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ORIGINAL ARTICLE

Microbiological Assessment of Poultry Droppings, Water and Soil Under Deep Litter (Dl) And Battery Cage (Bl) Systems Within Lagos, Nigeria

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Abstract

This study focuses on the evaluation of the microbiological profile of microbes found in water, soil, droppings, in selected poultry farms under intensive and semi-intensive management system within Lagos, Nigeria. Bacteria and fungi were isolated from poultry droppings, water and soil samples and identified by standard microbiological protocol. The data on the assessment of poultry production system were obtained with structured questionnaire. The bacterial and fungal counts ranged from 29 X 109 CFU/mL - 106 X 109 CFU/mL and 72 X 109 CFU/mL - 115 X 109 CFU/mL respectively. The microorganisms isolated were Streptococcus pluranimalium, Micrococcus sp., Pseudomonas aeruginosa, Salmonella sp., Staphylococcus arlettae, Escherichia coli, Staphylococcus saprophyticus, Bacilllus subtilis, Aeromonas hydrophila, Candida tropicalis, Saccharomyces spp, Sporendonema spp., Aspergillus fumigatus, Fusarium oxysporum, Kloeckera spp., Zygosaccharomyces spp and Aspergillus niger. Aspergillus niger (30.5%), Micrococcus sp. (17.6%) and Staphylococcus saprophyticus, (17.6%), occur most frequently while Candida tropicalis (4.6%) and Escherichia coli (5.8%) has the lowest percentage occurrence in poultry droppings. Aspergillus sp. (48.4%), Pseudomonas aeruginosa (28.6%) occur most frequently while Penicillium sp. (15.2%), Staphylococcus spp. (7.1%), has the lowest percentage occurrence in poultry soil samples. Fusarium sp. (38.8%) and Pseudomonas aeruginosa (28.6%), occur most frequently while Aspergillus sp. (7.7%), Staphylococcus spp. (7.1%) has the lowest percentage occurrence in water samples. Most of the isolated bacteria showed resistance to at least two different classes of antibiotics. There is strong evidence that poultry farms in Lagos have utilized many antibiotics and this may have contributed to antibiotic resistant pattern of some bacterial isolates to antibiotics.

Keywords: Antibiotic resistance, management systems, microbiological, poultry, questionnaire

Introduction

The term 'poultry' alludes to every single trained winged creature domesticated for egg, meat production and plumes. Poultry originates from the French word poul, which was gotten from Latin word pullus meaning little creatures (Eltanany et al., 2010). They include the following: Domestic chicken (fowls), Turkey, Guinea fowls, Duck and Geese (also called waterfowls), Quails, Pheasants, Ostriches, Pigeons and Doves (Gyang et al., 2019).

They have a place with the zoological class avers. The popularity of poultry birds in Nigeria is noteworthy and can be attributed to the numerous benefits associated with poultry production and other value chain (Eltanany et al., 2010). Birds are fully confined either in

homes or cages under two types of intensive systems; (a) Deep litter system: birds are fully restricted but can move around freely on the floor of the house, the floor is covered with wood shavings or a similarly absorbent (but non-toxic) material.

The fully enclosed system protects the birds from thieves and predators and is suitable for specially selected commercial breeds of egg or meat-producing poultry (layers, breeder, and broilers) (Folorunso et al., 2014). (b) Battery cage system: this is usually used for layers, which are kept throughout their productive life in cages. There is a high initial capital investment, and the system is mostly confined to large-scale commercial egg layer operations (Folorunso et al., 2014). Birds are in individual enclosures, made up of approximately 2 mm thick iron rods, these enclosures are made in such a way that bird can be kept inside easily, and they can be fed and water adjusted outside the enclosures (Folorunso et al., 2014). Hence, deep litter is an efficient method of labour-saving system that is required to keep poultry house clean and in perfect sanitary condition.

Poultry have been known to harbor distinctive nourishment borne pathogens. Numerous reports have indicated that Salmonella spp. and Campylobacter spp. are the most well-known reasons for human nourishment borne bacterial illnesses connected to poultry (Akond et al., 2009). These diseases are generally transmitted in feed, litter, and water. Drinking water is one of the foremost essential nutrients for the inhabitation of livestock. It functions in the digestion of food, transporting of nutrients, waste materials, hormones, and other chemical messengers along the gastrointestinal tract (Ezekiel et al 2011).

The quality of drinking water in poultry can be jeopardized because of diverse things, the source (well or pipe), poor cleaning and maintenance of drinkers, regurgitated feed by the birds, chicken feed, chicken conduct, rearing sites, faeces, antimicrobials, and knowledge of readers (Folorunso et al., 2014, Zaman et al.,2012). The use of top-quality water is basic importance to profitable poultry husbandry as a result large flocks of those species typically share identical supply of water. Low quality drinking water can adversely influence the livability and productivity of poultry birds. Contaminated drinking water plays a very important role in the transmission of many viral, bacterial, and protozoan infections in poultry (Zaman et al.,2012).

Development of antibiotic resistance among pathogenic bacteria could be a major public health concern, as it can cause danger to the people surrounding who have the common infections with those once treatable with antibiotics (Ezekiel et al 2011). The emerging resistant bacterial strains will adversely affect the efficacy of antibiotic chemotherapy for those that acquired the new strains of infectious disease.

Furthermore, it encourages the need for more expensive and toxic medications. Some resistant infections can cause death (Akond et.,al 2009, Apata et al., 2009). Therefore, this study will provide the information on the microbiological profile of organisms found in drinking water, soil, and poultry droppings at the selected poultry farms under deep litter (DL) and battery cage (BL) systems within Lagos metropolis, Nigeria.

Materials and Method

Sample Collection

The collected samples were drinking water, soil, and droppings from five poultry farms namely; Anifowose farm, Akinyemi farm, Olusunbo farm, Raji farm and Oladee farm. A total of twenty-three (23) samples were collected from each poultry farms in the month of October 2020. These samples were packaged in different sterile bags and labelled and transported to the Microbiology laboratory for analysis.

Microbiological Analyses

Nutrient agar (NEOGEN, Heywood, UK), Peptone water (Axiom Medical Ltd), Eosin methylene blue agar (Himedia laboratories, Vadhani, india), Potato Dextrose agar, (Himedia laboratories, Vadhani, india), MacConkey agar (Axiom Medical Ltd) and Muller-Hinton (Axiom Medical Ltd)

were used to determine the microbiological profile and prepared according to manufacturer's instruction.

Bacterial Isolation and Identification

Poultry droppings was collected using a sterilised spatula, one gram of the poultry dropping was introduced into 9 ml physiological peptone water in sterile bottle and mixed thoroughly. Serial dilution was made up to 10-10 aliquots (0.1ml) was inoculated into sterile Petri dishes, the appropriate dilutions were cultured by pour plate technique. Following incubation at 370C for 24 h, plates exhibiting 30-300 colonies were counted using the colony counter and total viable count was calculated according to ISO recommendation. The results of the total bacterial count were expressed as the number of organism or colony forming units per gram (CFU/g) of droppings samples (Sule et al.,2019).

Soil Samples

Surface of the soil sample sites was cleared and the soil samples in poultry environment were obtained using soil auger at depths of 6cm, samples were collected in sterile bags. One gram of each soil sample from each poultry farm location was diluted serially. An aliquot of 0.1ml was inoculated into sterile Petri dishes and the freshly prepared media was poured into the plate, using pour plate method. Plates were incubated at 370C for 24 h and observed for growth. Colonies of bacteria were recorded as colony forming units per gram of soil (CFU/g).

Drinking Water

Poultry drinking water was collected by disinfecting the cap of the tap with 100% ethanol and flame, the water was left open to run for a while, afterwards the water sample was collected in sterile and transported to the Microbiology Laboratory. 1ml of the poultry water was introduced into 9 ml of physiological peptone water in sterile bottle and mixed thoroughly. Appropriate dilutions were made and cultured by pour plate technique.

All isolates were sub-cultured repeatedly to obtain pure cultures and characterised using standard microbiological techniques (Cheesbrough et al., 2006).

Biochemical Tests

The following biochemical tests were carried out on the bacterial isolates for proper identification: Catalase test, Coagulase, Indole, Methyl red, Voges-Proskauer tests, Oxidase test, Citrate utilisation, and Triple Sugar Iron Agar Test (TSI) (Cheesbrough et al., 2006).

Isolation and Identification of Fungi

0.1 ml of the serially diluted aliquot for all samples was inoculated using pour plate technique on potato dextrose agar supplemented with streptomycin (Kemoi et al.,2013). After incubation, the colonies were counted and expressed in CFU/g, the fungi were characterized and identified by their macroscopic and microscopic features (Sule et al.,2019). Lactophenol cotton blue stain was used in the identification of fungi isolate. A drop of lactophenol solution was placed onto a clean slide, the wire loop was sterilised using Bunsen burner with blue fame, using the wire loop a small amount of the fungal culture was removed for the edge (younger colonies), the fungal culture was spread gently on the slide using the wire loop, the coverslip was gently placed on the slide, the slide was now examined under the microscope, the fungal elemental characteristic was detected, examined, and recorded (Sule et al.,2019).

Antibiotics Susceptibility Test

The antibiotics susceptibility test of the isolates was carried out using the Kirby-Bauer disk diffusion technique according to the methods recommended by Clinical Laboratory and Standards Institute (CLSI,2018). Discrete colonies of the isolates were inoculated into 5ml of normal saline standardized with 0.5 McFarland standard suspensions. Sterile cotton wool swab was used for the inoculation of the bacterial suspension to freshly prepared Mueller-Hinton agar plates prepared according to manufacturer's instructions. The antibiotic sensitivity discs were aseptically and spaciously placed (20mm away from each other) on the inoculated

Mueller-Hinton agar plates. The antibiotic discs used were: SXT; Septrin (30µg), R; Rocephin (25µg), AM; Amoxacillin (36µg); CN; Gentamycin (10µg), PEF; Pefloxacin (10µg), APX; Ampiclox (30µg), S; Streptomycin (30µg), E; Erythromycin (10µg) for Gram negative isolates. while SXT; Septrin (30µg), CH; Chloranphenicol (30µg), SP; Sparfloxacin (10µg), CPX; Ciprofloxacin (30µg), AM; Amoxacillin (30µg); AU; Augmentin (10µg), PEF; Pefloxacin (30µg), OFX; Tarivid (10µg) for Gram positive isolates. After incubation, the test plates were examined for confluent growth and zone of inhibition. The diameter of each zone of inhibition was measured in millimetre (mm) using a ruler on the underside of the plate. The interpretation of the measurement as sensitive, intermediate, and resistant were made according to CLSI manual (CLSI,2018).

Surveillance on Antibiotics Usage and Hygiene on Poultry Farm

A multiple-choice structured questionnaire was administered to all poultry attendants and farm manager that were on duty as at the time of sample collection. In the questionnaire, the year of experience of the poultry farmer, age of birds, antibiotics frequently employed, number of antibiotics that have been used were put into consideration and poultry farmer's personal hygiene (Oluwasil et al., 2015).

Statistical Analyses

Data were analysed using SPSS version 25.0. Prevalence of the bacterial isolates was expressed in simple descriptive statistics such as means and standard deviations. For CFU/ml values, one-way analysis of variance (ANOVA) was used, where the levels of significance were set at $P<0.05$, and the means between the samples were separated.

Result and Discussion

Table 1 to 10 shows the all the data and results in this study. Table 11 shows the bacterial and fungal counts obtained from the five farm sites used in this study. The farm C has the largest microbial counts both for bacteria (106 X 10^9 CFU/mL), and for fungi (115 X 10^9 CFU/mL). The farm A has the least bacterial count (40.67 X 10^9 CFU/mL), while farm 2 has the least fungal count (72 X 10^9 CFU/mL).

Table 1: Cultural and Morphological Characteristics of Fungal Isolates from Poultry droppings, water, and soil samples

Macroscopy	**Microscopy features**	**Probable fungi**
The colonies were creamish with glossy appearance; the reverse of plate also creamish at 72 hours of incubation.	The hyphae were breaking into rod shaped chained or arthrosporous. The arthrosporous were spherical and in chains.	***Candida tropicalis***
Rapid growing colonies, flat and filamentous, surface colour is greyish green while the reverse is pale yellow, powdery texture.	The zonation of yeast colonies was radially furrowed on the reverse, the revere colour was slightly creamy. Septate hyphae, long conidiophores with a rough texture below the vesicle, vesicles are spherical.	***Aspergillus Candidus***
A creamish colonial fungus. The yeast cell was mucoid, the reverse of the plate was whitish cream after 72 hours of incubation.	The yeast colonies were spindle shaped, pointed at both ends, long, thin walled.	***Kloeckera spp.***
A white yeast, big in size, non-mucoid with irregular edge, no spore	The yeast cells varied in shape some were oval, pointed at one end and arranged in	***Zygosaccharomyces spp.***

seen, reverse of the plate was whitish cream.	clusters and the asci of the yeast were bean shaped.	
A big woolly mould with whitsh tint on the surface. Reverse side of the plate was creamish and with concentric zones of dark and light reddish coloration after 72 hours of incubation.	The macroconidia were hyaline and sickle shaped; Microconidia were also produced. Sporulation of the spores were poor.	***Fusarium oxysporum***
A medium size filamentous mould with white margin and black spores seen at 72 hours.	Presence of double walled conidiopshores which was smooth and hyaline. Sporulation of the spores were heavy.	***Aspergillus niger***
A fungus growth with pinkish pigmentation at the reverse of the plate; appearance of woolly growth on the surface.	The conidial heads typically columnar, conidiospores short, green typically in the upper part, smooth walled.	***Aspergillus fumigatus***
A creamy irregular growth with whitish tint on the surface. Reverse side of the plate was creamish after 72 hours of incubation.	The yeast cells were round or spherical in shape.	***Saccharomyces* spp.**
Rapid growing colonies, flat and filamentous. Surface colour is dark greenish while reverse is light orange. Powdery and compact with a cottony texture.	Septate hyphae, branched conidiophores, ovoid conidia. Conidia are unicellular with unbranching chains.	***Penicillium* spp.**

Table 2: Occurrence of Fungal Isolates in the Poultry Droppings from Different Sampling Location

	Samplings location				
Fungal isolates	**A**	**B**	**C**	**D**	**E**
Candida tropicalis	**-**	**-**	**-**	**+**	**+**
Aspergillus candidus	**+**	**-**	**+**	**+**	**+**
Kloeckera spp.	**-**	**-**	**+**	**-**	**-**
Zygosaccharomyces spp.	**-**	**+**	**-**	**-**	**+**
Fusarium oxysporum	**+**	**-**	**+**	**-**	**+**
Aspergillus niger	**-**	**+**	**-**	**+**	**-**
Aspergillus fumigatus	**+**	**-**	**+**	**+**	**+**
Saccharomyces spp.	**+**	**+**	**-**	**+**	**+**

Key: A – Anifowose farm, **B** – Akinyemi farm, **C** – Olusunbo farm, **D** – Raji farm, **E** – Oladee farm, **+;** Isolated**, -;** Not Isolated**.**

Table 3: Occurrence Rate of Fungal Isolates in Poultry Droppings

Fungal Isolates	**Number of Occurrence**	**Rate of Occurrence (%)**
Candida tropicalis.	3	4.6
Aspergillus Candidus.	10	13.8
Kloeckera spp.	5	6.8
Zygosaccharomyces spp.	4	5.5
Fusarium oxysporum.	4	5.5
Aspergillus niger.	22	30.5
Aspergillus fumigatus.	11	15.2
Saccharomyces spp.	13	18.1
Total	72	100

Table 4: Occurrence of Fungal Isolates in the Poultry Soil from Different Sampling Location

	Samplings location				
Fungal isolates	**A**	**B**	**C**	**D**	**E**
***Fusarium* spp.**	**-**	**+**	**-**	**+**	**+**
***Aspergillus* spp.**	**+**	**+**	**+**	**+**	**+**
***Penicillium* spp.**	**+**	**-**	**-**	**+**	**-**
Saccharomyces spp.	**+**	**-**	**+**	**-**	**+**

Key: A – Anifowose farm, **B** – Akinyemi farm, **C** – Olusunbo farm, **D** – Raji farm, **E** – Oladee farm., **+;** Isolated**, -;** Not Isolated.

Table 5: Occurrence Rate of Fungal Isolates in Poultry Soil

Fungal Isolates	**Number of Occurrence**	**Rate of Occurrence (%)**
Aspergillus spp.	16	48.4
Penicillium spp.	5	15.2
Fusarium spp.	5	15.2
Saccharomyces spp.	7	21.2
TOTAL	33	100

Table 6: Occurrence of Fungal Isolates in the Poultry water from Different Sampling Location

	Samplings location				
Fungal isolates	**A**	**B**	**C**	**D**	**E**
***Fusarium* spp.**	**-**	**+**	**+**	**+**	**+**
***Aspergillus* spp.**	**-**	**-**	**+**	**+**	**+**
***Penicillium* spp.**	**-**	**+**	**+**	**-**	**-**

Key: A – Anifowose farm, **B** – Akinyemi farm, **C** – Olusunbo farm, **D** – Raji farm, **E** – Oladee farm., **+;** Isolated**, -;** Not Isolated

Table 7: Occurrence Rate of Fungal Isolates in Poultry Water

Fungal Isolates	**Number of Occurrence**	**Rate of Occurrence (%)**
***Aspergillus* spp.**	5	27.7
***Penicillium* spp.**	6	33.3
***Fusarium* spp.**	7	38.8
TOTAL	18	100

Table 8: Occurrence of Bacterial Isolates in The Poultry Droppings from difference locations

	Samplings location				
Bacterial isolates	**A**	**B**	**C**	**D**	**E**
Micrococcus sp.	**-**	**+**	**+**	**-**	**+**
Streptococcus pluranimalium	**-**	**+**	**-**	**-**	**+**
Pseudomonas aeruginosa	**+**	**-**	**-**	**+**	**-**
Staphylococcus sp.	**-**	**+**	**-**		**+**
Salmonella sp.	**+**	**-**	**-**	**+**	**-**
Staphylococcus arlettae	**+**	**-**	**-**	**+**	**-**
Escherichia coli	**-**	**-**	**+**	**-**	**-**
Staphylococcus saprophyticus	**-**	**+**	**+**	**-**	**+**

Key: A – Anifowose farm, **B** – Akinyemi farm, **C** – Olusunbo farm, **D** – Raji farm, **E** – Oladee farm., **+;** Isolated**, -;** Not Isolated

Table 9: Occurrence of Bacterial Isolates in Poultry water samples

	Samplings location				
Bacterial isolates	**A**	**B**	**C**	**D**	**E**
***Micrococcus* spp**	**+**	**-**	**-**	**+**	**-**
Pseudomonas aeruginosa	**+**	**+**	**-**	**+**	**+**
***Staphylococcus* spp**	**-**	**-**	**-**	**+**	**-**
Streptococcus pluranimalium	**+**	**-**	**+**	**-**	**-**
***Shigella* spp**	**+**	**+**	**+**	**+**	**+**

Key: A – Anifowose farm, **B** – Akinyemi farm, **C** – Olusunbo farm, **D** – Raji farm, **E** – Oladee farm, **+;** Isolated**, -;** Not Isolated

Table 10: Occurrence of Bacterial Isolates in Poultry soil samples

	Samplings location				
Bacterial isolates	**A**	**B**	**C**	**D**	**E**
***Shigella* spp**	**+**	**-**	**-**	**+**	**-**
Pseudomonas aeruginosa	**+**	**+**	**-**	**+**	**+**
***Staphylococcus* spp**	**-**	**-**	**+**	**-**	**-**
Streptococcus pluranimalium	**+**	**-**	**+**	**+**	**-**
Bacilllus subtilis	**+**	**-**	**-**	**+**	**-**
Aeromonas hydrophila	**-**	**+**	**-**		**+**

Key: A – Anifowose farm, **B** – Akinyemi farm, **C** – Olusunbo farm, **D** – Raji farm, **E** – Oladee farm., **+;** Isolated**, -;** Not Isolated

Table 11: Microbial loads of the poultry droppings, soil and water in each poultry farm

Site	Mean ± SD	
	Bacteria Count (10^9)	**Fungi Count (10^9)**
Farm A	40.67 ± 56.42	75.00 ± 96.26
Farm B	89.50 ± 88.55	72.67 ± 79.15
Farm C	106.00 ± 66.10	115.17 ± 110.74
Farm D	29.83 ±44.44	87.50 ± 91.31
Farm E	100.17 ± 70.13	86.33±64.03

Key: A – Anifowose farm, **B** – Akinyemi farm, **C** – Olusunbo farm, **D** – Raji farm, **E** – Oladee farm.

However, the data presented in Table 12 shows the differences between bacterial and fungal counts among the droppings used from the different farm sites. In all cases, the data showed that the bacterial and fungal counts obtained from droppings were greater than the soil and water microbial counts.

Similarly, just as expected the microbial counts in soil more than the microbial counts in water. Consequently, for each farm sites, there are significant differences between the counts in droppings and water; and droppings and soil ($p<0.05$). Same values written in subscript indicates the values with significant difference.

Table 12: Difference in bacterial and fungal counts among samples in different sites

Site	**Sample**	**Bacterial Counts (10^9) Mean ± SD**	**Fungal Counts (10^9) Mean ± SD**
Farm A	Droppings	113.0 ± 2.83^{a}	196.0 ± 22.6^{g}
	Water	0.5 ± 0.7^{a}	0.00 ± 0.00^{g}
	Soil	8.50 ± 12.0^{a}	29.0 ± 32.5^{g}
Farm B	Droppings	202.5 ± 3.5^{b}	169.5 ± 48.7^{h}
	Water	24.0 ± 7.1^{b}	25.0 ± 11.3^{h}

	Soil	42.0 ± 22.6 [b]	23.5 ± 26.1 [h]
Farm C	Droppings	157.5 ± 62.9 [c]	139.5 ± 38.9 [i]
	Water	31.0 ± 2.8 [c]	50.0 ± 2.8 [i]
	Soil	129.5 ± 14.8 [c]	201.0 ± 140.0 [i]
Farm D	Droppings	655.0 ± 77.0 [d]	202.5 ± 24.7 [j]
	Water	7.50 ± 3.5 [d]	25.0 ± 7.1 [j]
	Soil	16.5 ± 4.9 [d]	35.0 ± 35.4 [j]
Farm E	Droppings	160.0 ± 56.5 [e]	137.5 ± 31.8 [k]
	Water	19.0 ± 5.6 [e]	7.0 ± 4.2 [k]
	Soil	121.5 ± 10.6 [e]	114.5 ± 7.8 [k]
Total	Droppings	139.70 ± 62.3 [f]	169.0 ± 38.7 [l]
	Water	16.4 ± 12.12 [f]	12.4 ± 12.1 [l]
	Soil	63.6 ± 55.6 [f]	80.6 ± 88.2 [l]

*values that share the same letter are significantly different [a,b,c,d,e,f,g,h,l,j,k,l]

Key: A – Anifowose farm, **B** – Akinyemi farm, **C** – Olusunbo farm, **D** – Raji farm, **E** – Oladee farm

The results presented in Table 13 shows bacterial and fungal counts obtained from the five farm sites. The result shows the various poultry system used in each poultry farm; it shows that the battery cage system has the largest microbial counts for bacteria which ranged from (202 X 10^9 CFU/mL) - (655 X 10^9 CFU/mL). The deep litter system has the highest fungi counts (196 X 10^9 CFU/mL). Table 14 to 16 shows the data test from the analysis.

Table 13. Difference in bacterial and fungal counts for poultry droppings among the different poultry farm systems

Site	**Farm system**	**Sample**	**Bacterial Counts (10^9) Mean ± SD**	**Fungal Counts (10^9) Mean ± SD**
Farm A	Deep litter	Droppings	113.0 ± 2.83	196.0 ± 22.6
Farm B	Battery cage	Droppings	202.5 ± 3.5	169.5 ± 48.7
Farm C	Deep litter	Droppings	157.5 ± 62.9	139.5 ± 38.9
Farm D	Battery cage	Droppings	655.0 ± 77.0	202.5 ± 24.7
Farm E	Deep litter	Droppings	160.0 ± 56.5	137.5 ± 31.8

Key: A – Anifowose farm, **B** – Akinyemi farm, **C** – Olusunbo farm, **D** – Raji farm, **E** – Oladee farm

Table 14: Characterization and identification of bacterial isolates form poultry droppings

Bacterial isolate	Gram reaction	Cell shape	Oxidase	Citrate	H_2S	Gas	MR	VP	Catalase	Coagulase	Indole	Sucrose	Glucose	Lactose	Probable organism
1	**+**	**c**	**+**	**+**	**-**	**-**	**-**	**-**	**+**	**-**	**+**	**-**	**+**	**-**	***Micrococcus* spp**
2	**+**	**c**	**+**	**+**	**+**	**-**	**-**	**-**	**-**	**-**	**+**	**-**	**+**	**-**	***Streptococcus pluranimalium***
3	**-**	**r**	**+**	**+**	**-**	**-**	**-**	**-**	**+**	**-**	**-**	**-**	**+**	**-**	***Pseudomonas aeruginosa***
4	**+**	**c**	**+**	**+ / -**	**-**	**-**	**-**	**-**	**+**	**+**	**+**	**-**	**+**	**-**	***Staphylococcus spp***
5	**-**	**r**	**-**	**+**	**+**	**+**	**+**	**-**	**+**	**-**	**-**	**-**	**+**	**-**	**Salmonella *spp***
6	**+**	**c**	**+**	**-**	**-**	**-**	**-**	**-**	**+**	**-**	**-**	**-**	**+**	**-**	**Staphylococcus arlettae**
7	**-**	**r**	**-**	**-**	**-**	**+**	**+**	**-**	**+**	**-**	**+**	**+**	**+**	**+**	***Escherichia coli***
8	**+**	**c**	**+**	**+**	**-**	**-**	**-**	**-**	**+**	**-**	**-**	**-**	**+**	-	***Staphylococcus saprophyticus***

Key: + positive; - negative; c cocci; r rods; VP; Voges proskauer, MR; Methyl red

Table 15: Characterization and identification of bacterial isolates form poultry water

Bacterial isolate	Gram reaction	Cell shape	Oxidase	Citrate	H_2S	Gas	MR	VP	Catalase	Coagulase	Indole	Sucrose	Glucose	Lactose	Probable organism
1	**+**	**c**	**+**	**+**	**-**	**-**	**-**	**-**	**+**	**-**	**+**	**-**	**+**	**-**	***Micrococcus* spp**
2	**-**	**r**	**+**	**+**	**-**	**-**	**-**	**-**	**+**	**-**	**-**	**-**	**+**	**-**	***Pseudomonas aeruginosa***
3	**+**	**c**	**+**	**+ / -**	**-**	**-**	**-**	**-**	**+**	**+**	**+**	**-**	**+**	**-**	***Staphylococcus* spp**
4	**+**	**c**	**+**	**+**	**+**	**-**	**-**	**-**	**-**	**-**	**+**	**-**	**+**	**-**	***Streptococcus pluranimalium***
5	**+**	**c**	**-**	**-**	**-**	**-**	**-**	**+**	**+**	**-**	**-**	**-**	**+**	**-**	***Shigella* spp**

Key: + positive; - negative; c cocci; r rods; VP; Voges proskauer, MR; Methyl red

Table 16: Characterization and identification of bacterial isolates form poultry soil

Bacterial isolate	Gram reaction	Cell shape	Oxidase	Citrate	H_2S	Gas	MR	VP	Catalase	Coagulase	Indole	Sucrose	Glucose	Lactose	Probable organism
1	**+**	**c**	**-**	**-**	**-**	**-**	**-**	**+**	**+**	**-**	**-**	**-**	**+**	**-**	***Shigella* spp**
2	**-**	**r**	**+**	**+**	**-**	**-**	**-**	**-**	**+**	**-**	**-**	**-**	**+**	**-**	***Pseudomonas aeruginosa***
3	**+**	**c**	**+**	**+ / -**	**-**	**-**	**-**	**-**	**+**	**+**	**+**	**-**	**+**	**-**	***Staphylococcus* spp**
4	**+**	**c**	**+**	**+**	**+**	**-**	**-**	**-**	**-**	**-**	**+**	**-**	**+**	**-**	***Streptococcus pluranimalium***
5	**+**	**b**	**+**	**-**	**+**	**-**	**+**	**-**	**+**	**-**	**-**	**-**	**+**	**-**	***Bacilllus subtilis***
6	**-**	**r**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**-**	**+**	**-**	***Aeromonas hydrophila***

Key: + positive; - negative; c cocci; sr small rods; VP; Voges proskauer, MR; Methyl red; b bacilli; r rod

The result presented in Table 17a and 17b shows the information on personal hygiene among the poultry workers. The data presented shows that, 40% use overall before entering the poultry farms; 100% use a pair of boots before entering the poultry; 100% use nose mask before entering; 80% agree to the presence of disinfectant dip bath at the entrance of the farm; 100% ascertain that the workers often come in contact with poultry droppings; 80% agree to bathing after work period; non (0%) agree to eating during work period; while 100% agree to washing hands before eating.

On the basis of the responses provided by these data, it can be deduced that, the workers in the farm site practice personal hygiene to a large extent.

Table 17a. Personal Hygiene of Poultry workers

Items of hygiene	**Response**	
	Yes	**No**
	N(%)	**N(%)**
The use of overall before entering	8(40%)	12(60%)
The use of a pair of boots before entering	20(100%)	0(0%)
The use of a pair of gloves before entering	20(100%)	0(0%)
The use of nose mask before entering	20(100%)	0(0%)
Presence of disinfectant dip bath at entrance	16(80%)	4(20%)
Workers come in contact with poultry droppings	20(100%)	0(0%)
Bathing after work period	16(80%)	4(20%)
Eating during work period	0(0%)	20(100%)
Washing of hands before eating	20(100%)	0(0%)

Table 17b and 17c also shows that 80% of the workers affirm they wash their hands frequently, while 20% wash less frequently. Also, 60% of the workers affirm they frequently remove poultry droppings from the farm, while 40% do not. Table 18 shows the Antibiotics used in farm. Table 19a and b shows the Antibiotics Susceptibility Patterns of Bacteria Isolated from Poultry Dropping, Water and Soil in this study.

Table 17b. Personal Hygiene of Poultry workers

Items of hygiene	**Response**		
	Frequently	**Less frequently**	**Never**
	N(%)	**N(%)**	**N(%)**
Frequency of hand washing	19(80%)	4(20%)	0(0%)
Frequency of removal of poultry droppings	12(60%)	8(40%)	0(0%)

Table 17c. Methods of hand washing practiced by farm workers

Method of hand washing	**N**	**%**
Ordinary water	4	20.0
Detergent and water	4	20.0
Detergent, water and antiseptics	12	60.0

Table 18: Antibiotics used in farm

Antibiotics used	**Number of poultry farms**	**Rate of Occurrence (%)**
Chlortetracycline,Enrofloxacin, Gentamycin, Amoxacillin	5	10.6
Keproceryl, Gentamycin, Ciprofloxacin	4	8.5
Streptomycin, Doxycycline, Chlortetracycline	4	8.5
NCO, Gentamycin	4	8.5
Gentamycin, Keproceryl, Doxycycline	3	6.3
Keproceryl, Enrofloxacin, Neoceryl, NCO	3	6.3
Tylosin, Furazolidone, Neoceryl, Chlortetracycline	3	6.3
Chlortetracycline, Keproceryl, Enrofloxacin,	3	6.3
Gentamycin, Ciprofloxacin	2	4.3
Ciprofloxacin, Furazolidone, Gentamycin, Tylosin, Neoceryl	2	4.3
NCO, Chlortetracycline	2	4.3
Penicillin, Streptomycin, Tetracycline, Enrofloxacin 2	2	4.3
Chlortetracycline, Neoceryl Gentamycin, NCO, Enrofloxacin	2	4.3
Gentamycin, Ciprofloxacin, Chlortetracycline	2	4.3
Chlortetracycline	1	2.1
Penicillin, Streptomycin, Tetracycline	1	2.1
Keproceryl	1	2.1
NCO, Penicillin	2	4.3
NCO	1	2.1
Enrofloxacin	NU	NU
Total	47	100

Key: NU; Not in use, NCO- (Neomycine, Chloramphenicol, and Oxytetracycline), Neoceryl – (Neomycin, Erythromycin, Oxytetracycline, Streptomycin and Colistin), Keproceryl - (Oxytetracycline, Erythromycin, Colistin and Streptomycin).

Table 19a: Antibiotics Susceptibility Patterns of Bacteria Isolated from Poultry Dropping, Water and Soil

Gram positive bacteria	***Streptococcus pluranimalium***	***Staphylococcus* sp**	***Staphylococcus arlettae***	***Staphylococcus saprophyticus***	***Shigella* sp.**	***Bacilllus subtilis***
SXT	+	-	-	-	+	+
CH	-	-	-	+	-	-
SP	-	+	+	-	+	-
CPX	+	+	+	+	-	+
AM	-	+	-	+	+	-
AU	+	+	+	-	+	-
CN	-	-	-	+	-	+
PEF	+	+	-	+	+	-
OFX	+	+	-	+	+	+
S	-	-	+	+	-	+

Key: SXT; Septrin (30µg), CH; Chloranphenicol (30µg), SP; Sparfloxacin (10µg), CPX; Ciprofloxacin (30µg), AM; Amoxacillin (30µg); AU; Augmentin (10µg), CN; Gentamycin (30µg), PEF; Pefloxacin (30µg), OFX; Tarivid (10µg), S; Streptomycin (30µg); +; Susceptible, -; Resistant.

Table 19b: Antibiotics Susceptibility Patterns of Bacteria Isolated from Poultry Dropping, Water and Soil

Gram Negative Bacteria	***Pseudomonas aeruginosa***	***Salmonella* spp**	***Escherichia coli***	***Aeromonas hydrophila***
SXT	-	+	+	-
E	+	-	-	+
PEF	+	+	-	+
CN	-	+	+	-
APX	+	-	-	+
Z	-	+	+	+
AM	+	-	+	-
R	+	+	-	+
CPX	+	+	+	+
S	-	+	-	-

Key: SXT; Septrin (30µg), CH; Chloranphenicol (30µg), SP; Sparfloxacin (10µg), CPX; Ciprofloxacin (30µg), AM; Amoxacillin (30µg); AU; Augmentin (10µg), CN; Gentamycin (30µg), PEF; Pefloxacin (30µg), OFX; Tarivid (10µg), S; Streptomycin (30µg); - = Resistance, + = Susceptible.

Discussion

In this study, the microbial counts disclosed high contamination of poultry droppings, water, and soil. The fungal species isolated in this study were Candida tropicalis, Saccharomyces spp, Sporendonema spp., Aspergillus spp., Fusarium oxysporum, Kloeckera spp., and Zygosaccharomyces spp. In a similar research on diversity of fungi in fresh and aged poultry litter, Penicillium, Alternaria, Cladosporium, Aspergillus, Scopulariopsis and Trichosporon were isolated (Kemoi et al.,2013). The prevalence of Saccharomyces cerevisiae is inspired, as studies have shown that these organisms have helpful effects to poultry birds (Sule et al., 2019). (Abdul et al.,2012) Disclosed that Saccharomyces cerevisiae apart from being an excellent supply of amino acids for poultry is also a good supply of minerals and vitamin B complex.

As observed in this study, (Chat et al,2019) also revealed that poultry droppings are sources of Gram negative antibiotic resistant pathogens such as E. coli, Citrobacter spp., Enterobacter aerogenes, Klebsiella spp., Salmonella spp., Serratia marcescens, Shigella dysenteriae, Proteus spp., and Pseudomonas aeruginosa. It also showed that these enteric bacteria, Salmonella spp. and E. coli are also present in poultry soil, water and droppings in the farms visited.

This study agrees with the finding of (Gyang et al., 2019), who disclosed the incidence of Staphylococcus in poultry droppings. The presence of Staphylococcus aureus in poultry droppings and water will cause food poisoning in human through meat poultry meat consumption (Omoya et al., 2016). The Staphylococcus was being transmitted during the hatchery process, due to the farm environment and through fomites.

The infection of streptococci in poultry droppings could have from the respiratory route. Streptococcal and enterococcal infections in poultry will cause acute septicaemia and chronic infections in affected birds. Streptococcus pluranimalium is associated with valvular endocarditis and septicaemia in adult broilers (Zaman et al.,2012). Also in this study, Gram positive bacteria represent 60% of the bacterial isolates while the other 40% represent Gram negative bacteria. (Apata et al., 2009), obtained microbial populations in chicken litter up to 1010 CFU/g, and the predominance of Gram-positive bacteria that account for nearly 90% of the microbial diversity.

The antibiotic usage pattern ascertained during this survey showed that the poultry farmers in Lagos were heavily reliance on the antimicrobial medications. Most of them were multi-drug users and every farm used one or more antibiotics for therapeutic and prophylactic purposes (Omoya et al., 2016). This report shows that the antibiotics were most administered for therapy (65%) and prophylaxis (35%) among farmers in Lagos State. This report was like an earlier report by (Adebowale et al., 2016) that antibiotics were most administered for therapy (36.2%) and prophylaxis (29.3%).

As observed from this study, farmers use antimicrobials in poultry for varied purposes. Responses from questionnaires showed some poultry farmers do not get information on antibiotics they use from qualified personnel. Some of them admitted relying on directives from drug store vendors whereas others rely on their own experience on antibiotics administration. The administration of antibiotics without appropriate prescription on the quantity could be misapplied which may be harmful not to the poultry alone but conjointly to the public health (Ezekiel et al.,2011).

This could rely on a lot of factors which could include availability of information, educational levels, scale of farming and financial buoyancy (Adebowale et al., 2016). This study shows that poultry farms in Lagos, has utilized several antibiotics in their poultry farms but Amoxacillin, Enrofloxacin, NCO (Neomycine, Chloramphenicol, and Oxytetracycline), Chlortetracycline and Keproceryl were mostly used and may have contributed to antibiotic resistant pattern of some bacterial isolates to some antibiotics. Some isolated bacteria showed resistance to a minimum of one different classes of antibiotics.

Conclusion

In conclusion, this study shows that poultry droppings, water and soil contain numerous groups of bacteria and fungi, and all the poultry farms used one form of antibiotics or the other on their poultry farm. Since antibiotics utilized in poultry has greatly contributed to the emergence of antibiotic resistance towards pathogenic bacteria in poultry and then passed to man, various means of therapy like the use of probiotics and phytotherapy should be researched on to combat these resistance trends.

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