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Antibiotics resistance and phenotypic virulence factors of *Vibrio* species isolated from poultry litters at Obafemi Awolowo University, research farm.

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Abstract

The study investigates the antimicrobial resistance and phenotypic virulence factors of *Vibrio* isolates in poultry litter. Samples were enriched in 5 mL alkaline peptone water 1:10 (w/v) for 6 hours and cultured on Thiosulphate Citrate Bile salt (TCBS) agar. *Vibrio* isolates were identified through biochemical tests assay and were subjected to antibiotic susceptibility tests using standards disc diffusion technique. The phenotypic virulence factors, including hemolytic and proteolytic activity were determined. A total of 100 *Vibrio* isolates were identified as *Vibrio* parahaemolyticus (49%), *V. cholerae* (26%), *V. vulnificus* (11%), *V. alginolyticus* (9%), and *V. damsela* (5%). The *Vibrio* isolates showed a significant resistance to the selected antibiotics tested with 99% resistant to Gentamycin, 95% Cefixime, 96% Ofloxacin, 98% Augmentin, 96% Nitrofurantoin, 100% Ciprofloxacin, and 100% Cefuroxime. The *Vibrio* isolates showed varying levels of haemolysis with 17% alpha haemolysis, 61% beta haemolysis, 22% gamma haemolysis, and 29% proteolytic activity. The study reveals that poultry litter contains *Vibrio* isolates with antibiotic resistance and virulence factors, increasing their risk of severe infections. The high beta haemolysis prevalence highlights the interconnected relationship between animal, environmental, and human health, underscoring the need for effective management.

Keywords: Antibiotics resistance; Pathogenicity; Poultry liters; Vibrio; Virulence factors.

1. Introduction

Poultry litter is a mixture of feces, waste feeds, bedding material, and feathers, is a cost-effective organic soil fertilizer that enhances crop quality and productivity [1]. However, it can also be contaminated with pathogens such as bacteria, viruses, parasites, and fungi, posing risks to the environment [2, 3]. Poultry litter contains pathogenic organisms such as *Vibrio* species *E. coli*, *Salmonella* species and *Campylobacter* species posing a risk of transmission to animals, humans, and the environment [4, 5]. Poultry litter can serve as a potential reservoir for *Vibrio* species due to its rich organic content and can pose a risk of contamination to the environment if not properly managed [6].

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The *Vibrio* genus belongs to *Vibrionaceae* family, a group of Gram-negative bacteria, primarily flagellated, non-spore-forming rods, found in aquatic environments, particularly marine waters and estuaries. They are facultative anaerobes that obtain nutrients from mutualistic, parasitic, or pathogenic relationships [7, 8]. Over 140 *Vibrio* species are known, with 12 species being infectious to humans, with *V. cholerae* being the causative agent of cholera disease [9, 10]. Non-cholerae *Vibrio* species. are common foodborne infections from contaminated water, undercooked or raw seafood consumption [11]. Symptoms include gastroenteritis and diarrhea, sometimes associated with virulence factors like shiga-like cytotoxin, toxin-coregulated pilus, cytotoxins, siderophores, hemolysins, proteases, and heat-stable enterotoxin [12, 13].

Vibrio species pathogenicity is determined by virulent factors encoded by virulent genes, which influence infection severity and drug resistance [14, 15]. *Vibrio* species acquire external genetic material through horizontal gene transfer (HGT) and their adaptability in diverse environments is enhanced by shared genetic material encoding virulence factors such as toxin production, quorum sensing, lysogenic phage, hemolysin, and proteases [16, 17, 18, 19].

Vibrio species have developed resistance against antibiotics due to virulent factors and excessive use in human disease, agriculture, and aquaculture [20, 14, 15]. Their unique genetic makeup and competency have allowed them to adapt to adverse environmental conditions and resist the antibacterial agent [21]. This resistance poses a significant threat, potentially leading to antibiotic-resistant infections. Hence, this study aimed to determine the antibiotic resistance and phenotypic virulence factors of *Vibrio* species from poultry litters to understand potential health risks and the spread of antibiotic-resistant pathogens in the environment.

2. Materials and methods

2.1. Collection of samples

Poultry liters were collected from Obafemi Awolowo University's Research farm and transported in ice-pack to microbiology laboratory for microbial analysis over a four-week period.

2.2. Microbiological analysis

2.2.1. Enrichment of the samples

Exactly 1 g of the poultry liters were enriched in alkaline peptone water (APW, pH 8.4) in ratio 1:10 w/v and incubated at 37 ^{o}C, for 6 hours in an incubator shaker (ThermoFisher Scientific, Berkeley, MO, USA) before isolation.

2.2.2. Isolation of Vibrio isolates

The study used a modified method from Mishra *et al.* [22] to isolate presumptive bacterial colonies. The enriched samples were inoculated by streaking on thiosulfate citrate bile salts sucrose (TCBS) (Hi-media) agar and incubated at 37°C for 18-24 hours.

2.2.3. Identification of Vibrio isolates by biochemical tests assay

Vibrio isolates were identified through various biochemical tests, including oxidase, catalase, nitrate reduction, methyl red, Voges–Proskauer, arginine utilization, salt tolerance, ortho-nitrophenyl-b-D-galactopyranoside (ONPG), citrate utilization, ornithine utilization, and various carbohydrate tests (Hi-Media), and biochemical test strips. The isolates were prepared using a 12-well test strip, inoculated with 50 µL of the test sample, and incubated for 18-24 hours at 37°C and then analyzed for different *vibrio* spp. based on color changes. Probable *vibrio* species were confirmed using Bergey's Manual of Systematic Bacteriology [23] and Automated Biometric Identification System (ABIS online).

2.3. Determination of the virulence factors of the *Vibrio* isolates

2.3.1. Determination of hemolytic activity

The test organisms were inoculated on blood agar and incubated at 37°C for 24 hours. A clear zone around the colony confirmed a positive test, indicating the presence of hemolysis. Hemolytic reactions are assessed by observing partial red blood cell hydrolysis, resulting in a green zone (α-hemolysis), total red blood cell hydrolysis, producing a clear zone around bacterial colonies (β-hemolysis), or no reaction (γ-hemolysis).

2.3.2. Determination of proteolytic activity

The test organism was inoculated on skimmed milked agar containing 2.5g of Nutrient agar (Hi-media) and 10g of skimmed milk, then incubated at 37°C for 24 hours. The presence of a transparent zone around the colonies indicates caseinase activity.

2.4. Antibiotic susceptibility of the *Vibrio* isolates

2.4.1. Standardization of the Vibrio isolates

The *Vibrio* isolates were standardized using the National Committee for Clinical Laboratory Standards (NCCLS) [24]. A 0.2 mL of each bacterium's 18-hour-old culture was suspended in sterile universal bottles containing 20 mL of sterile nutrient broth (Hi media) and incubated for 5 hours at 37°C to obtain a logarithm growth phase. A sterile normal saline (0.9 %) was gradually added to compare turbidity to the McFarland Standard of 0.5, corresponding to approximately 1.0×10^8 CFU/mL.

2.4.2. Susceptibility test of the Vibrio isolates

Standards disc diffusion technique with Mueller–Hinton agar (MHA) (Hi- media) was used for antimicrobial susceptibility testing. Fresh cultures (18–22 h old) of the identified *Vibrio* isolates were introduced into 5 mL of 0.85% sterile saline with the suspension turbidity of 0.5 McFarland standards were inoculated uniformly with sterile swabs after each disc were aseptically added. The seeded plates were incubated for 18-24 hours at 37°C. Zones of inhibition were measured and interpreted as resistant, susceptible, or intermediate, following the CLSI guideline [25]. The antibiotics used were Gentamicin, Cefixime, Ofloxacin, Ceftaxidime, Augmentin, Nitrofurantoin, Ciprofloxacin, and Cefuroxime. The zone diameter interpretation was based on CLSI guideline [25]. The antibiotics used were Gentamicin (10 µg), Cefixime (5 µg), Ofloxacin (5 µg), Ceftaxidime (30 µg), Augmentin (30 µg), Nitrofurantoin (300 µg), Ciprofloxacin (5 µg), and Cefuroxime (30 µg).

2.5. Data and Statistical Analysis

The data obtained were statistically analyzed using Statical Package for Social Sciences (SPSS) version 22 and Microsoft excel (2016)

3. Results

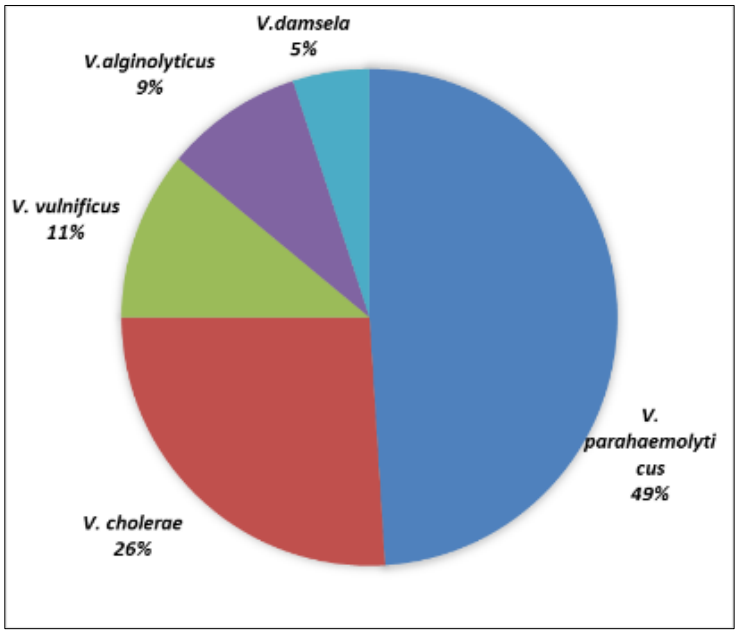

Figure 1 Percentage occurrences of the *Vibrio* isolates.

A total of 100 *Vibrio* isolates were obtained and identified as *V. parahaemolyticus* (49 %), *V. cholerae* (26 %), *V. vulnificus* (11 %), *V. alginolyticus* (9 %) and *V. damsela* (5 %). (Figure 1). The *Vibrio* isolates demonstrated species- or strain-

specific resistance to different antibiotics tested, indicating 99% were resistant to Gentamycin, 95% Cefixime, 96% Ofloxacin, 98% Augmentin, 96% Nitrofurantoin, 100% Ciprofloxacin, and 100% Cefuroxime (Figure 2).

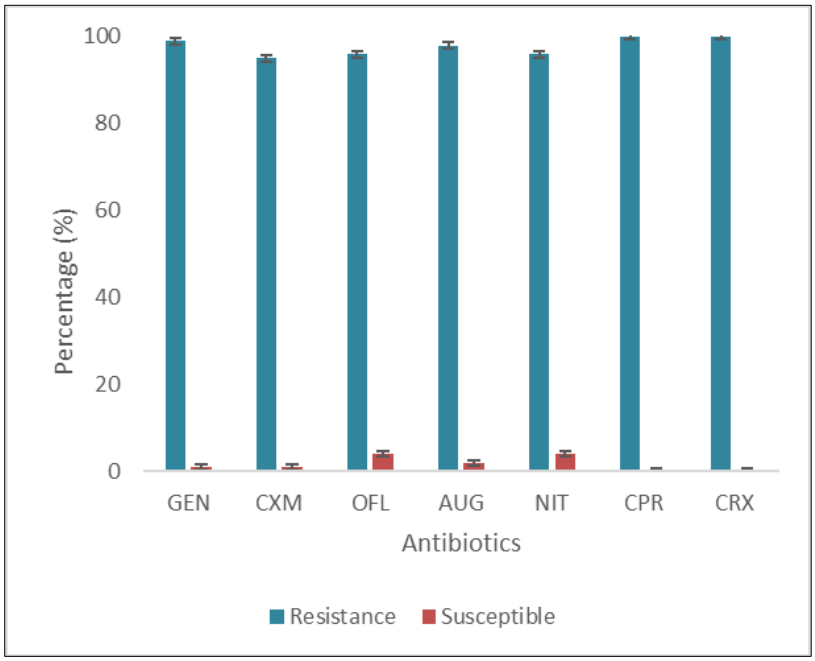

Figure 2 Antibiotic resistance pattern of the *Vibrio* isolates

Key

Antibiotics	**MIC**	**Resistance (R)**	**Intermediate (I)**	**Susceptible (S)**
Gentamicin (GEN)	10µg	≤ 12	13-14	≥ 15
Cefixime (CXM)	5µg	≤ 14	15-17	≥ 18
Ofloxacin (OFL)	5µg	≤ 12	13-15	≥ 16
Augmentin (AUG)	30µg	≤ 14	15-17	≥ 18
Nitrofurantoin (NIT)	300µg	≤ 14	15-16	≥ 17
Ciprofloxacin (CPR)	5 µg	≤ 15	16-20	≥ 21
Cefuroxime (CRX).	30µg	≤ 14	15-17	≥ 18

Among the isolates, 18% exhibited alpha hemolysis, 61% beta hemolysis, and 22% gamma hemolysis, while 29% exhibited proteolytic activity.

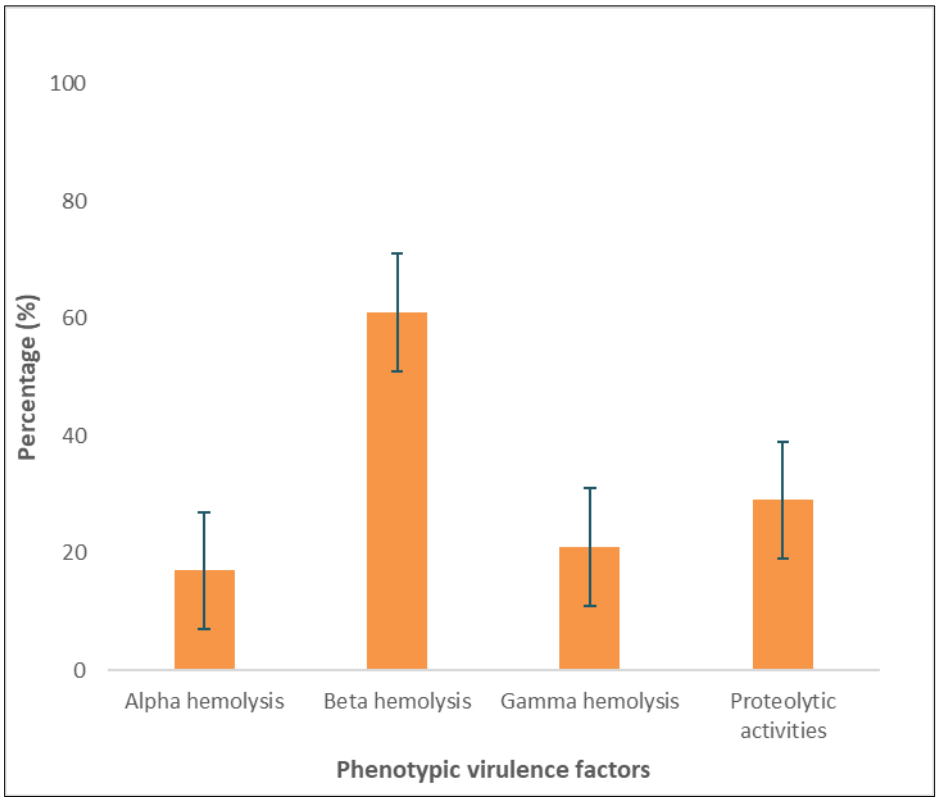

Figure 3 Phenotypic virulence factors of the *Vibrio* isolates

4. Discussion

The study revealed that a significant number of *Vibrio* isolates were resistant to the antibiotics tested, similar to Adeleke *et al.* [26] study on antibiotic resistance in poultry litters. This resistance may be due to excessive antibiotic use in poultry farms and the addition of antibiotics into their water and feed [27]. This resistance limits antibiotic effectiveness in treating *Vibrio* infections and can spread antibiotic-resistant bacteria to humans through contaminated food or direct contact [28]. The occurrence of *V. parahaemolyticus* and *V. cholerae* are prevalent in this study due to their ability to survive and multiply in the warm and saline environments commonly found in poultry farms [29]. These bacteria can easily contaminate the poultry products during processing, posing a significant risk to consumers if not properly managed [30].

The study reveals that the phenotypic virulence abilities of the *Vibrio* isolates including haemolysis and proteolytic, has the ability to complicate infection treatment by causing tissue damage and evading the immune system. These traits may be linked to the presence of antibiotic resistance genes, highlighting the interconnected nature of antimicrobial use and bacterial pathogenicity [15]. Microbial virulence factors are molecules produced by pathogenic bacteria that can evade host defense and cause infections. Some secrete microbial products that can enter host cells and aid in infection [31].

Virulence genes in *Vibrio* species have been used to initiate infections such as diarrhea, gastroenteritis, cholera, sometimes associated with virulence factors like shiga-like cytotoxin, toxin-coregulated pilus, cytotoxins, siderophores, hemolysins, proteases, and heat-stable enterotoxin [12, 13, 32].

5. Conclusion

The findings of this study underscore the One Health perspective by demonstrating that *Vibrio* isolates found in poultry litter exhibit substantial resistance to the antibiotics tested. Furthermore, many of these *Vibrio* species exhibited phenotypic virulence factors that can enhance their potential to cause disease. The observation of high beta haemolysis prevalence suggests that these isolates could pose a considerable risk for severe infections, emphasizing the interconnected relationship between animal, environmental, and human health within the One Health framework.

Compliance with ethical standards

Acknowledgement

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Disclosure of conflict of interest

The authors declare no conflict of interest.

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