VIRULENCE PHENOTYPE OF AVIAN PATHOGENIC *ESCHERICHIA COLI* (APEC) AND PREVALENCE OF COLIBACILLOSIS AMONG PAULTRY BIRDS IN BAUCHI METROPOLIS, NIGERIA

ABSTRACT

Background and Aim: Avian pathogenic *Escherichia coli* (APEC), a major cause of colibacillosis, are mainly associated with respiratory tract or systemic infections that lead to a variety of diseases. Avian pathogenic *Escherichia coli* infections include acute fatal Septicemia, and subacute pericarditis, which are responsible for severe economic losses world-wide in poultry industry. The study aimed to determine the virulence phenotype and prevalence of colibacillosis among poultry birds in Bauchi metropolis.

Methods: Faecal, internal organs and poultry house air samples were collected systematically from the birds in selected poultry farms within Bauchi metropolis. APEC were isolated and characterized using standard phenotypic conventional microbiological techniques.

Results: The study found that colibacillosis is caused by virulent strain of APEC with Haemolysin (59.6%) and type 1 fimbriae (40.4%) phenotypes, with high prevalence (100%) of colibacillosis in faecal samples, indicating faeces as a significant reservoir for APEC and a potential source of transmission. A moderate correlation (0.592) was observed among sample types. The occurrence of APEC was higher in faecal samples compared to internal organs. Birds with internal organ infections showed a 26.0% mortality rate, with younger (30.7%), female (69.3%), and layer birds (53.8%) being more vulnerable. The disease is more common (15.3%) in birds from the populated area of Federal low-cost housing estate in the study area. High prevalence with mortality cases was also found in younger birds (46.1%), mostly female (65.3%) and Layers (73.0%). But the highest mortality rates was observed in the adult **(**61.5%), Female (69.3%), Layers (53.8%).

Conclusion: It is recommended that further study should be conducted to trace the phylogenetic relationship of the virulent APEC isolates in the study area with other related sequences. This study also emphasizes the need for proper diagnosis of bac to decrease the prevalence of Colibacillosis in the area.

Keywords: APEC, Colibacillosis, Birds, Bauchi, *E. coli*

INTRODUCTION

Escherichia coli, also known as *Escherichia coli,* is a Gram-negative, facultative anaerobic, rod –shape, coliform bacterium of the genus Escherichia that is commonly found in the lower intestine of warm-blooded organisms. Most *Escherichia coli* strains are harmless, but some can cause serious food poisoning [1]. Avian pathogenic *Escherichia coli* (APEC), a major cause of colibacillosis, are mainly associated with respiratory tract or systemic infections that lead to a variety of diseases. Avian pathogenic *Escherichia coli* include acute fatal septicemia, subacute pericarditis, and airsaculitis, which are responsible for severe economic losses world-wide in poultry industry [2]. Although *E. coli* is present in the normal microflora of the intestinal tract, certain strains possessing specific virulence can cause colibacillosis[2].

Colibacillosis in chicken is an infectious disease of birds caused by *Escherichia coli*, which is considered as one of the principal causes of morbidity and mortality, associated with heavy economic losses to the poultry industry by its association with various disease conditions, either primary pathogen or as a secondary pathogen. It causes a variety of disease manifestations in poultry including yolk sac infection, omphalitis, respiratory tract infection, swollen head syndrome, septicemia, poly serositis, coli granuloma, enteritis, cellulitis and salpingitis. Colibacillosis of poultry is characterized in its acute form by septicemia resulting death and it in its sub-acute form by pericarditis, airsaculitis and peri-hepatitis [3].

APEC, the etiological agent of extra intestinal infections in birds, is a pathotype that belongs to the EXPEC group. Extra-intestinal infections caused by APEC are known as colibacillosis and characterized by fibrinous lesions around the Visceral organs [4]. Such as Septicemia, Arthritis, Granulomas, Omphalitis, Sinusitis, Enteritis/synovitis Peritonitis, Pericarditis, Perihepatitis, cellulitis and swollen bead syndrome [5]. APEC infections also lead to reduced yield, quality and hatching of eggs. The potential for zoonotic transmission must be considered, since poultry serve as the main host for APEC and the consumption of undercooked poultry may infect humans, which can serve as a reservoir of this pathotype [6].

The primary method of controlling APEC infections in poultry is to use a variety of antibiotics [7]. Meanwhile, the development of multidrug-resistant strains of APEC is a severe problem for global public health with a significant impact on animal health and food safety [8]. The emergence of antibiotic-resistant *Escherichia coli* strains has become a global issue due to overuse of antibiotics in the poultry industry [9]. The antibiotic-resistant forms of APEC strains were found to activate antibiotic resistance genes in other pathogenic *Escherichia coli* strains and such resistance genes could easily transmitted and spread between animals and humans [10].

Methodology

Study Design

This research is a clinico-laboratory investigation of tetracycline drug resistance pattern in Avian Pathogenic *Escherichia coli* (APEC) isolated from some poultry farms in Bauchi state, Nigeria. This work was carried out in the Microbiology Laboratory of Abubakar Tafawa Balewa University, Molecular Genetic and Infectious Disease Research Laboratory ATBUTH and the National Veterinary Research Institute (NVRI) Vom in Bauchi state. Bauchi state is located between latitudes, 9°3' and 12^{o}3' north of the equator. Longitudinally, it lies between 8^{o}5' and 11^{o} east of Greenwich meridian. Plateau state 9.2182^{o}N, 9.5179^{o}E.

Sample Size and collection

A total of 99 samples was collected as determined using formula derived by [11] with 95% confidence interval, at 5% desired absolute precision and previous prevalence of 20% from study by Charan & Biswas, (2013) was used. A total of ninety-nine samples was collected for this study, according to standard laboratory techniques. The sample was collected from diseased chicken aseptically which will comprise seven different types of samples, including air from the insides of poultry shade (n=31), faeces from sick bird (n=32), and the intestinal organs (trachea, intestine, liver, spleen, lung and egg yolk materials; n=36) of dead birds. Air sampling was done using the settle plate method.

Sample Processing

Culture Methods

The samples were inoculated unto MacConkey, and Eosin methylene blue (EMB) agar media (Oxford, UK) and incubated at 37℃ for 18-24 hours as described by Mahmud [13]. EMB) agar plates were exposed at 1m above the ground to different corners of the poultry shades for 10 minutes. Freshly dropped faecal samples was collected using sterile cotton buds from sick isolated groups of birds. The internal organs were collected during post-mortem examinations.

All the collected samples were given unique tag numbers and transported to the laboratory maintaining the cold chain. Immediately after arrival at the laboratory, faecal samples (1g) was seeded into test tubes containing 5ml of nutrient broth. Internal organs were cut into small pieces and then transferred into test tubes containing 5ml of nutrient broth. The EMB agar plates and the test tubes was incubated aerobically at 37℃ overnight [12].

Morphological Identification and Biochemical characterization of isolates

The plates were examined for typical morphological characteristics of *E. coli*. The isolates were identified also by Gram staining and microscopy. The isolates were further identified by basic sugar fermentation, indole and methyl red tests, as described by Mahmud [13]

Phenotypic detection of Virulence factors

(i) Haemolysin production test

The bacteria were inoculated onto 5% sheep blood agar and incubated overnight at 37ºC. Haemolysin production was detected by the presence of lysis, a zone of complete lysis of the erythrocytes around the colony and clearing of the medium.

(ii) Mannose resistant fimbrial haemagglutination (MRHA) test

This was carried out on the isolate's suspension on glass slide. The haemagglutination was considered to be mannose resistant when it occurred in the presence of D-mannose and it was considered to be mannose sensitive when it was inhibited by D-mannose [13].

RESULTS

Prevalence of Colibacillosis based on Sample sources

The correlation coefficient between of Samples analysed and number of positive *E. coli* isolates' is approximately 0.593, indicating a moderate positive correlation. The table presents the prevalence of *E. coli* in different sample sources. The data shows that faeces samples had the highest number of *E. coli* cases, with 32 out of 32 samples testing positive. Trachea and intestine samples also had relatively high numbers of *E. coli*, with 29 and 3 positive cases respectively out of the total samples. In contrast, air samples had 21 positive cases out of 31, and the internal organs such as the liver and lungs had lower prevalence, with 7 and 11 positive cases respectively. The egg yolk samples had the lowest number of *E. coli* at 6 positive cases out of 2 samples studied.

Table 1: Distribution of Colibacillosis according to Sample sources

Sample Source	No. of Samples Analyzed (n=99)	No. (%) of *E. coli* Isolated (n = 82)	No. (%) APEC Isolates (n=52)	Correlative Coefficient
Air	31	21(25.6)	04(7.6)	
Faeces	32	32(39.0)	08(15.3)	0.592
Internal organs				
Trachea	05	29(35.3)	19(36.5)	
Intestine	08	03(3.6)	02(3.8)	
Liver	07	07(8.5%)	05(9.6)	
Lungs	14	11(13.4)	09(17.3)	

Egg Yolk	02	06(7.3)	05(9.6)	

Distribution of APEC According to Sampling Location

The relationship between sample numbers and positive APEC isolates varies across sample types, showing a moderate correlation (0.89). Conversely, the correlation between sample numbers and negative APEC isolates by sample type is notably weak and negative (-0.05). When analysed by sample location, both positive and negative APEC isolates exhibit very strong correlations with sample numbers, with positive isolates showing a correlation of 0.89 and negative isolates a correlation of 0.94.Based on the data from the table, here is an overview of how APEC distribution varies across different sample characteristics: The samples were categorized into three types—Air (31%), Faeces (32%), and Internal organs (36%)—and were collected from various locations such as Yelwa (3.8%), Barracks (11.5%), Tudun Salmanu (5.7%), Fadaman Mada (9.6%), Kutum village (3.8%), GRA (13.4%), Gida Dubu (13.4%), Gombe Road (5.3%), Railway (17.3%), and Federal low cost (15.3%). Positive APEC isolates were found in 47 samples out of 99 overall samples. Key trends indicate significant variation in APEC isolate distribution across sample types and locations, with Faeces and Internal organs showing the highest positive rates, and Air samples generally exhibiting lower rates. Locations like Railway and Federal low-cost areas show relatively elevated rates for both positive and negative APEC isolates.

Table 2*:* Distribution of APEC According to Sampling Location

Sampling location	Sample Number (n=99)	No. of APEC Isolates (n= 52)	Percentage (%)	Correlative Coefficient
Yelwa	06	02	3.8	
Barracks	09	06	11.5	
Tudun Salmanu	05	03	5.7	
Fadaman Mada	08	05	9.6	0.89
Kuitum village	06	02	3.8	
GRA	13	07	13.4	
Gida Dubu	10	07	13.4	
Gombe Road	05	03	5.7	
Railway	22	09	17.3	
Federal low cost	15	08	15.3	

Prevalence of APEC infection According to Birds Characteristics

The distribution of APEC according to bird characteristics shows that for age groups, birds aged 1-5 weeks have 30 *E.coli* isolates and 24 APEC isolates, those aged 6-10 weeks have 10 *E.coli* isolates and 6 APEC isolates, and those aged 11-15 weeks have 42 *E.coli* isolates and 22 APEC isolates. In terms of sex, male birds have 40 *E. coli* isolates and 18 APEC isolates, while female birds have 42 *E. coli* isolates and 34 APEC isolates. Regarding breed, broilers have 34 *E. coli* isolates and 11 APEC isolates, layers have 51 *E. coli* isolates and 38 APEC isolates, and noilers have 7 *E. coli* isolates and 3 APEC isolates. The correlation coefficient of 0.886 indicates a strong positive correlation between *E. coli* Isolates and APEC Isolates based on the age of the birds. This means that as the number of *E. coli* isolates increases, the number of APEC isolates also tends to increase, and vice versa. And the correlation coefficient of 1.000 indicates a perfect positive correlation between *E. coli* Isolates and APEC Isolates based on the sex of the birds. This suggests that the relationship between *E. coli* and APEC isolates is directly proportional and perfectly linear when considering the sex of the birds. whereas the correlation coefficient of 0.907 indicates a strong positive correlation between *E. coli* Isolates and APEC Isolates based on the breed of the birds. Similar to the age characteristic, this means that an increase in *E. coli* isolates is strongly associated with an increase in APEC isolates across different breeds.

Table 3: Distribution of APEC Infection According to Birds Characteristics

Variables	No. of *E. coli* Isolates (n=82)	No. positive APEC isolates (n = 52)	Percentage positive (%)	Correlative Coefficient
Age (week)				
1-5	30	24	46.1	0.886
6-10	10	06	11.5	
11-15	42	22	42.3	
Sex				
Male	40	18	34.6	1.000
Female	42	34	65.3	
Breeds				
Broilers	34	11	21.3	0.907
Layers	51	38	73.0	
Noilers	07	03	5.7	

Mortality Rates of APEC-Infected birds from the Sample Location

Among birds across various sample characteristics. The table categorizes information into three columns: Variables, Sample Characteristics, and Rate of Mortality. Under Variables, sample types (Air, Faeces, Internal organs, Velva, Barracks, Tudun Salamu) and locations (Fadaman Mada, Kuitum village, GRA, Gida Dubu, Gombe Road, Railway, Fed. Low-cost) are listed. Sample Characteristics detail the number of birds sampled (n=99) for each variable. The Rate of Mortality column illustrates the percentage of birds that succumbed to APEC infection per sample type and location. Noteworthy findings include high mortality rates of 26% for birds sampled from Internal organs and 3% from Barracks, contrasting with 0% mortality observed in Air, Faeces, and Velva samples. This table provides valuable insights into the differential impact of APEC infection on birds, offering critical data for targeted strategies in avian disease prevention and management. The chi-square test shows a marginally significant relationship between the variables (sample types or locations) and mortality (p-value = 0.0581). This suggests there might be some association, but it's not strongly conclusive at the conventional 0.05 significance level. internal organs have a high mortality rate of 72.22% with a 95% CI of [58.33%, 86.11%], while air and faeces samples show no mortality.

Correlation between Number of Birds and Rate of Mortality for Sample Locations: 0.9450. There's a strong positive correlation (0.9450) between the number of birds and the rate of mortality for sample locations. This suggests that locations with more birds tend to have higher mortality rates

Table 4: Mortality Rate of Birds Infected with of APEC According to Sample Location

Sample Location	No. of Birds Sampled (n=99)	Number of Mortality (n =26)	Percentage Mortality (%)	CoC	x^2
Yelwa	06	02	7.6		
Barracks	09	03	11.5		
Tudun Salmanu	05	01	3.8		
Fadaman Mada	08	02	7.6		
Kuitum village	06	02	7.6	0.9450	0.058
GRA	13	04	15.3		
Gida Dubu	10	03	11.5		
Gombe Road	05	01	3.8		
Railway	22	07	26.9		
Fed. lowcost	15	03	11.5		

Chi-square statistic: 3.5898, p-value: 0.0581, Correlative Coefficient: 0.9450

Mortality rate of Birds Infected with of APEC According to Birds Characteristics

The table shows the rate of mortality of birds infected with APEC (Avian Pulmonary Edema and Congestion) according to various characteristics. It includes the number of sampled birds and the corresponding mortality rate for different age groups, sexes, and bird breeds (broilers, layers, and noilers). The data indicates that younger birds aged 1-5 weeks have a higher mortality rate of 8% compared to older birds, and female birds have a higher mortality rate of 18% compared to male birds at 8%. Among the breeds, noilers have the lowest mortality rate at 2%, while layers have the highest at 14%.

Based on these findings, it can be concluded that: the chi-square test yielded a statistic of 6.0 with a p-value of 0.199, indicating no statistically significant association between age groups and mortality rates at the conventional significance level of 0.05. Concerning Sex, the chi-square test resulted in a statistic of 0.0 with a p-value of 1.0, suggesting no significant evidence of an association between sex and mortality rates. Similarly, for Breed, the chi-square test yielded a statistic of 6.0 with a p-value of 0.199, mirroring the results observed for Age. This implies there is no statistically significant association between breed and mortality rates at the 0.05 significance level.

Table 5: Mortality rate of Birds Infected with of APEC According to Birds Characteristics

Variables	No. of Birds (n=99)	Number of Mortality (n = 26)	Percentage Mortality (%)	X^2
Age(weeks)				
1-5	35	08	30.7	0.199
6-10	10	02	7.6	
11-15	54	16	61.5	
Sex				
Male	40	08	30.7	1.000
Female	59	18	69.3	
Breeds				
Broilers	39	10	38.4	0.199
Layers	53	14	53.8	
Noilers	07	02	7.6	

Virulence Phenotype of *E. coli* Isolates from this Study

This table presents the virulence profile of bacterial isolates from a study. It compares the presence of three virulence factors, hemolysin and fimbrial adhesin, between the total *E. coli* isolates (n=99) and the APEC (Avian Pathogenic *E. coli*) positive isolates (n=52%). For each virulence factor, the table shows the number of isolates (out of 99) that possessed that factor, as well as the percentage of APEC positive isolates that had that factor. The data indicates that the virulence factors were highly prevalent in the overall *E. coli* isolates, but their frequencies were lower among the APEC positive subset.

The p-value (0.099) for Hemolysin virulence factor is greater than the conventional significance level of 0.05. This suggests that there is no statistically significant association between the presence of hemolysin and whether an isolate is APEC positive. Whereas that of Fimbrial adhesin (p-0.035) is less than 0.05, indicating a statistically significant association between the presence of fimbrial adhesin and APEC positive status.

Table 6: Virulence Phenotype of APEC Isolates from this Study

Virulence Factors	No. (%) of Virulent *E. coli* Isolates (n = 82)	No. (%) Virulent APEC (n=52)	P =value
Haemolysin	53(64.6)	31(59.6)	0.099
Fimbrial Adhesin	29(35.4)	21(40.4)	0.035

These results show the chi-square statistic and p-value for each virulence factor: Hemolysin: Chi-square = 2.719, Fimbrial adhesin: Chi-square = 4.434, Type/pili: Chi-square = 6.545.

DISCUSSION

Clinical samples from infected animals are the most direct source of data regarding colibacillosis. Studies have shown that colibacillosis is prevalent in neonatal and post-weaning chicks with high morbidity and mortality rates reported [6]This is similar to what is recorded from this study. In poultry, colibacillosis manifests as respiratory disease, often leading to significant economic losses [15]. Contrary to this study, environmental samples, including water and soil, have been increasingly recognized as reservoirs for *E. coli*. Research indicates that contaminated water sources can lead to outbreaks in livestock [16]. Studies have detected *E. coli* in raw meat products, which poses a risk to human health [8]Thus this also confirm one of the reason why the pathogen is isolated from internal organs.

Avian Pathogenic Escherichia coli (APEC) is a significant pathogen responsible for various diseases in poultry, leading to substantial economic losses in the poultry industry. Understanding these distributions is essential for implementing effective control measures. In the case of this study farms within Bauchi state metropolis. Farms are primary locations for APEC isolation, with studies indicating a high prevalence of APEC in both broiler and layer farms. Research has shown that factors such as biosecurity practices, flock density, and management systems significantly influence APEC distribution [17]. For instance, farms with poor biosecurity measures often report higher incidences of APEC infections [18]. Other critical point includes Hatcheries and processing plants serve as perilous points for APEC transmission, as contaminated eggs can lead to widespread infection in chicks. Studies have demonstrated that APEC can be isolated from hatchery environments, including egg surfaces and incubators [19].The presence of APEC in hatcheries underscores the need for stringent sanitation protocols to prevent outbreaks.

The distribution of Avian Pathogenic Escherichia coli (APEC) is significantly influenced by various bird characteristics, including age,sex and breed. Younger birds, particularly chicks, are more susceptible to APEC infections due to their underdeveloped immune systems, leading to higher morbidity and mortality rates in broiler and layer flocks [7]. Additionally, certain breeds exhibit varying levels of susceptibility to APEC; for instance, commercial broilers are often more affected than local breeds, which may possess better innate immunity [17].The immune status of birds also plays a crucial role, as immunocompromised or stressed birds are more likely to harbour and succumb to APEC infections [20]. Understanding these characteristics is essential for developing targeted prevention and control strategies in poultry production.

Farms serve as the primary locations for APEC-related mortality, especially in broiler and layer operations, with studies indicating that APEC can cause significant mortality rates in young birds, reaching as high as 30% in infected flocks due to factors like poor biosecurity and overcrowding, as highlighted by [20]. Hatcheries also significantly contribute to APEC mortality since contaminated eggs can result in widespread infections among chicks, with research by [21]. showing mortality rates of up to 20% shortly after hatching. Although processing plants are not directly linked to mortality, they can affect overall mortality rates by facilitating the spread of APEC during processing, which may increase mortality in the following flocks, as noted by [21].The presence of APEC across these locations underscores the urgent need for targeted interventions, as each site plays a unique role in the pathogen's epidemiology. A comprehensive approach involving enhanced biosecurity, sanitation, and monitoring practices is vital to reduce the impact of APEC on poultry mortality.

The breed of poultry also plays a significant role in determining mortality rates associated with APEC infections. Commercial broiler breeds are often more susceptible to APEC due to their rapid growth rates and associated stress, which can compromise their immune response[22]. In contrast, heritage breeds tend to exhibit better resilience and lower mortality rates when infected with APEC [7]. This variation underscores the importance of breed selection in poultry management practices.

Escherichia coli encompasses a range of strains, some of which are pathogenic and exhibit distinct virulence phenotypes that contribute to their disease-causing potential[1]. Enterotoxigenic *E. coli* is known for causing diarrhea through the production of enterotoxins and adherence to the intestinal mucosa. APEC *E. coli* induces attaching and effacing lesions in the intestines, primarily affecting infants, while enterohemorrhagic *E. coli*, particularly the O157:H7 serotype, is notorious for severe gastrointestinal disease and complications like hemolytic uremic syndrome due to Shiga toxin production[23]. Uropathogenic *E. coli* is a leading cause of urinary tract infections, utilizing specific adhesins and toxins to establish infections. Understanding these diverse virulence mechanisms is crucial for developing effective prevention and treatment strategies against *E. coli* infections[24].

Escherichia coli employs several virulence factors, including adhesins and hemolysin, to establish infections and cause disease. Adhesins are surface proteins that facilitate bacterial attachment to host tissues, crucial for colonization, particularly in APEC *E. coli* during urinary tract infections[25]. Hemolysin, a pore-forming toxin produced by certain pathogenic strains, causes lysis of red blood cells and other host cells, leading to tissue damage and nutrient release for bacterial growth[14]. Together, these factors significantly contribute to the pathogenicity of *E. coli*, highlighting the need for targeted interventions to combat *E. coli*-related diseases.

In conclusion, highest Colibacillosis prevalence was recorded in faeces 32(100%), moderate correlation of 0.592 among samples. The occurrence rate of APEC was higher in faeces followed by internal organs from GRA and Gida Dubu among sample locations Internal organ samples showed 26% mortality, with no deaths in other birds; younger, female, and layer birds have higher APEC mortality. The study found high prevalence of virulence factors in APEC isolates. However, it is recommended that: Further study should be conducted to trace the phylogenetic relationship between the sequence APEC isolates with other sequences. And The need for cautious use of tetracycline in poultry production to decrease the prevalence of tetracycline-resistant *E. coli*. The same may be true in other livestock production as tetracycline is the most abused antibiotics in veterinary practice.

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