PREVALENCE AND MULTI-DRUG RESISTANCE OF GRAM-NEGATIVE BACTERIA IN POULTRY WASTE IN ETHIOPE EAST LOCAL GOVERNMENT AREA DELTA STATE

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Abstract

The spread of resistant strains of bacteria and infectious micro organisms in the environment through poultry waste remains a global health challenge. This is due to the potential risk of transfer of antimicrobial resistance from poultry and poultry products to the human population, making it difficult to treat infections caused by these resistant strains. This study was aimed at identifying the multi-drug-resistance/sensitivity patterns of gram-negative bacteria, prevalent in poultry waste obtained from poultry farms in Ethiope East Local Government Area of Delta State. Samples of poultry waste was collected from different locations in Ethiope East Local Government Area of Delta State and transported to the Microbiology laboratory for analysis. All samples were inoculated on McConkey and Deoxycholate citrate agar for bacteriological examination following standard procedures. Relevant biochemical tests were carried out for identification of the isolates. Also, antimicrobial susceptibility test was carried out using the Kirby-Bauer disc diffusion method. Organisms isolated were *Proteus spp*, *Escherichia coli*, *Salmonella spp* and *Shigella spp*.*Proteus spp* demonstrated the highest resistance against all the antibiotics. *Escherichia coli* demonstrated a high resistance rate against cephalexin (75%), nalidixic acid (100%), septrin (trimethoprim and sulfamethoxazole) (75%), and ampicillin (100%). *Shigella* and *Salmonella* spp demonstrated high resistance to septrin (trimethoprim and sulfamethoxazole) and ampicillin ranging from 71% - 86%. Totally 85% of the isolates showed multi-drug resistance. This study clearly demonstrates that poultry birds may serve as the reservoirs formulti-drug resistant strains of gram-negative bacteria that infect humans. Also, that bacteria organism from poultry waste can contribute significantly to the spread of multidrug-resistant organisms, due to the indiscriminate use of antibiotics in poultry feeds by farmers. This study recommends proper information dissemination to poultry farmers on the dangers of antibiotics resistant strains in order to curb the spread of multidrug-resistant strains to humans causing gastrointestinal infections. Also, strict bio-security regulations should be put in place for proper waste disposal and its use as manure.

Keywords: Multi-drug resistance, Antibiotics, Gram-negative bacteria.

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1. INTRODUCTION

Antibiotic resistance is currently a major topic of interest for researchers and physicians. Antibiotic resistance has been referred to as "the silent tsunami facing modern medicine" [1]. Multi-drug resistance is the ability of bacteria to resist different classes of antibiotics (three or more than three classes of antibiotics) which are structurally different and have different molecular targets [2]. Antibiotic resistance is also described as the ability of microorganisms to grow in the presence of an antimicrobial agent at a concentration that will normally kill or inhibit their growth [3].

It is a natural process that begins when microorganisms are exposed to susceptible bacteria or antibiotics, sometimes they are killed or inhibited while bacteria that are naturally resistant or that have acquired antibiotic resistant traits have a greater chance to survive and multiply [4].

Poultry production can be a tool for economic development. As low-income and developing countries grow, the demand for quality sources of poultry products will increase. As a result, livestock production systems will continue to shift from agricultural practices to intensive food production that involves the systematic use of antimicrobials [5].The poultry industry is one of the largest and fast growing agro structures in Africa and the world. This can be attributed to the increasing demand for poultry meat and egg products. The increment of human demand poses a concern to the producers on the issue of care and keeping chicken health. In doing this, poultry farmers employ the use of antibiotics at therapeutic doses to prevent diseases and increase efficiency of feed utilization and growth performance [3]. The amounts and frequency of use of antimicrobials in food animals is a major causal factorfor the propagation of resistance [6]. Resistance to antibiotics can either be naturall y occurring fora particular organism/drug combin ation or acquired resistance,where misuse of anti microbials results in a population being exposed to an environment in which organisms th at have genesconferring resistance (either spontan eously mutated or throughDNA transfer from othe r resistant cells) have been able to flourish and spread[7]. Resistant bacteria can be transferred from poultry products to humans. This transfer could be direct or indirect. Direct contact with contaminated poultry products such as unwashed and uncooked poultry meat and indirect through consumption of contaminated surface water, ground water and crops grown with poultry waste used as manure which contain resistant bacteria. Once these pathogens are in the human system, they could colonize the gut and resistant genes could be shared or transferred to endogenous gut flora causing infections and jeopardizing future treatments of infections caused by these organisms. The extensive and misuse of antibiotics in animals for growth promotion and disease prevention triggers high selection pressure among microbial agents which might contribute to the emergence of multi-drug-resistant bacteria and put humans at risk of becoming infected with these transferred zoonotic resistant bacteria [8].

Antibiotics misuse clearly encourages the evolution of antimicrobial resistance [9]. With the advent of the antibiotic era, the overuse and inappropriate consumption and application of antibiotics in poultry farms for Prophylaxis, treatment of diseases and as growth promoters has driven the rapid emergence of multidrug-resistant pathogens. Antimicrobial resistance represents a huge global health crisis and one of the most serious threats humans face today.Although the use of antimicrobial agents is appropriate and reasonable, it is known to contribute to the development of resistance, but its indiscriminate use worsens the situation [10]. Antimicrobialresistance especially multi-drug resistance is a serious problem caused by indiscriminate use of antibiotics at poultry farms. Despite its benefits, the indiscriminate use of antibiotics leads to the emergence of antibiotic-resistant bacteria, and this situation has threatened the current and future efficacy of advanced modern treatment [8].

Antimicrobial resistance increases the morbidity, mortality, length of hospitalization and healthcare costs worldwide. The unfeasibility of reversing antimicrobial resistance back towards susceptibility and the critical need to treat bacterial infection in modern medicine have burdened researchers and pharmaceutical companies to develop new antimicrobials effective against these difficult-to-treat multidrug-resistant pathogens. However, it can be anticipated that antibiotic resistance will continue to develop more rapidly than new agents to treat these infections become available and a better understanding of the molecular, evolutionary and ecological mechanisms governing the spread of antibiotic resistance is needed. The only way to curb the current crisis of antimicrobial resistance will be to develop entirely novel strategies to fight these pathogens such as combining antimicrobial drugs with other agents that counteract and obstruct the antibiotic resistant mechanisms expressed by the pathogen. Furthermore, as many antibiotics are often inappropriately prescribed, a

more personalized approach based on precise diagnosis tools will ensure that proper treatments can be promptly applied leading to more targeted and effective therapies. However, in more general terms, also the overall use and release of antibiotics in the environment needs to be better controlled [11].

The spread of multidrug-resistant bacteria is an ever-growing concern, particularly among Gram-negative bacteria due to their inherent resistance and the rate at which they acquire and spread new resistance mechanisms. Some bacterial strains have acquired resistance to almost all antibiotics. Generally, Gram-negative bacteria are more resistant than Gram-positive bacteria and cause significant morbidity and mortality worldwide [12]. These bacteria present in poultry waste can be transferred to humans causing mild to severe infections. Infections caused by Gram-negative bacteria are a challenge for doctors and increases patient mortality and the cost of care globally. Actions must be taken to reduce the resistance of these pathogens to multiple antibiotics either by controlling the antibiotic use or studying their mechanism of resistance and developing new antibiotics to counter these mechanisms. This study will identify multi-drug resistant gram-negative bacteria species in poultry waste which will aid the development of new antibiotic agents and the exploration of alternative treatment options for treatment of multi-drug resistant gram-negative bacteria species affecting humans.

2. MATERIALS AND METHOD

Glass wares and other apparatus: Test tubes, beakers, Petri dishes, measuring cylinder, Pasteur pipette, Bijou bottle, cotton wool, masking tape, Disposable gloves, face masks, aluminum foil, Bunsen burner, spatula, Sterile swap stick, Sterile 5ml and 2ml Syringes, test tube rack, white laboratory coat, weighing balance, refrigerator, incubator, forceps, Sterile universal containers, Durham tubes, Autoclave, oil immersion microscope, microscope slides.

Media and reagents: McConkey agar, Nutrient agar, Mueller Hilton agar, Deoxycholate citrate agar, Mannitol salt agar, peptone water, kovac's reagent, Methyl red, glucose, lactose, sucrose, hydrogen peroxide, safranin counter stain, crystal violet, lugol iodine, Ethanol, oil immersion, oxidase reagent, urease reagent, Disinfectant, distil water and sterilized water.

2.3 Antibiotic sensitivity disk: OPTUDISC multi disc coated in various antibiotics which include Ofloxacin, gentamycin, ciprofloxacin, cephalexin, streptomycin, septrin, Ampicillin, Nalidixic acid, perfloxacin, Augmentin.

METHOD

Study area and sample collection: The study was carried in Ethiope east LGA Delta State. Faecal Droppings were collected from different poultry farms within Ethiope east LGA. A total of 200 samples were obtained from different poultry farms in different villages. These samples were transported to the laboratory within an interval of 3 to 4 hours of collection in a sterilized universal container.

Preparation of culture media: Each of the media was prepared according to the directions of their labels in the container.

Nutrient agar: 7.2g of Nutrient agar was weighed out and dissolved in 200mls of distilled water in a beaker, the medium was mixed properly and sterilized by autoclaving at 121°C for 15 minutes. It was then allowed to cool to about 50-55°C before aseptically pouring into sterile Petri dishes prior to solidification. After solidification, swab sticks were used to smear each sample on each agar plate. The plates were then incubated for 24 hours at 37°C. Presence of colonies indicated bacteria growth on the plate.

McConkey agar: 11g of McConkey agar powder was weighed and dissolved in 200mls of distilled water in a beaker, the medium was properly mixed and sterilized by autoclaving at 121°C for 15 minutes. It was then allowed to cool to about 50-55°C before aseptically pouring into sterile petri dishes prior to solidification (This medium is used as a selective medium due to its preference for gram negative organisms in this study). After solidification, swab sticks were used to smear each sample on each agar plate. The plates were then incubated for 24 hours at 37°C. Presence of colonies indicated bacteria growth on the plate.

Deoxycholate citrate agar: 14.2g of Nutrient agar was weighed out and dissolved in 200mls of distilled water in a beaker, the medium was mixed properly and sterilized by boiling. The agar was sterilized by boiling not autoclaving because excess heat can destroy it. It was then allowed to cool to about 50-55°C before aseptically pouring into sterile petri dishes prior to solidification. After solidification, swab sticks were used to smear each sample on each agar plate. The plates were then incubated for 24 hours at 37°C. Presence of colonies indicated bacteria growth on the plate.

Preparation of slants: This is done to better preserve cultureorganisms. After culturing the different samples growth occurred and about 42 organisms was obtained. Slant agar was then prepared using 42 bijou bottles. Nutrient agar was weighed and dissolved in water. The agar was poured into each bottle and positioning in a slant position to ensure it solidifies and forms a slant shape which was later inoculated with the 42 organisms obtained. The slants showed significant growth after incubation at 37°C for 24 hours.

Preparation of broth culture: 3.75g of peptone water powder was dissolved in 250mls of water in a conical flask and shared equally into test tubes, sterilized and properly labeled. Then each organism from the slant was inoculated into the broth and the test tubes incubated at 37°C for 24 hours.

Biochemical tests

Biochemical tests were carried out to confirm the identity of the test organisms obtained for the study. They include:

Catalase test: This test is used to identify an organism that produces the enzyme, catalase. This enzyme breaks down hydrogen peroxide into water and oxygen gas. Thus there is formation of gas bubbles in the medium indicating a positive result but absence of bubbles indicates a negative result.

About 2-3 drops of 3% hydrogen peroxide was added to the isolates in different test tubes using a sterile pipette and they were observed for effervescence/bubble formation within the liquid media of the test tube. Tubes with bubbles were recorded as catalase positive.

Citrate utilization test: This is used to test the organism's ability to utilize citrate as a source of energy. Bacteria that can grow on this medium, produce an enzyme which capable of converting citrate to pyruvate. Pyruvate can then be used in the organism's metabolic cycle for the production of energy. A positive test is indicated by a change in colour from green to blue.

To a citrate agar was weighed and dissolved in distilled water and then transferred into a bottle. The agar was sterilised by autoclaving at 121°C for 15 minutes. The molten agar was allowed to cool and then poured into petri dishes and allowed to solidify. A sterilised wire loop was used to inoculate the broth culture by streaking on the Simon citrate agar plate. The inoculated plates were incubated for 24 hours at 37°C. It was then observed for colour change from green to prussian blue indicating a positive result but if the colour remain green, the result is negative.

Indole test: Indole test is used to determine the ability of the organism to break down amino acid tryptophan to form a compound indole. The production of indole is detected by kovac's reagent (containing hydrochloric acid and para-dimethyl amino benzaldehyde in amyl alcohol. The solution turns from yellow to red. The colour will form in an oily layer at the top of the broth culture.

About 4-5 drops of kovac's reagent was aseptically added to the test tubes containing the broth culture. It was then observed for appearance of a pink to red colour ring at the top layer which indicates a positive test result. Negative indole test result is shown by a light yellow to brown coloured ring at the top layer.

Oxidase test: The oxidase test is based on the principle that certain bacteria produce indophenol blue from the oxidation of dimethyl-p-phenylenediamine and alpha-naphthol. In the presence of the enzyme cytochrome oxidase (gram-negative bacteria), the N, N-dimethyl-p-phenylenediamine oxalate and alpha-naphthol react to indophenols blue.

Nutrient agar culture plate was prepared and streaked with inoculums from the nutrient agar slant culture and incubated at 37°C for 24 hours. After incubation a smear of the organism was placed on a filter paper while a drop of oxidase reagent was placed on the filter paper. Formation of purple colour in 10 seconds indicates oxidase positive while oxidase negative was recorded in the absence of purple colour change immediately or within 10 seconds.

Methyl red test: It depends on if the bacteria has the ability to utilize glucose with the production of a stable acid, the colour of the methyl red broth changes from yellow to red when added to the broth culture.

Methyl red broth was prepared and poured aseptically into test tubes and sterilized, the test tubes were inoculated with test organisms. The test tubes were covered immediately with aluminum foil to prevent contamination and incubated at 37°C for 48 hours in ambient air. After which 2ml of methyl red was added into each test tube and observed for colour change. Red colour change indicates a positive result.

H2S test: It depends on the organism's ability to produce hydrogen sulphite.

Lead acetate paper was added to an overnight broth of the organism. When the paper turns to black or shows black precipitates it is a positive result but if it remains the same it is negative.

Urease test: Urease is the product of decarboxylation of amino acids. Hydrolysis of urea produces ammonia and carbon (iv)oxide. The formation of ammonia makes the medium alkaline and the shift in PH is detected by the colour change of phenol red from the light orange to magenta (pink) colour. Rapid urease-positive organisms turn the entire medium pink within 24 hours.
Urease broth was inoculated with the inoculums in the test tube and incubated at 37°C for 48 hours. The urease-positive organisms showed pink colour after 24-48 hours while urease-negative organisms retained the colour of the urease broth even after 48 hours.

Gram staining: The basic principle of gram staining involves the ability of the bacterial cell wall to retain the crystal violet dye during solvent treatment. Solvent dehydrates the gram-positive cell walls with the closure of pores preventing diffusion of violet-iodine complex and thus, bacteria remain stained but in the case of gram-negative bacteria it turns pink or red.

Motility test: It is a differential medium used to determine whether an organism is motile or non-motile. Generally, if the entire test tube is turbid, this indicates that the bacteria has moved away from the stab mark (motile). If however, the stab mark is clearly visible and the rest of the tube is not turbid, the organism is likely non-motile.
With the aid of a wire loop, overnight broth were inoculated by inserting the loop halfway into the broth in the tube. Presence of turbidity indicates growth and a positive result while an absence of turbidity indicates no growth and a negative result.

Fermentation test: The principle of carbohydrate fermentation states that the action of an organism on a carbohydrate substrate results in acidification of the medium, detected by a PH indicator dye. Carbohydrate fermentation is the process micro organisms use to produce energy. Most micro organisms convert glucose to pyruvate during glycolysis; however, some organisms use alternate pathways. A fermentation medium consists of a basal medium containing a single carbohydrate (glucose, sucrose and lactose etc.) for fermentation. However the medium also contains PH indicators to detect the production of acid from fermentation. A Durham tube is placed in each tube to capture gas produced by metabolism.
To 5ml of peptone water and 2ml of sugars (glucose, sucrose and lactose) in a test tube, 2 drops of phenol red was added after which inverted Durham tubes were introduced into the tube ensuring no air bubbles was present in it. The test tubes were sterilized and the inoculums introduced and incubated for 48 hours at 37°C. After incubation, it was then observed for colour changes and acid production. The test tubes with colour changes to yellow were taken as positive while those still red were negative. Presence of bubbles in the Durham tubes were taken as gas producers while those without were recorded as well.

ANTIBIOTIC SUSCEPTIBILITY TEST
Antibiotics susceptibility test for each organism was carried out using a multi disk containing several antibiotics. The OPTUDISC multi disk used consists of Ofloxacin, gentamycin, ciprofloxacin, cephalexin, streptomycin, septrin, Ampicillin, Nalidixic acid, perfloxacin and Augmentin.
Kirby-Bauer disk diffusion technique was used.
Mueller Hilton agar was prepared and sterilized according to the directions on the label and poured into sterile petri dishes prior to solidification. Then the agar plates were labelled. The test organisms were inoculated all over the surface of the plate with the aid of sterile swab stick. Multi disk was then placed in the plate with the edges touching the agar in the plates. The plates were then incubated at 37°C for 24 hours in an inverted position. Results were recorded as figures in form of millimeters based on zone of Inhibition of the various organisms.

3. RESULTS

TABLE 1.0 BIOCHEMICAL TESTS

S/N	CODE	CAT	CIT	IND	OX	MR	H2S	URE	GRAM STAIN	MoT	G	L	S	INFERENCE
1	N EU	+	+	+	-	+	+	+	- Rod	+	A	A	A	*Proteus spp*
2	N K	+	+	+	-	+	+	+	- Rod	+	AG	A	A	*Proteus spp*
3	N OK	+	-	+	-	+	-	-	- Rod	+	AG	AG	A	*Escherichia coli*
4	N AB	+	+	+	-	+	+	+	- Rod	+	A	A	A	*Proteus spp*
5	N OR	+	+	+	-	+	+	+	- Rod	+	AG	AG	A	*Proteus spp*
6	N IS	+	+	+	-	+	+	+	- Rod	+	A	AG	A	*Proteus spp*

7	N EK	+	+	+	-	+	+	+	- Rod	+	A	AG	A	*Proteus spp*
8	M EUP	+	-	+	-	+	-	-	-Rod in chains	+	AG	AG	A	*Escherichia coli*
9	M EUCr	+	-	-	-	+	+	-	- Rod	+	A	AG	AG	*Salmonella spp*
10	M KP	+	-	+	-	+	-	-	- Rod	+	A	A	AG	*Escherichia coli*
11	M KCr	+	-	-	-	+	+	-	- Rod	+	AG	AG	A	*Salmonella spp*
12	M OKP	+	-	+	-	+	-	-	- Rod	+	AG	A	A	*Escherichia coli*
13	M OKCr	+	-	-	-	+	+	-	- Rod	+	A	AG	AG	*Salmonella spp*
14	M ABP	+	-	+	-	+	-	-	- Rod	+	AG	A	A	*Escherichia coli*
15	M ABCr	+	-	-	-	+	+	-	-Rodinchains	+	AG	AG	AG	*Salmonella spp*
16	M ORP	+	-	+	-	+	-	-	- Rod	+	A	A	AG	*Escherichia coli*
17	M ORCr	+	-	-	-	+	+	-	- Rod	+	AG	AG	AG	*Salmonella spp*
18	M ISP	+	-	+	-	+	-	-	- Rod	+	AG	AG	A	*Escherichia coli*
19	M ISCr	+	-	-	-	+	+	-	- Rod	+	A	AG	AG	*Salmonella spp*
20	M EKP	+	-	+	-	+	-	-	-Rod	+	AG	AG	A	*Escherichia coli*
21	M EKCr	+	-	-	-	+	+	-	- Rod	+	AG	AG	AG	*Salmonella spp*
22	M EUC	+	-	-	-	+	-	-	- Rod	-	A	AG	AG	*Shigella spp*
23	M KC	+	-	+	-	+	-	-	- Rod	-	A	A	AG	*Shigella spp*
24	M OKC	+	-	+	-	+	-	-	- Rod	-	A	A	AG	*Shigella spp*
25	M ABC	+	-	+	-	+	-	-	- Rod	-	A	AG	AG	*Shigella spp*
26	M ORC	+	-	-	-	+	-	-	-Rod	-	AG	A	AG	*Shigella spp*
27	M ISC	+	-	+	-	+	-	-	- Rod	-	A	AG	A	*Shigella spp*
28	M EKC	+	-	-	-	+	-	-	- Rod	-	AG	AG	A	*Shigella spp*
29	DC EUB	+	-	-	-	+	+	-	-Rod	+	AG	AG	AG	*Salmoella spp*
30	DC EUC	+	-	+	-	+	-	-	-Rod	-	AG	AG	A	*Shigella spp*
31	DC KB	+	-	-	-	+	+	-	- Rod	+	AG	AG	AG	*Salmonella spp*
32	DC KC	+	-	+	-	+	-	+	- Rod	-	A	AG	AG	*Shigella spp*
33	DC OKC	+	-	-	-	+	-	-	- Rod	-	A	AG	A	*Shigella spp*
34	DC OKB	+	-	-	-	+	+	-	- Rod	+	A	AG	A	*Salmonella spp*
35	DC ABC	+	-	-	-	+	-	-	- Rod	-	A	AG	AG	*Shigella spp*
36	DC ABB	+	-	-	-	+	+	-	- Rod	+	AG	A	AG	*Salmonella spp*
37	DC ORC	+	-	+	-	+	-	-	- Rod	-	AG	A	AG	*Shigella spp*
38	DC ORB	+	-	-	-	+	+	-	- Rod	+	AG	AG	AG	*Salmonella spp*
39	DC ISC	+	-	-	-	+	-	-	- Rod	-	A	AG	A	*Shigella spp*
40	DC ISB	+	-	-	-	+	+	-	- Rod	+	AG	AG	AG	*Salmonella spp*
41	DC EKC	+	-	+	-	+	-	-	-Rod	-	A	AG	AG	*Shigella spp*
42	DC EKB	+	-	-	-	+	+	-	-Rod	+	AG	AG	AG	*Salmonella spp*

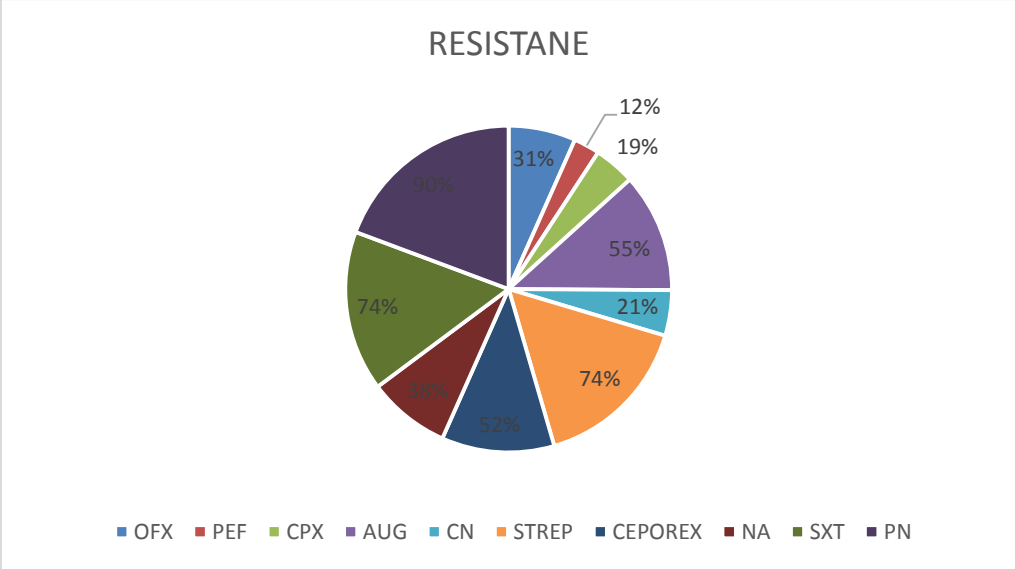
KEYS;
N= Nutrient agar, M= McConkey agar, DC= Deoxycholate citrate agar, CAT= Catalase test, CIT= Citrate test, IND= Indole test, OX= Oxidase test, MR= Methyl Red test, H2S= Hydrogen Sulphide test, URE= Urease test, MoT= Motility test, G= Glucose, L= Lactose, S= Sucrose
+; positive, -; negative, P= Pink, C & Cr= Cream, B= Black head.

TABLE 2.0; ANTIBIOTICS SUSCEPTIBILITY TEST ZONE OF INHIBITION

S/N	CODE	OFX	PEF	CPX	AUG	CN	S	CEPOREX	NA	SXT	PN	INFERENCE
1	N EU	-	11	20	-	-	-	-	-	-	-	*Proteus spp*
2	N K	17	-	17	-	18	-	17	18	-	-	*Proteus spp*
3	N OK	-	12	-	-	-	-	-	-	-	-	*Escherichia coli*
4	N AB	-	-	28	-	14	-	-	-	-	-	*Proteus spp*
5	N OR	-	15	20	-	20	-	18	17	18	-	*Proteus spp*
6	N IS	-	-	-	-	-	-	-	-	-	-	*Proteus spp*
7	N EK	15	16	-	-	17	-	10	-	20	-	*Proteus spp*
8	M EUP	20	25	23	-	18	-	-	-	-	-	*Escherichia coli*
9	M EUCr	25	21	18	18	18	-	15	14	11	-	*Salmonella spp*
10	M KP	22	20	22	15	17	-	-	-	17	-	*Escherichia coli*
11	M KCr	20	22	20	17	20	-	-	17	-	-	*Salmonella spp*
12	M OKP	17	12	17	-	11	-	-	-	-	-	*Escherichia coli*
13	M OKCr	21	24	17	21	22	-	14	15	-	-	*Salmonella spp*
14	M ABP	20	12	27	14	-	-	-	-	-	-	*Escherichia coli*
15	M ABCr	13	22	25	-	21	-	-	-	11	11	*Salmonella spp*
16	M ORP	20	18	23	17	-	-	20	-	18	-	*Escherichia coli*
17	M ORCr	25	23	22	16	18	-	-	-	-	11	*Salmonella spp*
18	M ISP	-	15	17	-	-	-	16	-	-	-	*Escherichia coli*
19	M ISCr	27	21	20	-	17	-	18	17	-	-	*Salmonella spp*
20	M EKP	20	20	27	-	-	-	-	-	-	-	*Escherichia coli*
21	M EKCr	27	20	22	15	20	-	17	-	-	-	*Salmonella spp*
22	M EUC	17	18	-	20	21	14	18	18	-	11	*Shigella spp*
23	M KC	18	17	-	-	18	15	20	21	11	-	*Shigella spp*

24	M OKC	-	-	12	-	-	-	-	-	-	-	*Shigella spp*
25	M ABC	-	25	-	17	17	17	18	20	15	-	*Shigella spp*
26	M ORC	15	18	11	18	15	16	21	18	-	-	*Shigella spp*
27	M ISC	17	17	17	-	20	18	20	-	-	11	*Shigella spp*
28	M EKC	-	-	18	11	22	12	21	-	12	-	*Shigella spp*
29	DC EUB	25	20	17	17	15	-	-	12	-	-	*Salmoella spp*
30	DC EUC	-	19	-	-	25	11	18	15		-	*Shigella spp*
31	DC KB	21	22	21	18	17	-	-	14	11	-	*Salmonella spp*
32	DC KC	20	18	20	-	21	18	22	11		-	*Shigella spp*
33	DC OKC	22	20	26	-	23	-	-	-	14	-	*Shigella spp*
34	DC OKB	23	18	20	17	20	-	12	11	-	-	*Salmonella spp*
35	DC ABC	17	17	18	14	18	17	17	-	-	-	*Shigella spp*
36	DC ABB	-	18	24	15	21	-	14	-	-	-	*Salmonella spp*
37	DC ORC	14	20	-	-	20	15	-	-	-	-	*Shigella spp*
38	DC ORB	21	25	20	-	18	-	17	-	-	-	*Salmonella spp*
39	DC ISC	-	16	10	-	-	-	-	-	-	-	*Shigella spp*
40	DC ISB	-	19	27	-	15	-	-	-	-	-	*Salmonella spp*
41	DC EKC	18	18	17	11	18	20	-	12	-	-	*Shigella spp*
42	DC EKB	20	21	22	18	17	-	15	-	-	-	*Salmonella spp*

KEYS +; susceptible, -; resistant
NB; inhibition lines between 11 and 14 is considered intermediate +/-
OFX; Ofloxacin
PEF; Perfloxacin
CPX; Ciprofloxacin
AUG; Augmentin
CN; Gentamycin
S; Streptomycin
Ceporex; Cephalexin
NA; Nalidixic Acid
SXT; Septrin (Trimethoprim/Sulfamethoxazole)
PN; Ampicillin



4. DISCUSSIONS

The bacteria pathogens isolated were gram-negative bacteria with *Salmonella spp* and *Shigella spp* having the highest prevalence followed by *Escherichia coli* and *Proteus spp.* The frequency of each isolate is seen in table 1 which explains the distribution of different isolates in the poultry farms. *Proteus spp*(6) (14%), *Escherichia coli* (8) (19%), *Salmonella spp* (14) (33%), a*nd Shigella spp* (14) (33%). The sensitivity results in table 2 shows that most of the isolates were resistant to at least 2 or more conventional antibiotics used. *Proteus spp*had the highest resistance to all the antibiotics tested. *Escherichia coli, Salmonella spp and Shigella spp* showed intermediate resistance to the conventional antibiotics. The high resistance pattern of the isolates suggests that most of the isolates have lost sensitivity to conventional antibiotics used in the study. Although isolates are part of the normal flora found in the gastrointestinal tract of poultry birds, they are of great importance to public health as they are major pathogens responsible for most gastrointestinal infections in humans which are becoming difficult to treat recently. The use of

poultry droppings as manure to boost crop production increases the possibility of antibiotics resistant gene transfer when the crops are consumed. High level of resistance against Ampicillin was observed with all the isolates being 90%. Resistance to streptomycin and septrin (trimethoprim and sulfamethoxazole was also high (74%). An intermediate resistance was observed with Augmentin (amoxicillin and clavulanic acid potassium), cephalexin, ofloxacin, and nalidixic acid ranging from 55-16% for all the isolates. However most of the isolates showed significant sensibility to the fluoroquinolones (ofloxacin, perfloxacin and ciprofloxacin). With regards to the antibiotic resistance pattern of bacteria species to individual antibiotics, Proteus spp demonstrated the highest resistance being that it was 100% resistant against Augmentin (amoxycillin /clavulanate potassium), streptomycin and Ampicillin. *Escherichia coli* had a high resistance rate against cephalexin (75%), Nalidixic acid (100%), septrin (75%). Salmonella and Shigella spp demonstrated high resistance to septrin and ampicillin ranging from 71%- 85%. All the species of isolates showed low resistance to the fluoroquinolones and gentamycin ranging from 14% -42%. This indicates that more than half of the isolates were multi-drug resistant organisms as resistance to three or more different classes of the antibiotics used.

5. CONCLUSION

From the results obtained resistance of the isolates to several antibiotics was observed confirming the prevalence of multi-drug resistant strains of gram-negative bacteria in the poultry waste samples. This demonstrates that the gastrointestinal tract of poultry birds serves as the reservoirs for multi-drug resistant strains of gram-negative bacteria that contribute to infections when consumed by humans. Infections could range from mild to severe diarrhoea or other life-threatening infections which are difficult to treat in the health sector. The result also suggested that bacteria organisms from poultry can spread resistant genes which they carry. This could arise from the indiscriminate use of antibiotics by poultry famers and feed producers. This study therefore recommends that poultry farmers should be enlightened on the dangers of resistant gene transfer and the means to prevent it, policies and regulations should also be put in place on the proper poultry waste disposal as well as its use as manure in crop production. These means will help curb the transfer of resistant gene strains to the human population thereby reducing the risk of infections by these resistant strains.

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