.

Table 3 shows the morphological characteristics of the bacteria isolates for poultry feed and poultry water respectively. The morphological features were grouped based on their color, shape, margin and elevation on the plates.

Table 3. Morphological characteristics of the bacteria isolates from poultry feed and water samples.

Poultry FeedIsolates	Shape	Color	Elevation	Margin
A	Irregular	Creamy	Flat	Entire
B	Circular	Milky	Convex	Entire
C	Circular	Creamy	Flat	Entire
D	Circular	Creamy	Convex	Entire
E	Irregular	Creamy	Flat	Entire
Poultry Water Isolates	**Shape**	**Color**	**Elevation**	**Margin**
F	Circular	Milky	Convex	Entire
G	Circular	White	Convex	Entire
H	irregular	Creamy	Flat	Entire
I	Circular	Creamy	Flat	Entire
J	Circular	Creamy	Convex	Entire

The biochemical properties of the bacterial isolates are presented in Table 4. The probable organisms were identified as *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Salmonella* spp.

Table 4. Biochemical properties of bacterial isolates from the poultry feed and poultry water samples.

Isolates	Gram rxn	Form	I	C	MR	VP	U	C	M	G	F	Probable organism
A	+	Cocci	-	+	+	+	+	+	-	A/G	A/G	*Staphylococcus* sp
B	-	Rods	-	+	+	-	-	+	+	A/G	A/G	*Escherichia coli*
C	+	Cocci	-	+	+	+	+	+	-	A/G	A/G	*Staphylococcus* sp
D	-	Rods	-	+	-	+	+	+	-	A/G	A/G	*Klebsiella* sp
E	+	Cocci	+	+	+	+	+	+	-	A/G	A/G	*Staphylococcus* sp
F	-	Rods	+	+	+	-	-	+	+	A/G	A/G	*Escherichia coli*
G	-	Rods	-	+	-	-	+	+	+	A/G	A/G	*Klebsiella* sp
H	+	Cocci	-	+	+	+	+	+	-	A/G	A/G	*Staphylococcus* sp
I	+	Cocci	-	+	+	+	+	+	-	A/G	A/G	*Staphylococcus* sp
J	-	Rods	-	+	+	-	-	-	+	A/G	A/G	*Salmonella* sp

Key: I : Indole; C : Catalase; MR= Methyl red; VP = Voges Proskauer; U : Urease; C : Citrate; M : Motility; G : Glucose; F : Fructose + = Positive; - = Negative;; A/G + Acid and gas production; rxn = reaction; A,B,C,D,E = Poultry feed bacterial isolates; F,G,H,I,J = Poultry water bacterial isolates.

Fungi isolated were grouped based on the macroscopic and microscopic identification s presented in Table 5, which specifies their fungi type, color, underside color, texture, hyphae, and spore formation.The identified fungi include *Aspergillus spp.*, *Mucor spp.*, *Penicillium spp. and Candida spp*.

Table 5. Microscopic and macroscopic properties of the fungal isolates from poultry feed (PFS) and watersamples (PWS).

PFS	Fungi type	Color	Underside color	Texture	Hyphae	Spore former	Probable organism
A	Mould	Black	Brown	Cottony	Aseptate	Sporangiospore	*Aspergillus* sp.
B	Mould	White	Black	Woolly	Aseptate	Sporangiospore	*Mucor* sp.
C_1	Mould	Black	Brown	Cottony	Aseptate	Sporangiospore	*Aspergillus* sp.
C_2	Mould	Green	Yellow	Wrinkled	Aseptate	Sporangiospore	*Penicillium* sp.
D	Mould	White	Black	Woolly	Aseptate	Sporangiospore	*Mucor* sp.
E	Mould	Black	Brown	Cottony	Aseptate	Sporangiospore	*Aspergillus* sp.
PWS	**Fungi type**	**Color**	**Underside color**	**Texture**	**Hyphae**	**Spore former**	**Probable organism**
F	Mould	white	Black	Woolly	Aseptate	Sporangiospore	*Mucor* sp.
G	Yeast	Cream	Cream	Smooth	Septate	Conidiophore	*Candida* sp.
H	Yeast	Cream	Cream	Smooth	Septate	Conidiophore	*Candida* sp.
I_1	Mould	Green	Yellow	Wrinkled	Aseptate	Sporangiospore	*Penicillium* sp.
I_2	Mould	Black	Brown	Cottony	Aseptate	Sporangiospore	*Aspergillus* sp.
I_3	Mould	White	Black	Woolly	Aseptate	Sporangiospore	*Mucor* sp.
J	Mould	Black	Brown	Cottony	Aseptate	Sporangiospore	Aspergillus sp.

3.3. Antibiotic susceptibility characteristics.

Table 6 shows the antibiotic susceptibility test results for Gram-negative Bacteria in millimeters. Table 7 shows the antibiotic susceptibility test results for Gram-positive Bacteria in millimeters. In Table 6 for gram-negative bacteria, reflecine showed the highest zone of inhibition for *E. coli*, while nalixidic acid showed the least zone of inhibition for *Klebsiella pneumoniae.* In Table 6 for gram-positive bacteria, amoxil showed the highest zone of inhibition while streptomycin showed the least zone of inhibition. All bacteria were resistant to some antibiotics.

Table 6. Antibiotic susceptibility test result for gram-negative bacteria (mm).

Organisms	OFX	NA	PEF	CN	AU	CPX	SXT	S	PN	CEP
Escherichia coli	12	-	22	-	-	20	13	15	12	-
Klebsiella pneumoniae	13	-	20	15	-	19	-	-	-	11
Salmonella sp.	10	-	19	16	-	20	12	16	-	14

Key: -= Resistance; mm = millimeters; OFX = Ofloxacin (10 µg); NA = Nalidixic acid (30µg); PEF = Reflecine (10µg); CN = Gentamycin (10µg); AU = Augmentin (30µg); CPX = Coprofloxacin (10µg); SXT = Septrin (30µg); S = Streptomycin (30µg); PN = Amplicin (30µg); CEP = Ceporex (10µg).

The results showed that all the poultry feed and poultry water samples were contaminated with microorganisms that pose public health concerns. From the morphological characteristics, microscopic and biochemical tests of the selected bacterial isolates, three genera of Gram-negative bacteria were encountered. These include *E. coli, Klebsiella* sp.and *Salmonella* sp. The Gram-positive bacterium obtained in the study is *Staphylococcus aureus* and this aligns with the findings of [14], who worked on Bacterial Contaminants Associated with commercial poultry feeds in Enugu, Nigeria. The presence of *Staphylococcus aureus* had been discovered to excrete toxins in food which is poisonous to humans and animals. staphylococcal infection can cause food poisoning in man when contaminated poultry meat is consumed. Presence of the pathogens in food products imposes potential hazard for poultry animals and causes grave economic loss. *Klebsiella* causes healthcare-associated infections,

which can take the form of pneumonia, sepsis; wound infections and urinary tract infections. This aligns with the findings of previous research by [15], who isolated similar organisms from cassava granules.

Table 7. Antibiotic susceptibility test results for gram-positive bacteria (mm)

Organism	Antibiotics	Zone of inhibition (mm)
Staphylococcus aureus	CPX (10ug)	20
	E (10ug)	-
	LEV (20ug)	18
	CN (10ug)	22
	APX (20ug)	12
	RD (20ug)	13
	AMX (20ug)	23
	S (30ug)	11
	NB (10ug)	10
	CH (30ug)	-

Key: - = resistance, CPX = ciproflox, E = erythromycin, LEV = levofloxacin, CN = gentamycin, APX = smpiclox, RD = rifampicin, AMX = amoxil, S = streptomycin, NB = norfloxacin, CH = chloramphenicol

Some species of Aspergillus have been implicated in the secretion of aflatoxins, which are carcinogenic to humans and animals when consumed. Aspergillus is the most common fungi found in poultry feed and poultry water. In this study, it was the most frequently occurring fungal isolate. This finding is consistent with previous research by [16], who investigated fungal contamination and mycotoxin levels of poultry feeds in Lagos, Nigeria.The total plate count per unit source indicates varying levels of contamination, reflecting the hygiene practices and storage conditions of the poultry feed and poultry water samples. For instance, the results of this research show that Sample B had the highest colony count, suggesting that the hygienic processes employed in that poultry operation were less stringent than those in other samples. Some of the fungi isolated in this research align with the findings of [16], who studied fungal contamination and mycotoxin levels of poultry feeds in South Africa.

The highest and lowest colony counts of bacteria in poultry water in the results of this research differ from those in a study by [17], who examined the antibiotic susceptibility profile of bacterial isolates from commercial poultry farms in Ile-Ife, Nigeria. In this research, Staphylococcus was the most prevalent bacterial isolate, which is consistent with previous research by [18], who investigated *Staphylococcus aureus* infections. Additionally, *Aspergillus spp*. was the most prevalent fungal isolate in this study, consistent with previous research by [19], who studied the global burden of chronic pulmonary aspergillosis complicating sarcoidosis. The bacterial isolates in this study exhibited susceptibility to most of the antibiotics and resistance to some of them. Amoxil showed the highest zone of inhibition for Staphylococcus aureus, indicating its effectiveness against this bacterium, similar to the report by [20]. Some of the antibiotic resistance pattern in this study were found to align with the findings of [1, 10], who investigated the antibiotic susceptibility pattern of microorganisms from poultry water and abattoirs in Awka respectively. Their study found that most of the isolates were resistant to erythromycin, while a few percentage were resistant to chloramphenicol. nalidixic acid and ceporex were found to be resistant to *Escherichia coli* in this study, in line with previous research by [10], who studied antimicrobial resistance and plasmid profiling of *Escherichia coli* isolated from poultry farms in Bangladesh. Their study

found that 62.5% of the isolates were resistant to nalidixic acid, while 50% were resistant to ceporex.

4. Conclusions

The research has proven that poultry feed and poultry water showed varying levels of contamination and may pose serious health risks to the poultry. Amoxil, levofloxacin, ciproflox, reflecine and ofloxacin are recommended for use as antibiotics to treat diseases that may be caused by some of these pathogens.

Competing Interest

The authors hereby declare that no competing interests exist regarding the publication of this research.

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