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Distribution, Prevalence, Virulence and Antimicrobial Resistance profile of *Enterococcus* species isolated from Chicken and Poultry Droppings in Ojo, Lagos, Nigeria

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Abstract
Enterococcus species are important commensals and opportunistic pathogens associated with animals and the environment. This study aimed to investigate the distribution, prevalence and antimicrobial resistance of Enterococcus species isolated from chicken and poultry droppings in Ojo, Lagos. Two hundred faecal samples were collected from various poultry farms in the study area. Enterococcus species were isolated, and identified through standard microbiological methods, biochemical tests and assessed for antimicrobial susceptibility using the Kirby-Bauer's disk diffusion method. The results revealed 230 isolates with a widespread presence of Enterococcus spp. 100 (43.48%) and others: Streptococcus spp. 40 (17.39%), Staphylococcus spp. 40 (17.39%), Salmonella spp. 26 (11.30%) and Pseudomonas spp. 24 (10.43%) in poultry droppings. The prevalence rates of Enterococci varied among the different poultry birds: broilers, layers, turkeys, breeders, and geese with 14, 28, 20, 26 and 12 isolates respectively. Varying levels of virulence factors were observed, with aggregation substance being the most prevalent (70%) and cytolysin the least prevalent (55%). Antimicrobial resistance of Enterococcus spp. to erythromycin was 92%, Streptococcus spp. to erythromycin (86.6%), Staphylococcus spp. to rocephin (75%)., Salmonella spp. to levofloxacin (84.6%) and Pseudomonas spp. to nalidixic acid (75%) was observed and they were susceptible to ciprofloxacin (63%), ciprofloxacin (76.6%), erythromycin (82.5%), augmentin (84.6%) and levofloxacin (87.5%) respectively. The high antimicrobial resistance by the bacterial isolates portends a potential public health concern considering the likely problem associated especially with multiple antibiotic-resistant enterococci in the poultry environment. These findings provide insights into the dissemination of Enterococcus species in poultry settings and their resistance patterns, thus underscoring the need for prudent antimicrobial use and effective hygiene practices in poultry farming so as to mitigate potential risks to both animal and human health.

Keywords*: Enterococcus, Resistance, Susceptibility, Antibiotic test, Poultry droppings, Poultry birds*

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I. Introduction

Enterococcus species, belonging to the lactic acid bacteria group, are prevalent in various ecological niches, including the gastrointestinal tracts of animals and humans, as well as in environmental sources such as soil, water, and food [1]. While traditionally considered commensals, certain *Enterococcus* species have gained notoriety due to their role as opportunistic pathogens associated with a range of infections in humans, particularly those involving the urinary tract and bloodstream [2]. Moreover, their remarkable capacity to acquire and transfer antibiotic resistance genes has elevated them to a central position in the global challenge of antimicrobial resistance [3].

In the context of the poultry industry, characterized by intensive farming practices and the frequent use of antibiotics, *Enterococcus* species often flourish within poultry flocks and associated environments [4]. Poultry, including chickens, are recognized reservoirs for enteric bacteria, and the extensive use of antibiotics for growth promotion and disease prevention provides a conducive environment for the development and spread of antimicrobial resistance [5].

Enterococcus faecium are commonly found in the guts of poultry, they can cause infections in poultry that can lead to significant economic losses for the industry. Enterococcal infections in poultry can result in decreased growth rates, reduced feed efficiency and increased mortality rates [6]. Poultry and food products of poultry origin are the most consumed worldwide [7]. Enterococci can contaminate poultry products and pose a risk to human health if consumed [8]. As avian *Enterococcus* strains are known to share genetic material with human strains, the dissemination of antibiotic-resistant [9]. Antibiotic resistance in Enterococci is also a concern for the poultry industry, as the use of antibiotics in poultry production can contribute to the development and spread of antibiotic-resistant strains [10]. Therefore, the presence of antimicrobial-resistant Enterococci, especially multidrug resistance *Enterococcus* species, in poultry is of public health concern as it may serve as a pool from which antimicrobial resistance genes are disseminated.

Enterococcus faecium has transitioned from a commensal organism to an ESKAPE (*E. faecium*, *Staphylococcus aureus*, Klebsiella pneumoniae, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species) pathogen. ESKAPE is an acronym for a group of life-threatening nosocomial pathogens that successfully evade the effect of antimicrobial drugs and represent a model for pathogenesis, transmission, and resistance [11].

Given the increasing importance of addressing antimicrobial resistance at a global level, investigating the dynamics of *Enterococcus* species in poultry settings is not only critical for animal health but also for safeguarding public health. Vancomycin-resistant Enterococci have been reported worldwide [12], including in Zambia [13]. However, they have not been given the same attention as other commensals of the GIT such as *Staphylococci*, *Salmonella*, *Shigella*, *Campylobacter* and *Escherichia coli*. A comprehensive understanding of the prevalence and resistance patterns of *Enterococcus* species in poultry environments can inform policies on antibiotic use in agriculture, and strategies to minimize the dissemination of antibiotic resistance genes [14].

In light of the dynamic interplay between *Enterococcus* species, antimicrobial resistance, and poultry farming, this study aims to contribute valuable insights into the distribution, prevalence, and antimicrobial resistance profiles of *Enterococcus* species isolated from chicken and poultry droppings. By shedding light on the intricate relationship between this area, this research seeks to provide a foundation for evidence-based interventions that address the growing challenge of antibiotic resistance in both animal and human health.

II. Materials and Methods

Study Area

Ojo Local Government Area of Lagos State, Nigeria is situated in the southwestern part of the country, specifically within the Lagos metropolitan area and lies on the Latitude 6° 27' 59.99" N and Longitude 3° 10' 60.00" E.

Sample Collection

A total of 200 fresh poultry droppings were collected from layers, broilers, turkeys, turkeys and geese. Five different visits were made to selected poultry farms where 40 faecal samples each were collected at random from birds of different health status using sterile cotton wool swabs and stored in ice at 4 °C for experiment.

Isolation of Enterococci

Conventional microbiological assays were performed to detect and identify *Enterococcus* species as described by Facklam and Collins [15]. Briefly, 1 g of poultry droppings was suspended in 9 mL buffered peptone water (BPW) (HIMEDIA, India), mixed and incubated at 37 °C for 24 h. A loopful of the BPW suspension was streaked on Bile Esculin Agar (BEA) (HIMEDIA, India) and incubated at 37 °C for 24 h. Following this, colonial traits were noted and smears of suspect colonies (small black shiny colonies on BEA) were made and stained using Gram's color staining kit. Gram-positive cocci appearing in chains, doubles or singles were characteristic of Enterococci.

Identification of Bacteria isolates

Colonies were identified and characterized using biochemical tests such as catalase, coagulase, indole, urease, oxidase, citrate utilization, motility, nitrate reduction, methyl red and Voges Proskauer tests according to CLSI [16].

i. Gelatinase Assay:

Gelatinase production was detected by inoculating the Enterococci onto freshly prepared NA containing 3% gelatin (Merck, Germany). Plates were incubated for 18 to 24 hours at 37°C and then cooled to ambient temperature for 2 hours. The appearance of a turbid halo or zone around the colonies indicated the production of gelatinase. The production of gelatinase was assessed using a method described by Semedo [17].

ii. Haemolytic Activity:

Haemolysins activity was detected in blood agar base (CMO271, Oxoid, UK) plates, with 5% of defibrinated sheep blood after incubation at 37°C for 24 h and 50°C for 48 h. The presence of a viridant halo round the colonies indicated haemolysis, while the presence of a translucent halo indicated β-haemolysis. Haemolysin activity was evaluated on blood agar plates as per the procedure outlined by Marra [18].

iii. Cytolysin Production:

Phenotypic assays for cytolysin were conducted using blood agar base (Becton Dickinson, MA) with 5% cattle blood according to Marra [18].

iv. Aggregation Substance:

Phenotypic expression of the asa1 gene was investigated using the method of Macovei and Zurek (2006). Enterococci were grown for 6 h at 37°C in Todd-Hewitt broth (Becton, Dickinson, MA). The broth was then centrifuged at 6,000 rpm for 10 min, and the pheromone-containing supernatant that induces pheromone plasmids was removed and autoclaved for 15 min. Tested isolates were then grown in Todd-Hewitt broth (5 ml) for 6 h at 37°C. After incubation, 1 ml of the supernatant was added to each tube and incubated at 37°C overnight on a shaker at 150 rpm. Isolates that showed clumping were considered positive for aggregation substance. *Enterococcus faecalis* served as a positive control. The expression of the asa1 gene and detection of aggregation substance followed the procedure of Macovei [19].

Determination of Antimicrobial Resistance Levels

Susceptibility to amoxicillin (30 µg), gentamycin (10 µg), sulphamethoxazole/trimethoprim (SXT) (30 µg), rocephin (25 µg), zinnacef (20 µg), pefloxacin (10 µg), ciprofloxacin (10 µg) and erythromycin (10 µg) for gram positive bacteria and cefurixime (10 µg), nalidixic acid (10 µg), amoxicillin (30 µg), ceftriaxone (45 µg), cefotaxime (25 µg), levofloxacin (5 µg), imipenem (10 µg) gentamycin (10 µg), ofloxacin (5 µg), cefexime (5 µg) for Gram negative bacteria was determined using the disk diffusion method according to the CLSI [16]. The disks used for susceptibility testing were manufactured by HIMEDIA, India. Diameters of the zones of inhibition were recorded in millimeters (mm) and interpreted as susceptible, intermediate or resistant. In this study, intermediate results were taken as resistant.

III. Results

A total of 200 birds including broilers (40), layers (40), turkeys (40), breeding hen (40) and geese (40) were sampled during this study and the prevalence rates of E nterococci varied among the different poultry birds with 14, 28, 20, 26 and 12 isolates respectively. Table 1 presents the distribution of *Enterococcus* spp. and other bacterial I solates among different poultry birds, highlighting the percentage distribution of *Enterococcus* spp. Table 2 show varying levels of presence of virulence factors, with aggregation substance being the most prevalent (70%) and cytolysin the least prevalent (55%).

The results revealed 230 bacterial isolates with a widespread presence of *Enterococcus* spp. 100 (43.48%) and others: *Streptococcus* spp. 40 (17.39%), *Staphylococcus* spp. 40 (17.39%), *Salmonella* spp. 26 (11.30%) and *Pseudomonas* spp. 24 (11.43%) in poultry droppings (Figure 1). *Enterococcus* spp. was resistant to all antibiotics with erythromycin being the highest (92%) except ciprofloxacin (63%) (Figure 2).

Streptococcus spp. displayed high resistance to erythromycin (86.6%) and susceptible to ciprofloxacin (76.7%) (Figure 3). *Staphylococcus* spp. showed a high resistance to rocephin at (75%), sulphamethoxazole-trimethoprim (SXT) (67.5%) and pefloxacin (57.5%) but highly susceptible to erythromycin (82.5%), ciprofloxacin and streptomycin with 75% respectively (Figure 4). *Salmonella* spp. showed high resistance to levofloxacin with 84.6% and was susceptible to augmentin with 84.6%, cefexime (73.1%) and gentamycin (65.4%) (Figure 5). *Pseudomonas* spp. was resistant to nalidixic acid with 75% and highly susceptible to levofloxacin with 87.5%, augmentin, gentamycin and ofloxacin with 75% respectively (Figure 6).

Table 1: Frequency of *Enterococcus* spp. and other bacterial isolates in poultry birds

Total Number of bacterial Isolates	**Other bacterial isolates**	**Poultry birds**	**Number of *Enterococcus* spp. (%)**	
Enterococcus spp: 100	86	Broilers: 40	14 (6.09)	
Streptococcus spp: 40	12	Layers: 40	28 (12.17)	
Staphylococcus spp: 40	20	Turkeys: 40	20 (8.70)	
Salmonella spp: 26	0	Breeding hens: 40	26 (11.30)	
Pseudomonas spp: 24	12	Geese: 40	12 (5.22)	
TOTAL: 230	**130**	**200**	**100 (43.48)**	

Table 2: Virulence factors among *Enterococcus* spp. (n=100).

Virulence factors	**Negative (%)**	**Positive (%)**
Gelatinase	40	60
Cytolysin	45	55
Haemolytic activity	34	66
Aggregation substance	30	70

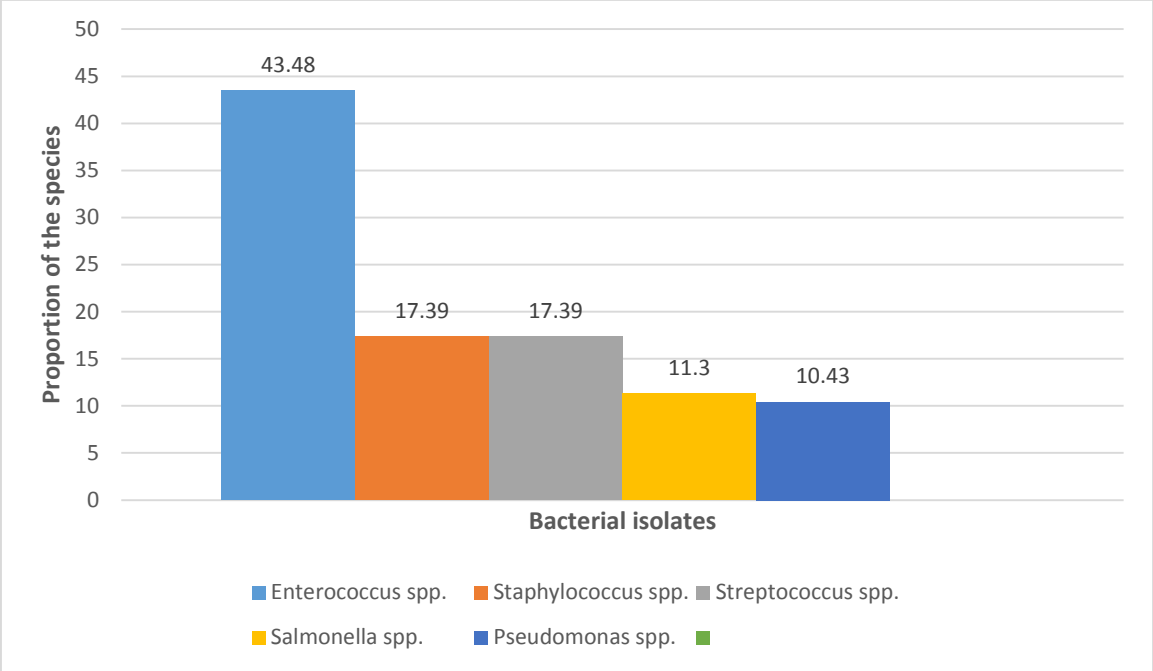

Figure 1: Percentage Distribution of Bacterial Isolates from Specimen

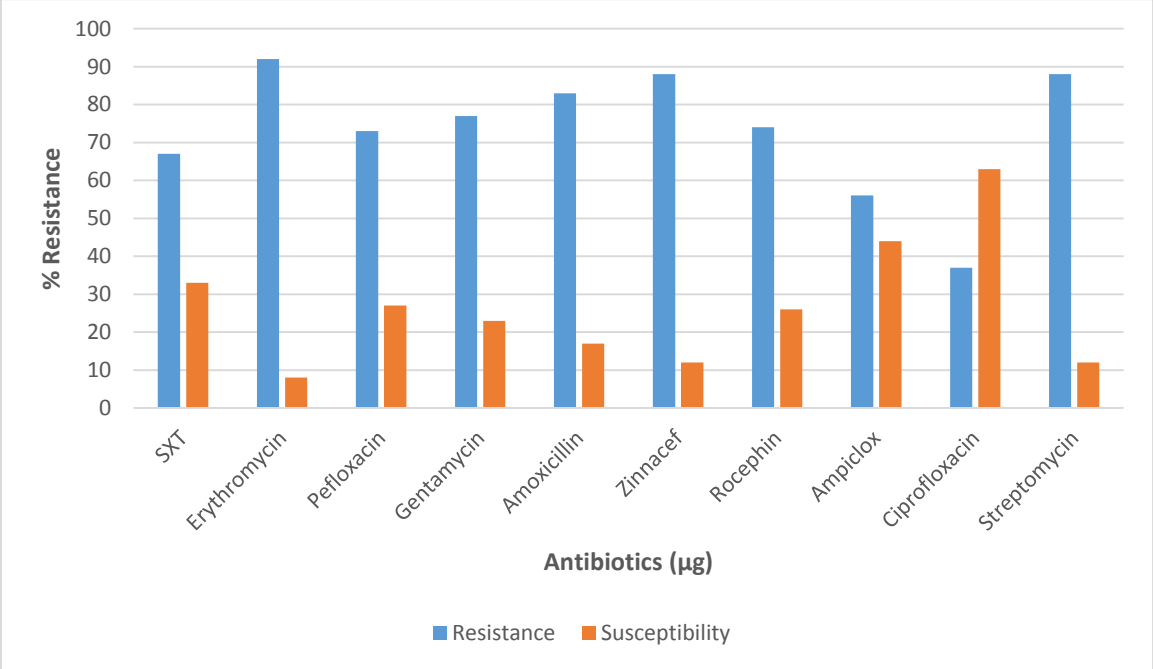

Figure 2: Antibiotic Resistance Pattern of *Enterococcus* spp.

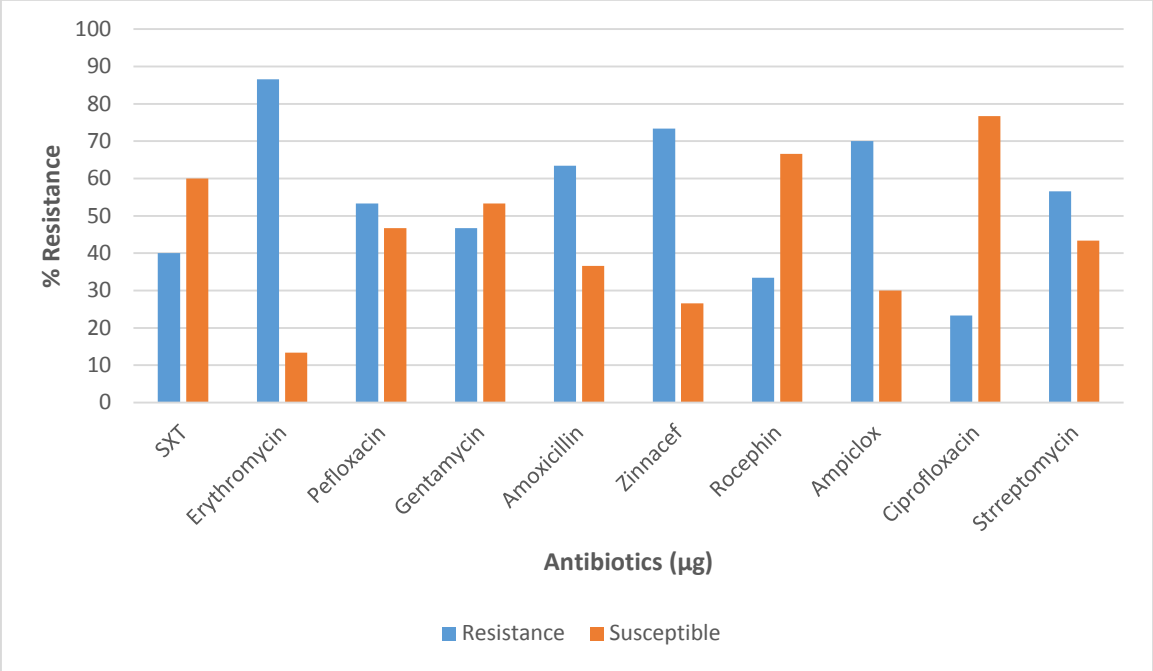

Figure 3: Antibiotic Resistance Pattern of *Streptococcus* spp.

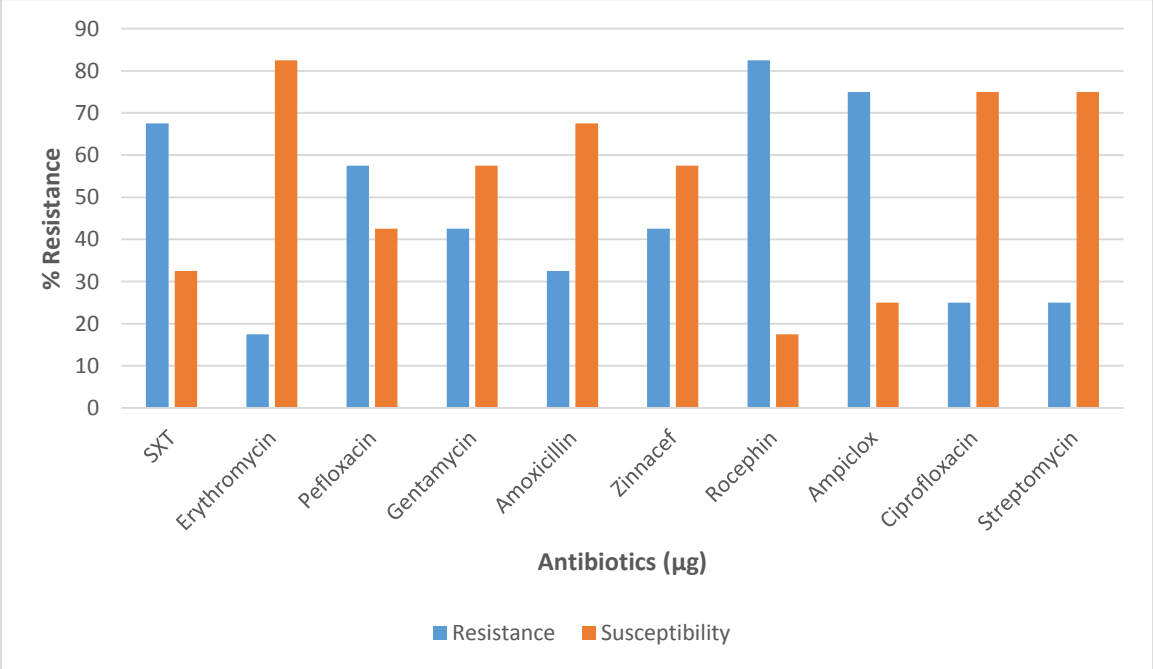

Figure 4: Antibiotic Resistance Pattern of *Staphylococcus* spp.

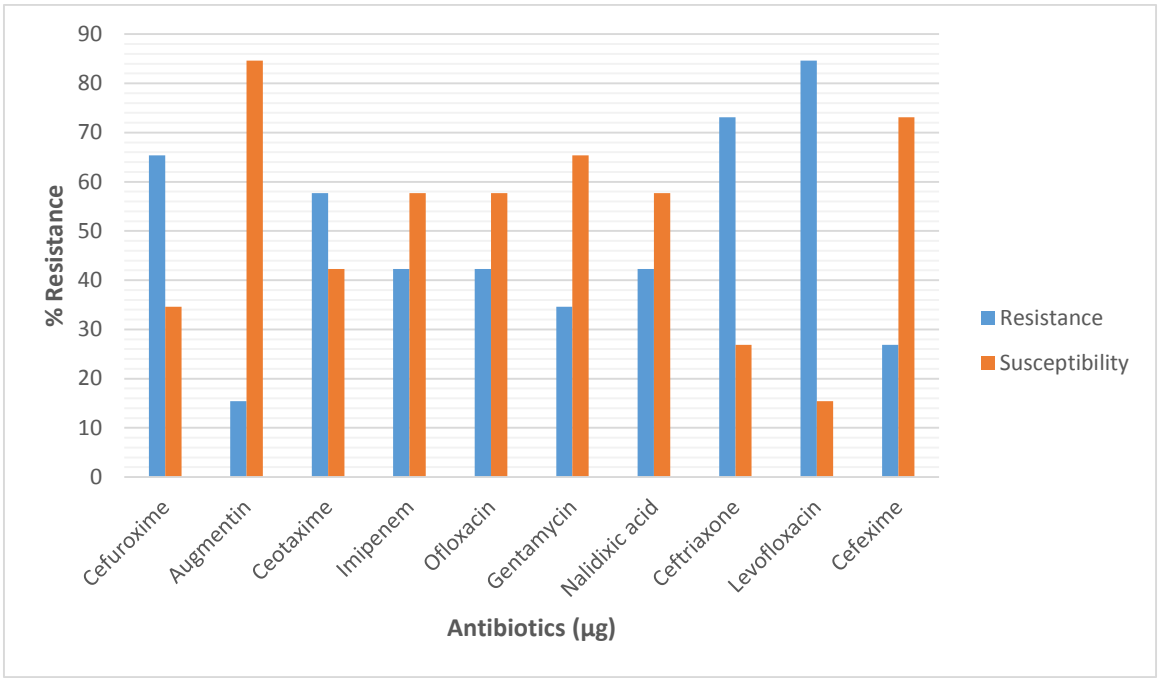

Figure 5: Antibiotic Resistance Pattern of *Salmonella* spp.

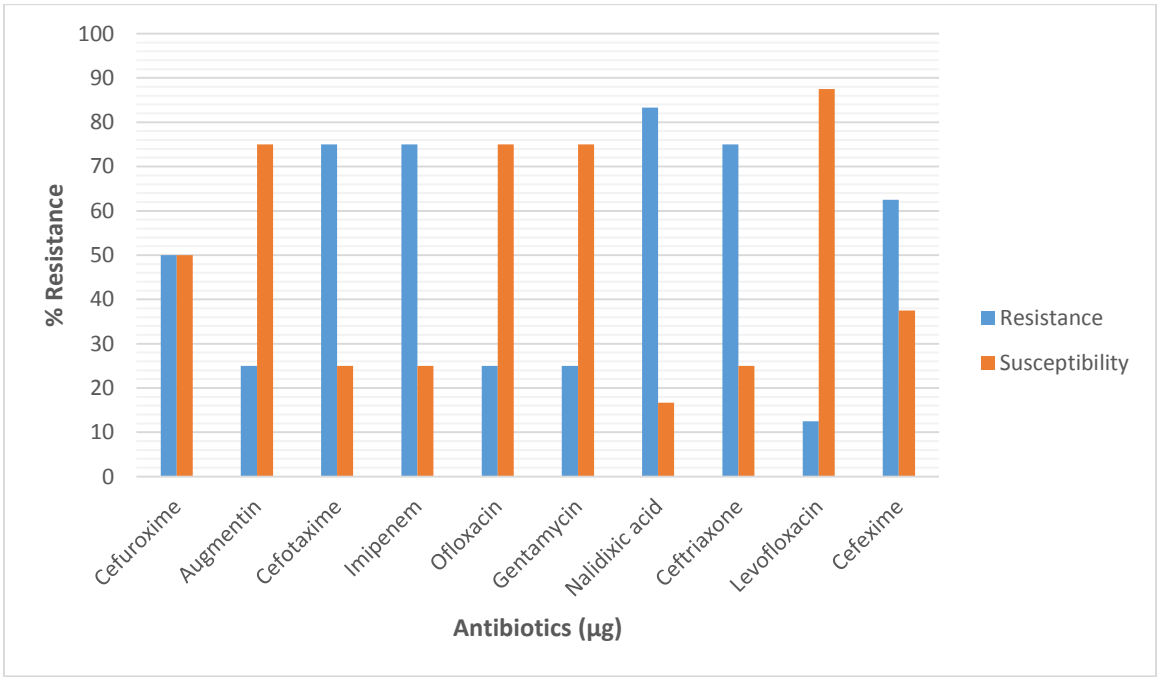

Figure 6: Antibiotic Resistance Pattern of *Pseudomonas* spp.

IV. Discussion

Enterococcus spp. were the major organisms isolated from this study constituting 43.48% of the total bacterial isolates, *Streptococcus* spp. and *Staphylococcus* spp., (17.39%) respectively, *Salmonella* (11.30%) and *Pseudomonas* (10.43%) were also isolated. This distribution of bacterial isolates in poultry is in conformity with a study carried out by Wahidullah [20], where 134 chicken samples were analysed and the frequency of isolated bacteria were Enterococci (50%), *Staphylococcus* (20%), *Salmonella* (17%), *Pseudomonas* (5%). This shows that the direct addition of poultry dropping to the field without any form of treatment poses some public health problems since they contain pathogenic microorganisms that can contaminate the surrounding crops and vegetables and become the source of infection especially when such crops or vegetables are eaten raw or taken home where they can contaminate other materials.

Enterococci were detected in broilers (14%), turkeys (28%), layers (20%), breeders (26%) and geese (12%). These results are in consonance with the result put forward by Arias [21], in a survey of the microflora of

poultry droppings. The reason behind this could be attributed to the similarity in the mechanism used in the handling process by the farm handlers. Enterococci which showed high frequency among the isolates is a very worrisome occurrence. Despite their small share in the microbiota of the microorganism, an increase in the clinical significance of these opportunistic pathogens was observed. It is mainly associated with arthritis, spondylitis, osteomyelitis, spondylolisthesis and femoral head necrosis in broiler and breeder flocks. *Enterococcus faecalis* has been linked to endocarditis in chickens, hepatic granulomas in turkeys, ascites in hens and pulmonary hypertension in broilers [22].

Gelatinase, an enzyme that degrades gelatin and other proteins, was found in 60% of the isolates. This enzyme plays a significant role in tissue invasion and infection propagation by breaking down host tissue barriers [17].

Cytolysin, a toxin that can lyse a wide range of cell types, was present in 55% of the isolates. Haemolysins were detected in 66% of the isolates, indicating a high potential for these bacteria to cause haemolysis. Haemolysins disrupt red blood cells, leading to the release of haemoglobin, which can provide a nutrient source for the bacteria and facilitate further infection [23]. The presence of haemolytic activity in a significant portion of isolates suggests a robust mechanism for host tissue invasion and nutrient acquisition.

The highest prevalence was observed for aggregation substance, with 70% of the isolates testing positive. Aggregation substance facilitates bacterial adhesion to host tissues and other bacteria, promoting biofilm formation and enhancing the bacteria's ability to colonize and persist in the host. This virulence factor is particularly significant in the context of persistent infections and biofilm-related resistance to treatments [24].

The high prevalence of the virulence factors among *Enterococcus* spp. isolated from the poultry birds indicates a substantial pathogenic potential, which can impact poultry health. Infected birds may suffer from various health issues, including systemic infections, reduced growth rates, and increased mortality, leading to economic losses in the poultry industry [25]. Furthermore, these virulent strains can spread to humans through the food chain, posing a public health risk [26]. These findings are consistent with other studies that have demonstrated the presence of multiple virulence factors in *Enterococcus* spp. isolated from different sources. Eaton and Gasson [27] reported similar levels of gelatinase and cytolysin production in *Enterococcus* strains from dairy products. Another study by Huycke [28]. highlighted the role of haemolysins and aggregation substance in the pathogenicity of *Enterococcus faecalis*.

Enterococcus spp. were resistant to all antibiotics with erythromycin being the highest (92%) except ciprofloxacin (63%), making it the foremost antibiotic to consider in the treatment of enterococcal infections. This result conforms with a study carried out by Moro [29], where Enterococci showed the most resistance to erythromycin with 61.7%, but Islam [30] reported that resistance of Enterococci to erythromycin was 72.%. Faced with these results, ciprofloxacin presents bactericidal activity against *Enterococcus* spp. However, in the context of the results, there is the possibility that some species could be tolerant to ciprofloxacin. *Streptococcus* spp. also displayed high resistance to erythromycin with 86.6% and susceptible to ciprofloxacin with 76.7% which is higher compared to a study carried out by Jordan [31] where *Streptococcus* spp. was resistant to erythromycin at 62.5%. This is in disagreement with the study carried out by Islam [30] where ciprofloxacin showed low activity against *Streptococcus* spp. at a rate of 48.6%. The course of antibiotic resistance to erythromycin could be as a result of consistent use of the antibiotic for treatment because it is easier sought for over the counter.

Staphylococcus spp. indicated high resistance to rocephin at (75%), amoxicillin, SXT and pefloxacin but highly vulnerable to erythromycin at 82.5%, ciprofloxacin and streptomycin at (75%) respectively. Ciprofloxacin is an active antibiotic agent and has shown potency against gram positive bacteria [30]. The presence of *Streptococcus* and *Staphylococcus* spp. in this proportion agrees with the study carried out by Landoni [32], that they may be part of the transient flora of poultry birds, bird health status, management practices, and contamination sources. The presence of *Staphylococcus* spp. at this level could have medical implications, particularly in terms of disease transmission risk, zoonotic potential and antimicrobial resistance. Both species can also cause opportunistic infections, particularly in individuals with weakened immune systems. The presence of these bacteria in poultry droppings could be a concern for people with compromised immunity, such as the elderly, children, or individuals with underlying health conditions.

Salmonella spp. showed high resistance to levofloxacin with 84.6% and was susceptible to augmentin at 84.6% and gentamicin. augmentin and gentamicin are broad spectrum antibiotics and have been shown in literature to present high effectivity against gram negative bacteria [33]. The detection of *Salmonella* in the study is an indication of improper hygiene practices carried out by the handlers as all species are known to be pathogenic and can cause food borne illnesses and spoilage. This agrees with Kwon [34]. that 26% of *Salmonella* spp. isolated could be a source of faecal contamination; when proper hygiene measures are not implemented, as over-crowded or stressful conditions in poultry farms can weaken the immune systems of birds, thereby making them more susceptible to *Salmonella* infections, Poor sanitation practices in the poultry environment, including improper waste disposal and cleaning procedures, can contribute to the persistence of *Salmonella* spp. [35]. *Salmonella* spp. are responsible for a significant proportion of food-borne infections in humans. Consumption of contaminated poultry products, such as eggs and meat, can lead to gastroenteritis, characterised by symptoms like diarrhoea,

abdominal cramps, fever, and vomiting. In severe cases, *Salmonella* infections can lead to hospitalization, especially in vulnerable populations such as the elderly, young children, and individuals with compromised immune systems [36].

Pseudomonas spp. was resistant to nalidixic acid with 75% and highly susceptible to levofloxacin at 87.5%, augmentin, gentamycin and ofloxacin with 75% respectively. The 24% prevalence of *Pseudomonas* spp. could result from contamination originating from the external environment, feed, water, or other sources, as *Pseudomonas* spp. may interact with other microorganisms present in the droppings, which can inadvertently affect their prevalence. *Pseudomonas* spp. are opportunistic pathogens and can cause infections, especially in individuals with compromised immune systems or underlying health conditions. While the presence of *Pseudomonas* spp. in poultry droppings does not necessarily imply direct human health risks, it is essential to consider potential pathways of transmission, such as through contaminated water, surfaces, or food [37], to avert untoward associated consequences.

V. Conclusion

A high prevalence of Enterococci and other pathogenic bacteria in poultry droppings and the increased resistance of *Enterococcus* spp. to a wide range of antibiotics were observed in this study. The findings also highlight the importance of monitoring antibiotic susceptibility and resistance in poultry populations. The varying resistance patterns observed across different poultry categories emphasise the need for targeted antibiotic management strategies tailored to specific species. It is crucial for poultry producers, veterinarians, and policy makers to consider these findings when developing antibiotic usage protocols to ensure effective disease management and minimise the development of antibiotic-resistant bacteria in poultry production.

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Conflicts of Interest

The authors declare no conflict of interest.

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