Genotypic Characterization and Virulence Features of Bacteria Associated with Chicken Meat in Rivers State, Nigeria

ABSTRACT

Microbial investigation of live poultry birds were carried out in three different locations (Aluu, Elioparanwo and RSU) in Ikwerre, Obio-Akpor and Port Harcourt Local Government Areas, Rivers State, Nigeria. In this study a total of 125 samples of themuscle, breast, intestine, wing and leg of poultry birds were analysed using molecular techniques and evaluating there virulence features. The birds were processed and microbiological analysis was done using standard procedures, molecular identification of the isolates was carried out. The mean total heterotrophic bacterial count of the muscle, breast, intestine, wing and leg in the three different locations ranged from 16.6±2.3 to 25.2±3.5, 1.6±0.2 to 24.8±8.6, 0.11±0.1 to 8.9±2.7, 1.1±0.8 to 10.6±1.8 and 3.4±0.1 to 4.9±0.9 CFU/g, respectively. The mean total coliform counts of the three locations ranged from 2.1±0.3 to 2.1±0.2, 1.8±0.2 to 0.9±0.6, and 0.32±2.5 to 0.32±0.25, 2.6±0.3 to 7.7±0.9 and 2.3±0.4 to 2.9±0.3 CFU/g, respectively. Virulence features of *A.caviae* (CPO72326) shows proteolytic activity, DNase, motility and biofilm formation. *A. baumanii* (ON60630, OL455994) showed Lipolytic activity, catalytic, starch hydrolysis and biofilm formation. *C. braakii* (ON557391) was positive for Motility, catalytic and proteolytic. *C. freudii* (motile, catalytic, proteolytic and biofilm formation. *M. odoratiminus* (Lipolytic, motile, catalytic, DNase, proteolytic, ureolytic, heomolytic and biofilm formation. The study revealed that microbial contamination of Chicken meat could arise from microorganism present in the environment and on birds. These findings, thus underscored the urgent need for improved biosecurity measures, enhanced hygiene practices to safeguard both poultry health and public health. There should therefore be stringent implementation of food safety measure throughout the poultry value chain from farm to table.

Keywords: Chicken meat, Public health, Molecular identification, Virulence.

1. INTRODUCTION

Poultry farming in Nigeria has now assumed a significant source of animal protein [1]. Household and commercial poultry production now complement imported poultry meat, although the former is more widespread due to cost [2]. Consumption of chicken overshadows other poultry meat in Nigeria [3]. Chicken provide good quality protein and low fat poultry meat at lower cost; hence it is preferable. With high acceptance of chicken meat, due in part to it not having religious restriction, their demand is ever increasing [4]. Yet, poultry meat is seen as being expensive foran average Nigerian. In villages, eating of poultry meat is kept for important events, meat are gotten from household flocks. Those living in cities consume larger quality of poultry due to their relatively higher income and they have quick access to fresh or frozen products in markets and fast food outlets. Poultry are used for all kinds of ceremonies/events such as social-cultural and religious events in Nigeria, causing demand for poultry meat to spike around Christmas, New year, and Easter [5].

As nutritious and healthy as poultry meat, its production and processing for consumption can introduce both pathogenic and spoilage microorganisms into them [6, 7]. Human illness may follow from handling of raw meat, undercooking or mishandling of the cooked product. Foodborne illnesses from poultry consumption are of serious public health concern [8]. These pathogens can come from farms, during transportation, slaughter and particularly when processing, although modern practices requires sterility of the final products [9]. The presence of few pathogens in uncooked meats raises no objection as they can be handled through cooking before ingestion.

Meat can be processed under hygienic conditions andproperly stored yet not immune to contamination from natural microflora which maybe pathogenic. Pathogenic bacteria contaminate organic poultry as they do conventional [10], concluded whatever differences are observed between organic and conventional might come from choice of breed, which ultimately embroil meat quality such as appearance and nutritive value. Since chicken protein is a good media supporting microbial growth, any unsanitary condition during the rearing, processing and retailing of poultry meat which would have an effecton the bacterial load of the poultry meat. The presence of pathogens of any kind in food is undesirable, hence the setting up of standards. It is in the public interest that food sold in the open market be regularly monitored for presence of pathogens like *Myroides* spp, *Enterobacter* spp, *Citrobacter* spp, *Acinetobacter* spp and *Aeromonas* spp.

In recent years, the health benefits of meat as a regular part of the human diet is offset by risks. Meat, like any food, transmit certain diseases [11], but complete cooking and avoiding recontamination reduces this possibility. According to [12], food-borne disease is any illness that results from eating contaminated food or meats. Food- borne diseases associated with the consumption of poultry meat and its processed products are of public health significance worldwide [13]. It is caused by pathogenic bacteria, viruses, parasites that contaminate food, as well as chemical or toxins. Ensuring safe food supply has been one of the major challenges and concerns for producers, consumers and public health officials in both developing and developed countries. This is because foods excessively contaminated withpathogenic and spoilage microorganisms are undesirable and can cause food borne illnesses [14, 15]. Such

illnesses cost billions of dollars in medical care and sometimes even result in death. Several epidemiological reports have implicated foods from animal origin as major vehicles associated with illnesses caused by food borne pathogens [14, 15]. Therefore, this study was aimed to determine virulence features and molecularly characterize bacterial isolates from Chicken meat.

2. MATERIALS AND METHODS

2.1. Description of Study Area

The study was conducted in three Local Government Areas (Obio Akpor, Port Harcourt and Ikwerre Local Government Area) in Rivers State Nigeria. Three poultry farms were chosen for this study Aluu, Elioparanwo and Rivers State University demonstration farm. Samples were collected trimonthly (Quarterly) for a period of one year from each of the farms.

2.2. Collection of Samples

A total of One Hundred and Twenty-Five (125) samples of Muscle, Breast, Wing, Intestine and Leg, were obtained from the three different farms namely; Aluu, Elioparanwo and RSU Farm and transported aseptically to the Department of Microbiology Laboratory, Rivers State University for bacteriological analysis.

2.3. Preparation of Samples

The meat samples were prepared for bacteriological analysis as described by [16]. The muscle, breast, wing, intestine and leg were collected with the aid of a sterile knife.

2.4. Enumeration of Bacterial Isolates

Collected samples were analysed using standard microbiological method. Samples were streaked on Nutrient, MaCconkey, and Eosin methylene blue agar plate. Ten grams (10g) of each chicken parts were weighed and transferred into different conical flask containing 90ml of sterilized normal saline. The flasks were swirled to dislodge the microbial contents under study [17]. Subsequent 10-fold serial dilution were carried out by transferring 1 ml into test tubes containing 9ml sterile normal saline until 10^{-6} dilution. Analiquot(0.1ml)wascollectedfromthetesttubewitha dilutionfactorof10^{-3} and 10^{-6} usingapipetteandinoculatedondriedmedium.Usingaspreader,the aliquotwasevenlyspreadacrossthemedium.

Theinoculatedplateswereinvertedandincubated at 37°C for24 hours after which plates were counted and recorded. Pure bacteria isolates were suspended in 10% (v/v) glycerol at -4^{o}C [18].

2.5. Characterisation of Bacterial Isolates by Conventional and Molecular methods

The bacterial isolates were characterised based on their colonial/morphological and biochemical characteristics [19].

Isolates were also subjected to analytical processes using simple molecular tools such as polymerase chain reaction (PCR) and genome sequencing approach to classify and identify the isolates from their Deoxyribonucleic acid (DNA) [20].

2.6. Test for Virulence

2.6.1. Biofilm Formation Assay

This test was done to ascertain the ability of the known bacteria to produce biofilms. The possibility of the biofilm development from the recovered bacteria was determined using the Congo red agar method as adopted by [21], was employed in this study is a simple qualitative and quantitative analysis that involved the use of Brain Heart Infusion agar supplemented with agar-agar, sucrose and congo red stain.

2.6.2. DNase Assay

The DNase test was done to determine the ability of the bacterial isolates to hydrolyze Deoxyriboseoxiribonuclase acid and utilize it as a source of carbon and energy for growth. DNase is an extracellular enzyme that is secreted outside a bacterial cell membrane. When the enzyme isexpressed, it is an indicative of a virulent property of bacteria which enables it to cause disease. The test procedure was strictly carried out as described by [22]. In sorting this feature, the bacterial isolates were streaked on a freshly prepared (DNase) medium and incubated at 37°C for 24 hours. After incubation, growth colonies were observed, and the plates were then flooded with diluted hydrochloric acid. DNase positive cultures showed a distinct clear zone around the streak while negative cultures showed no clear zone.

2.6.3. Lipase Test

The investigation was done to know if the bacterial isolates can breakdown the lipids of its host. This breakdown is made possible by lipase which the bacterium possesses, and from the breakdown process, the bacterium is able to derive carbon as its source of energy for growth and survival. Thus, when the bacterium survives, its harmfulness is expressed. This assay was carried out according to [23]. The bacterial isolates were

streaked onto a freshly prepared lipid agar medium and for 24 hours at 37°C. After incubation, growth was observed and a blue spirit indicator chemical was used to flood the medium, and observed within seconds. The blue spirit indicator, on observation, was deep blue in appearance which later changed to a light blue opaque medium, signifying a positive result, while in the absence of a change to a light blue opaque medium it indicated a negative result.

2.6.4. Catalase Activity

The assay was to investigate the production of catalase (an enzymes that speeds the release of oxygen from hydrogen peroxide) in the test organism. Catalase production is a virulent determinant property, which a bacterium elaborates to survive the effect of hydrogen peroxide released by phagocytes. The procedure employed to express this activity in bacteria wasaccording to [24], and involves transferring a small fraction of bacterial isolate onto a clean microscope slide and adding a loopful of hydrogen peroxide. After a few seconds, where gas bubble ensued it indicated positive result while absence of gas bubble indicated negative result.

2.6.5. Motility Test

This test was done to spot the presence of flagella in bacteria. More recently, it has become evident that flagellum, a structure for movement in some bacteria also have other biological functions [25]. Flagella has been reported to function as adhesions during bacterial invasion of a host cell; an action which signifies its virulence. The procedure for motility test as done by [25], required the preparation of emulsified nutrient medium, followed by pouring into a test tube and then with the aid of a sterile

needle, the isolate is inoculated in the medium by piercing. The tube was then incubated for 24 hours at 37°C. A diffused growth spread within the tube and not along the stab line indicated motility/virulence of the bacterial isolate.

2.6.6. Capsule Staining

The presence of a capsule in a bacterium is implicative of its virulence because of the capsule can impede phagocytotic response of the host cell. Capsule staining test was done to determine the presence of capsule in the bacterium. The procedure was carried by adding a few drops of crystal violet onto the test bacteria on a clean microscope slide, then stirred and viewed under a light microscope. A light blue appearance signified encapsulated cell, while the reverse signified that the cell was not capsulated [26].

2.6.7. Hemolytic Activity

Hemolytic activity of the isolates was investigated. This was to fiqure out if the bacterial isolates have the ability to digest red blood cells. Heamolytic activity was determined by streaking the isolates onto freshly prepared blood agar medium as described by [24], and incubated at 37°C for 24-48 hours. Positive cultures showed a distinct clear zone around the streak showing the ability of the bacteria to produce toxin while negative result revealed no clear zone around the streak.

2.6.8. Ureolytic Activity

Urease is a virulence property found in some bacteria, and its presence in a bacterium aid it in colonizing its host organism with a toxic effect on the host. The ureolytic test is

aimed at determining the ability of the bacteria to produce urease. The urease so produced, reduces urea to ammonia that is toxic to the host. The procedures for the test according to [24] involved inoculation of the test bacteria into urease broth medium, followed by incubation at 35°C for 18-24 hours. A positive result indicated by change of the medium colour from red to pink while a negative result was indicated by retention of the original colour of red.

2.6.9. Proteinase Activity

Protease activity of the bacterial isolate was screened using casein agar. In screaning, the isolates were streaked onto casein agar medium, followed by incubation at 37°C for 48hours. positive plates were identified with zones of proteolysis, in which the casein is hydrolysed leaving a clear zone around the growth area of the isolates and negative plates observed without clear zone on the plate [27].

2.6.10. Starch Hydrolysis

The assay was aimed at observing if the bacterial isolate can breakdown starch in the host by the production of amylase enzyme. The procedure for the test required preparing a starch agar medium and streaking of the isolate onto it,followed by inoculation at 35°C for 48hours.The process was then followed by flooding the agar plate with gram's iodine. Apurple-blue colouration around the organism signified the presence of amylase while an absence signified no amylase [28].

2.7. Statistical Analysis

The enumerated colonies were calculated using descriptive statistics. One-way and Two-way ANOVA was used to check for significant differences. A p-value of less than 0.05 was considered to indicate a statistically significant difference.

3. RESULTS

3.1. Total Heterotrophic Bacterial Load of Chicken parts for the three Sampled farms

Results of the total heterotrophic bacterial load of chicken parts for the three farms are presented in Table 1. Results showed that the mean range of the total heterotrophic bacterial counts for Breast, Wing, Leg, Intestine and Muscle (thigh) of the farms were 16.6±2.3 to 25.2±3.5, 1.6±0.2 to 24.8±8.6, 0.11±0.1 to 8.9±2.7, 1.1±0.8 to 10.6±1.8 and 3.4±0.1 to 4.9±0.9 CFU/g, respectively.

More so, results showed that there were significant differences across the poultry parts surfaces in the respective farms and across the farms. Poultry parts such as the intestines, breast and wings of samples obtained from the RSU were significantly higher than those recorded in Aluu and Elioparanwo poultry parts.

Table 1: Mean Total Heterotrophic Bacterial Counts from Chicken Parts from the three Sampled Farms

Samples	Aluu ($\times 10^6$)	Elioparanwo ($\times 10^6$)	RSU ($\times 10^6$)	P-value
Breast (CFU/g)	16.6±2.3^{b}	9.9±7.0 ^{a}	25.2±3.5 ^{b}	<0.01
Wing (CFU/g)	1.6±0.2 ^{a}	20.1±1.5 ^{b}	24.8±8.6 ^{b}	0.004
Leg (CFU/g)	0.11±0.1 ^{a}	23.3±1.8 ^{c}	8.9±2.7 ^{b}	0.02
Intestine (CFU/g)	1.1±0.8^{a}	0.59±0.8^{a}	10.6±1.8^{b}	<0.001
Muscle (thigh) (CFU/g)	3.4±0.1^{a}	3.2±0.1^{a}	4.9±0.9^{a}	0.182

***Means with similar superscript (alphabet) across the group show no significant difference (P>0.05)**

3.2. The Mean Coliform Counts for Chicken Parts from the three Sampled Farms

Results of the mean coliform counts for chicken parts from the three sampled farms is presented in Table 2. Results showed that the mean coliform counts of the chicken parts in Aluu, Elioparanwo and RSU farms ranged from 2.1±0.3 to 2.1±0.2, 1.8±0.2 to 0.9±0.6, 0.32±2.5 to 0.32±0.25, 2.6±0.3 to 7.7±0.9 and 2.3±0.4 to 2.9±0.3 CFU/g, respectively.

Table 2: Mean Total Coliform Counts for Chicken Parts from the three Sampled Farms

Samples	Aluu ($\times 10^3$)	Elioparanwo ($\times 10^3$)	RSU ($\times 10^3$)	P-value

Breast (CFU/g)	2.1±0.3[a]	1.8±0.2 [a]	2.1±0.2 [a]	0.331
Wing (CFU/g)	1.8±0.2 [a]	1.8±0.2 [a]	0.97±0.6 [a]	0.331
Leg (CFU/g)	0.32±2.5 [a]	1.8±0.2 [a]	0.32±0.25 [a]	0.611
Intestine (CFU/g)	2.6±0.3[a]	7.7±0.9[b]	7.7±0.9[b]	0.022
Muscle (thigh) (CFU/g)	2.3±0.4[a]	2.1±0.9[a]	2.9±0.3[a]	0.441

***Means with similar superscript (alphabet) across the group show no significant difference (P>0.05)**

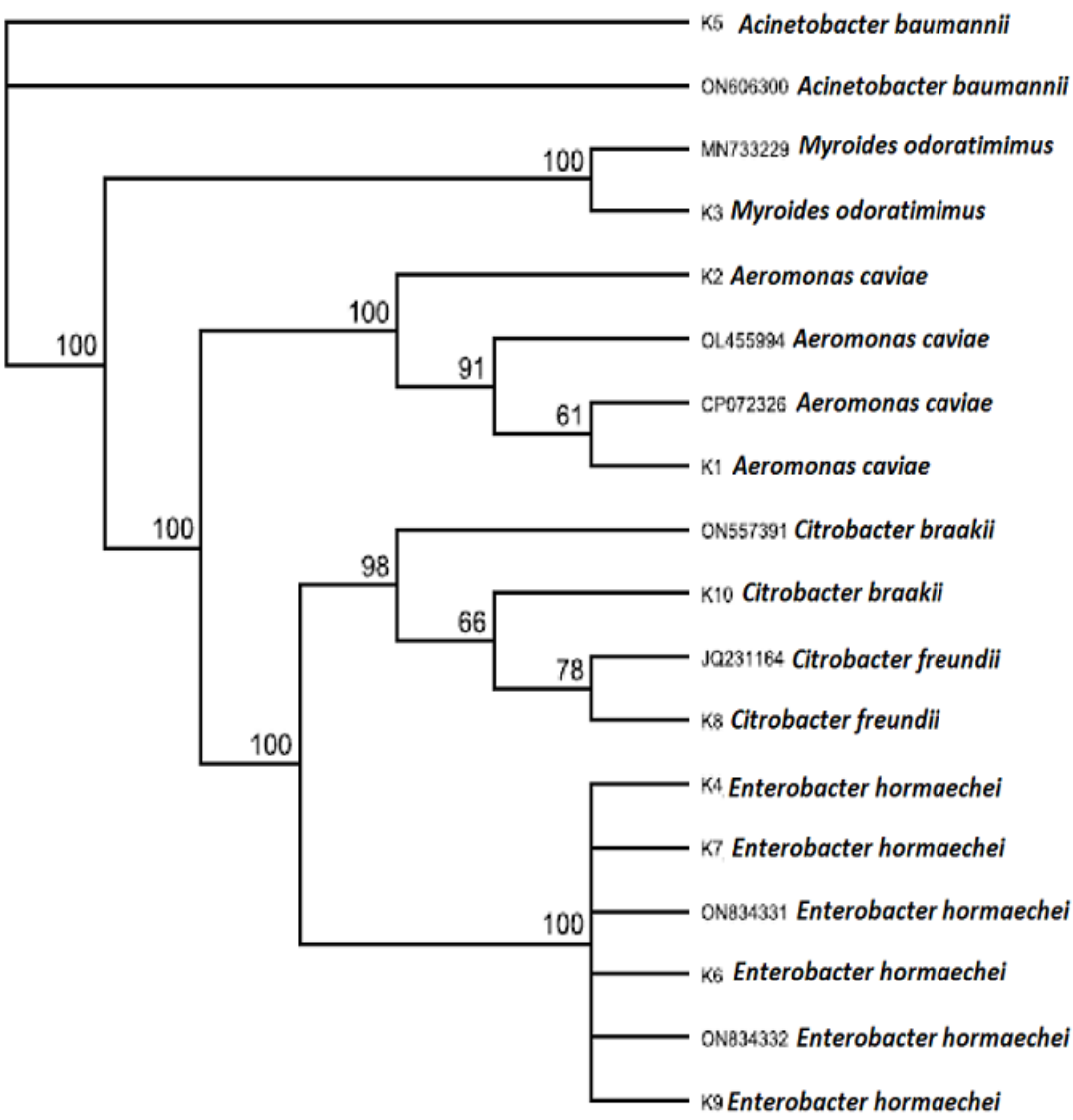

Fig. 1: Phylogenetic Tree showing the Evolutionary Distance between the Bacterial Isolates

3.3. Phylogenetic Analysis

The obtained 16s rRNA sequence from the isolate produced an exact match during the megablast search for highly similar sequences from the NCBI non-redundant nucleotide (nr/nt) database. The 16S rRNA of the isolates showed a percentage similarity to other species at 100%. The evolutionary distances computed using the Jukes-Cantor method were in agreement with thephylogenetic placement of the 16S rRNA of the isolates and revealed close relatedness to *Citrobacter braaki, Citrobacter freundii* and *Enterobacter hormaechei* (Fig.1).

3.4.Agarose Gel Electrophoresis

The Agarose gel electrophoresis showing the plasmid bands. Lane 1-10 showing the16SrRNA bands at 1500bp while lane L represents the 500bp molecular ladder in Plate 1

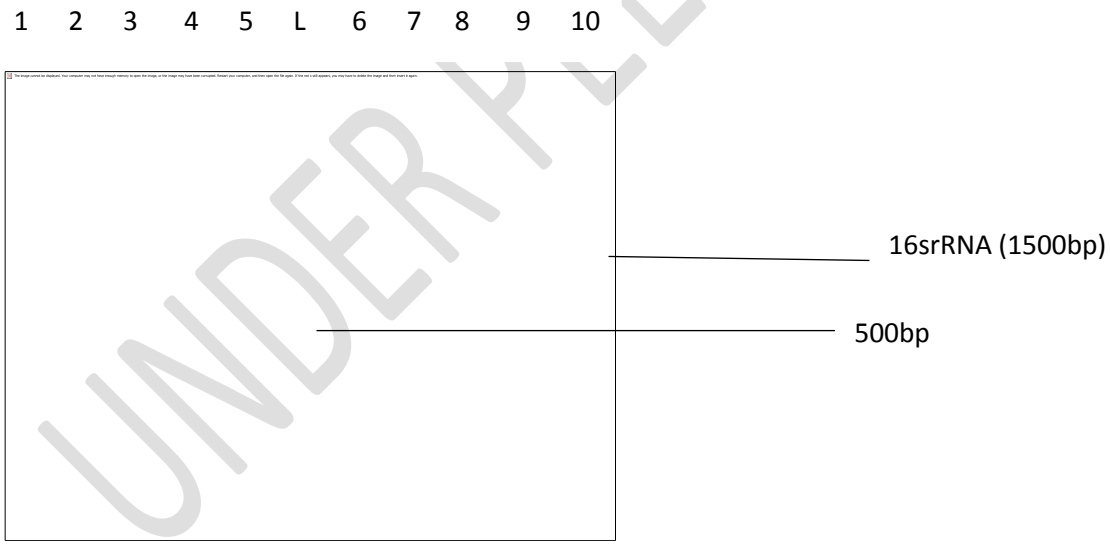

Plate 1: Amplified 16S rRNA gene bands at 1500bp on Agarose gel after electrophoresis

3.4. Results of the virulence features from Aluu Farm

Results of the virulence features of *A. cavie, A. baumanii, C. braakii, C. freundii, E. hormaechei* and *M. odoratiminus* from Aluu farm is presented in Table 3. Results showed that the percentage of *A. baumanii* and *M. odoratiminus* to lipolytic activity was 100% while other isolates showed no lipolytic activity. *A. cavie, C. braakii, C. freundii, E. hormaechei* and *M. odoratiminus* is presented in Table 3. Results showed that the percentage of *A. caviae, C. braakii, C. freundii, E. hormachei* and *M. odoratiminus* were 100% motile. The possession of DNase by bacterial isolates showed that *A. cavie* and *M. odoratiminus* were 100% positive for DNase enzyme while *A. baumanii, C. braakii, C. freundii,* and *E. hormaechei* lack the DNase enzyme. The ability to hydrolyze starch by the bacterial isolates showed that only *A. cavie, A. baumanii* and *E.hormaechei* isolates were able to hydrolyze starch while for proteolytic activity, isolates of *A. cavie, C. braakii, C. freundii* and *M. odoratiminus* were the only isolates with proteolase enzyme. In the virulence test for ureolytic and haemolysin activity, only the isolates of *M. odoratiminus* possessed the enzymes. The ability to produce biofim was also investigated and only isolates of *A. cavie, A. baumanii, C. freundii, E. hormaechei* and *M. odoratiminus* formed biofilm while *C. braakii* and *M. odoratiminus* are the only isolates that possessed capsule.

3.5. Results of Virulence Features from Elioparanwo Farm

Results of the virulence features of *A. cavie, A. baumanii, C. braakii, C. freundii, E. hormaechei* and *M. odoratiminus* from Elioparanwo farm is presented in Table 4. Results was similar to the virulence attributes of similar isolates reported in the Aluu

farm. The exception was just the number of isolates which was higher than those reported in the Aluu farm. For instance, *A. baumanii* and *M. odoratiminus* to lipolytic activity was 100% while other isolates showed no lipolytic activity. *A. cavie, C. braakii, C. freundii, E. hormaechei* and *M. odoratiminus* is presented in Table 4. Results showed that the percentage of *A. caviae, C. braakii, C. freundii, E. hormaechei* and *M. odoratiminus* were 100% motile. The possession of DNase by bacterial isolates showed that *A. cavie* and *M. odoratiminus* were 100% positive for DNase enzyme while *A. baumanii, C. braakii, C. freundii,* and *E. hormaechei* lack the DNase enzyme. The ability to hydrolyze starch by the bacterial isolates showed that only *A. cavie, A. baumanii* and *E. hormaechei* isolates were able to hydrolyze starch while for proteolytic activity, isolates of *A. cavie, C. braakii, C. freundii* and *M. odoratiminus* were the only isolates with proteolase enzyme. In the virulence test for ureolytic and haemolysin activity, only the isolates of *M. odoratiminus* possessed the enzymes.

3.6. Results of Virulence Features from RSU Farm

Results of the virulence features of *A. cavie, A. baumanii, C. braakii, C. freundii, E. hormaechei* and *M. odoratiminus* from RSU farm is presented in Table 5. Similar to the results in Table 3 and4, *A. cavie, A. baumanii, C. braakii, C. freundii, E. hormaechei* and *M. odoratiminus* produced virulent attributes including the possession of biofilm and capsules.

Table 3: Virulence Features of Bacterial Isolated from Aluu Farm

Bacteria	No. of Isolates	Lipolytic Activity n (%)	Motility n (%)	Catalytic Activity n (%)	DNAse n (%)	Starch hydrolysis n (%)	Proteolytic n (%)	Ureolytic n (%)	Hemol n (%)	Biofilm n (%)	Capsule formation n (%)

Key: n (%) =Number of bacteria positive (percentage positive), *A. caviae=Aeromanas caviae*, *A. baumanii=Acinetobacter baumanii*, *C. braakii=Citrobacter braakii*, *C. freundii=Citrobacter freundii*, *E. hormaechei=Enterobacter hormaechei*, *M. odoratiminus=Myroides odoratimumus,* Hemol=Hemolysin.

A. cavie	41	0 (0)	41 (100)	30 (73.2)	41 (100)	41 (100)	33 (80.5)	0 (0)	0 (0)	41 (100)	0 (0)
A. baumanii	40	40 (100)	0 (0)	40 (100)	0 (0)	34(85)	0 (0)	0 (0)	0 (0)	32 (80)	40 (100)
C.braakii	15	0 (0)	15 (100)	10 (66.7)	0 (0)	0 (0)	15 (100)	0 (0)	0 (0)	0 (0)	0 (0)
C. freundii	22	0 (0)	22 (100)	18 (81.8)	0 (0)	0 (0)	17 (77.3)	0 (0)	0 (0)	22 (100)	0 (0)
E. hormaechei	44	0 (0)	44 (100)	0 (0)	0 (0)	40 (90.9)	0 (0)	0 (0)	0 (0)	38 (86.4)	0 (0)
M. odoratiminus	21	21 (100)	21 (100)	15(71.4)	21 (100)	0 (0)	21 (100)	21 (100)	21 (100)	21 (100)	21 (100)
Total	183										

Table 4: Virulence Features of Bacterial Isolated from Elioparanwo Farm

Bacteria	No. of Isolates	Lipolytic Activity n (%)	Motility n (%)	Catalytic Activity n (%)	DNAse n (%)	Starch hydrolysis n (%)	Proteolytic n (%)	Ureolytic n (%)	Hemol n (%)	Biofilm n (%)	Capsule formation n (%)
A.cavie	53	0 (0)	53 (100)	40(75.5)	53 (100)	43 (81.1)	40 (75.5)	0 (0)	0 (0)	53 (100)	0 (0)
A.baumanii	42	42 (100)	0 (0)	42 (100)	0 (0)	42 (100)	0 (0)	0 (0)	0 (0)	21 (50)	42 (100)
C.braakii	28	0 (0)	28 (100)	20 (71.4)	0 (0)	0 (0)	22 (78.6)	0 (0)	0 (0)	0 (0)	0 (0)
C. freundii	33	0 (0)	33 (100)	33 (100)	0 (0)	0 (0)	25 (75.8)	0 (0)	0 (0)	28 (84.8)	0 (0)

E. hormaechei	71	0 (0)	71 (100)	0 (0)	0 (0)	62 (87.3)	0 (0)	0 (0)	0 (0)	60(84.5)	0 (0)
M. odoratiminus	20	20 (100)	20 (100)	20 (100)	20 (100)	0 (0)	20 (100)	20 (100)	10 (50)	20 (100)	20 (100)
Total	247										

Key: n (%) =Number of bacteria positive (percentage positive), *A. caviae=Aeromanas caviae*, *A. baumanii=Acinetobacter baumanii*, *C. braakii=Citrobacter braakii*, *C. freundii=Citrobacter freundii*, *E. hormaechei=Enterobacter hormaechei*, *M. odoratiminus=Myroides odoratimumus,* Hemol=Homolysin.

Table 5: Virulence Features of Bacterial Isolated from RSU Farm

Bacteria	No. of Isolates	Lipolytic Activity n (%)	Motility n (%)	Catalytic Activity n (%)	DNAse n (%)	Starch hydrolysis n (%)	Proteolytic n (%)	Ureolytic n (%)	Hemol n (%)	Biofilm n (%)	Capsule formation n (%)
A.cavie	29	0 (0)	29 (100)	29 (100)	29 (100)	17(58.6)	20 (68.9)	0 (0)	0 (0)	29 (100)	0 (0)
A.baumanii	29	29 (100)	0 (0)	22(75.9)	0 (0)	27 (93.1)	0 (0)	0 (0)	0 (0)	17 (58.6)	29 (100)

C. braakii	17	0 (0)	17 (100)	17 (100)	0 (0)	0 (0)	17 (100)	0 (0)	0 (0)	0 (0)	0 (0)
C. freundii	23	0 (0)	23 (100)	20 (86.9)	0 (0)	0 (0)	21 (91.3)	0 (0)	0 (0)	18 (78.3)	0 (0)
E. hormaechei	42	0 (0)	42 (100)	0 (0)	0 (0)	21 (50)	0 (0)	0 (0)	0 (0)	21 (50)	0 (0)
M. odoratiminus	29	29 (100)	29 (100)	27 (93.1)	29 (100)	0 (0)	29 (100)	29 (100)	29 (100)	29 (100)	29 (100)
Total	169										

Key: n (%) =Number of bacteria positive (percentage positive), *A. caviae=Aeromanas caviae*, *A. baumanii=Acinetobacter baumanii*, *C. braakii=Citrobacter braakii*, *C. freundii=Citrobacter freundii*, *E. hormaechei=Enterobacter hormaechei*, *M. odoratiminus=Myroides odoratimumus,* Hemol=Hemolysin.

4. DISCUSSION

4.1. Bacterial Isolates Associated with Poultry

Bacterial associated with poultry meat in Rivers State was investigated. The world's consumption of poultry meat is rising significantly; according to the most recent figures, it reached 14.2 kg per person per year [29]. Therefore, in light of the rising consumption and production of poultry meat, assuring the microbiological safety of poultry meat products is a crucial concern. In actuality, poultry parts like the legs, intestines, muscles, wings and breasts including processed meat products are contaminated during and after slaughter by the germs from animal microbiota, the slaughterhouse environment, and the equipment employed [29]. Also, microbial contamination of poultry meat could arise from the type of microorganisms present in that environment as well as from the microorganisms on the bird (which could be the normal or transient flora) that gets into the poultry meat via openings or cuts either during killing or processing [30]. This agreed with the present study which showed uneven distribution of enteric bacterial isolates across the various parts of the poultry meat. More so, during the preparation and storage of food, several of these bacterial contamination might proliferate or persist [29]. Cross contamination of poultry meat is also very possible especially during scalding and defeathering of the poultry [31]. The muscles of healthy live birds are considered to be sterile while the digestive tract, the lungs, feathers and skin are considered to accommodate microbial diversities [30]. Thus, the presence of bacterial isolates in the muscles could be attributed to cross contamination during processing of the poultry meats. It has been noted that microorganisms from the environment as well as the microbiota of the poultry could contaminate the poultry meat [32, 29]. More so, it has been noted that while bacteria primarily come from the exterior of the animal and its

intestinal tract during slaughter, dressing, and cutting, more are introduced via knives, clothes, the air, workers, carts, crates, and equipment in general [33].

4.2. Virulence of Isolates

The virulence of the bacterial isolates which were evaluated showed that all bacterial isolate possessed certain degree of virulence. In particular, DNases may play a role in bacterial proliferation, biofilm formation, and the capacity of bacteria to evade the immune system [34]. DNases are enzymes that hydrolyze nucleic acids to produce oligonucleotides [35]. Dnase could therefore suggest these isolates' capacity to evade the immune system's activity, spreading and producing disease within the host. DNases are frequently cited as contributing to the pathogenicity of *Staphylococci* or *Streptococci* [21]. Indeed, studies have revealed that DNase can aid bacteria in eluding the structures that neutrophils create called extracellular traps (NETs) in order to capture and destroy pathogens [36]. The pathogenicity of most diseases varies, and virulence is a parameter that effectively separates pathogenic from non-pathogenic strains [37].

The presence of pili, capsular polysaccharide, DNase, coagulase, fibrinolysin, proteolytic, haemolysin, bacteriocin production, haemagglutination, serum sensitivity, attachment of epithelial cells, hydrophobicity, lipase, antiphagocytic factor (coagulase), biofilm, extracellular enzyme production, presence of surface layer, lysine decarboxy [38]. Accordingly, the existence of these virulent characteristics (Dnase, lecithin, motility, amylase, haemolysin and capsule) in the bacterial isolates of the present study could imply that they are pathogenic and could cause infections or disorders when given the proper infective dose. According to [39], pathogenic bacteria use many strategies to infect human hosts and cause sickness. They also release a variety of compounds that

bind to host cell targets and enable a range of host responses. According to [40], the formation of biofilms is a crucial component of virulence because it promotes bacterial colonisation by facilitating cell adhesion to epithelial cells and intestinal villi, decreasing bacterial susceptibility to antibiotics, and lessening immune system recognition of the bacteria.

5. CONCLUSION

The survey of microorganism associated with poultry in three local government areas in Rivers State has provided valuable insights into the potential risks associated with these bacteria in the poultry farming ecosystem. The following conclusions can be drawn from the study:

The presence of pathogens in poultry raises concerns about food safety and potential zoonotic transmission to humans. Proper cooking and handling of poultry products, as well as strict adherence to hygiene measures in processing facilities, are essential to reduce the risk of foodborne illnesses.

The study underscores the importance of implementing improved management practices in poultry farming, including enhanced biosecurity, waste management, and water source protection. These measures can help reduce the prevalence and transmission of bacteria, ultimately improving the health and productivity of poultry.

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