BIOCHEMICAL, CLINICAL AND MOLECULAR CHARACTERIZATION OF AVIAN PATHOGENIC *Escherichia coli* (APEC) IN COMMERCIAL BROILER FLOCKS IN THE PHILIPPINES USING THE PLASMID PATHOGENICITY-ASSOCIATED ISLAND PREDICTORS

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ABSTRACT

Avian pathogenic *Escherichia coli* (APEC) poses a significant dual threat to the economic viability and health of the broiler industry. A thorough analysis of 18 commercial broiler flocks across diverse regions in the Philippines was aimed at confirming the presence of APEC, utilizing the plasmid pathogenicity-associated island predictors. The affected flocks, aged one to five days, exhibited various clinical signs, including lethargy, increased mortality, omphalitis, pericarditis, perihepatitis, enteritis, and delayed growth. All isolated APEC strains distinctly demonstrated typical staining and cultural characteristics as Gram-negative rods with gamma hemolysis. Biochemical profiles yielded positive results for catalase, indole, lactose, methyl-red (MR), nitrate reduction, saccharose, and glucose, as well as gas production; negative results were observed for citrate, hydrogen sulfide (H_2S), oxidase, urease, and Voges-Proskauer (VP). Among the 18 farms analyzed, 50% tested positive for all APEC virulence genes—*iroN* (83%), *iss* (94%), *ompT* (94%), *iutA* (61%), and *hlyF* (89%). This virulence gene profile, revealed through PCR analysis using the plasmid pathogenicity-associated island predictors, highlights the role of these genes, encoding protectins, iron acquisition systems, and toxins. The findings have enhanced the understanding of Philippine APEC strains, emphasizing their biochemical and genetic profiles. The current data are crucial for developing diagnostic kits and formulating effective preventive and control protocols against APEC in the country.

Keywords: APEC, broilers, *Escherichia coli,* Philippines, virulence genes

INTRODUCTION

The continuous reliance of the public on safe and healthy poultry products is reflected in the most recent data generated by the Philippine Statistics Authority (PSA); wherein, Filipinos chose poultry products as primary source of protein with an average of 6.89 kg per capita consumption (PSA, 2021). Additionally, this statistic is also reflected in

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the number of poultry products produced and imported annually. Data from the PSA during the 2nd quarter of the year 2023, showed a significant improvement with a 3.2 % increase at 477.76 thousand metric tons (PSA, 2023). Although the Philippine poultry industry is slowly recovering from the lethal effects of the highly pathogenic avian influenza (HPAI) infection back in 2021, there are still various factors that prevent the industry from prospering. Hafez and Attia (2020) cites the following reasons: challenges in the chicken's overall health, immunity, food safety, disease surveillance, and the public hazard brought about by various zoonotic pathogens associated with poultry products.

Among the most common diseases of poultry, infections with APEC can cause a plethora of clinical signs and associated disorders that may affect a diverse range of poultry species and wide age groups in different stages of production. Due to the extra-intestinal pathogenic characteristics of this bacteria, it can affect organs outside of the gastrointestinal system; resulting in the development of airsacculitis, omphalitis, synovitis, and egg peritonitis (Chen *et al.,* 2021; Kathayat *et al*., 2021). Due to the listed clinical symptoms and other contributing factors, elevated morbidity and mortality rates are observed causing a substantial loss in production in terms of carcass quality, low hatch rates, and high carcass condemnation in slaughterhouses. Aside from the direct effects of APEC in poultry production, the threat of zoonosis arises as similarities in the genetic makeup of APEC and *E. coli* strains that commonly affect humans are found. Furthermore, the issues in food security and consumer welfare come to light as APEC can reach the consumers through inappropriate and ineffective mitigation protocols starting from the farm and food processing facilities.

To infect its hosts, APEC uses a variety of virulence genes and pathogenesis factors, such as toxins*,* adhesins*,* iron acquisition systems, invasins, protectins*,* two-component systems, quorum sensing system, transcriptional regulators, secretion systems, and metabolism-associated genes (Kathayat *et al.*, 2021). These factors aid in the development of infection in poultry by facilitating cell adhesion, tissue colonization, cell proliferation, apoptosis, sequestration of metal ions from body fluids, resistance to environmental degradation, and biofilm formation (Kathayat *et al.*, 2021).

Through the detection of several pathogenic genes by PCR, APEC could be differentiated from non-APEC organisms (Johnson *et al*., 2008a). These diagnostic markers include plasmid-based genes such as *iss, iroN, ompT, iutA,* and *hlyf* that correspond to various virulence and pathogenesis factors associated with the bacteria namely: *protectins* that safeguard APEC from the detrimental attacks from the immune system of the chicken (*ompT* and *iss*); *iroN* acquisition systems responsible for the sequestration of required iron for bacterial growth (*iutA* and *iroN*) and toxins that induce injury to infected tissues usually causing the formation of outer membrane vesicles, motility, and colonization *(hlyF)* (Johnson *et al*., 2006, Johnson *et al*., 2008, Murase *et al*., 2016 and Kathayat *et al*., 2021)

Information on APEC prevalence or virulence in the Philippines is lacking. The rise in antimicrobial-resistant APEC organisms necessitates the need for an effective and efficient nucleic acid-based confirmation to mitigate the potential spread of these bacteria from farm to table. This study was performed to characterize the clinical and virulence genetic profile of APEC in broiler chickens from select regions of the Philippines using PCR to aid in the development of rapid diagnostic techniques and preventive and control measures against the disease.

MATERIALS AND METHODS

This study was approved by the Institutional Animal Care and Use Committee (IACUC) of the College of Veterinary Medicine, University of the Philippines Los Baños (UPLB) with assigned protocol number 2019-0027. Field sampling was executed with authorization obtained through a written permit issued by the Director of the Bureau of Animal Industry.

Study Population

A total of 18 commercial poultry flocks that were willing to participate in the study were selected from different regions in the Philippines. Samples were collected via convenience sampling method wherein the samples that were obtained depended on the availability and accessibility of study units at the time of sample collection. Farm records such as the farm location, farm size, population of the affected flock, age at onset of disease, morbidity rate, mortality rate, and clinical signs were all obtained for the characterization of the clinical profile of the disease.

Table 1. Farm history and clinical profile of APEC strains from the Philippines

Farm Code	Farm location	Year	Farm size	Clinical Signs	Age (days)
PHBR6B1 219B10	Bacolod	2019	20,000	weak chicks with omphalitis	3
PHBR6B1 219B19	Bacolod	2019	20,000	weak chicks with omphalitis	4
PHBR6B1 219B21	Bacolod	2019	20,000	weak chicks with omphalitis	5
PHBR3P1 119R1	Pampanga	2019	180,000	lethargy, swollen abdomen, pasty vents	1
PHBR3P1 119R2	Pampanga	2019	180,000	lethargy, swollen abdomen, pasty vents	2
PHBR3P1 119R3	Pampanga	2019	180,000	lethargy, swollen abdomen, pasty vents	1
PHBR3P0 619R1	Pampanga	2019	180,000	wet feces, pasty vents, elevated mortality	3
PHBR3P0 619R2	Pampanga	2019	180,000	wet feces, pasty vents, elevated mortality	2
PHBR3P0 619R3	Pampanga	2019	180,000	wet feces, pasty vents, elevated mortality	1
PHBR3P0 619R4	Pampanga	2019	180,000	wet feces, pasty vents, elevated mortality	3
PHBR3P0 619R5	Pampanga	2019	240,000	wet feces, pasty vents, elevated mortality	2
PHBR3P0 619R6	Pampanga	2019	240,000	wet feces, pasty vents, elevated mortality	2
PHBR3P0 819R1	Pampanga	2019	80,000	weak chicks, wet feces	1
PHBR3B0 619R1	Bulacan	2019	200,000	elevated mortality, weak chicks	1
PHBR3B0 619R2	Bulacan	2019	200,000	lethargy, pasty vents	2
PHBR3B0 619R3	Bulacan	2019	200,000	elevated mortality, weak chicks	1
PHBR3B0 619R4	Bulacan	2019	200,000	wet feces, weak chicks	2
PHBR3B0 619R5	Bulacan	2019	200,000	wet feces, weak chicks	1

Sample Collection

A total of five to ten birds per flock with clinical signs suggestive of APEC such as omphalitis, pericarditis, perihepatitis, and enteritis was also collected from each broiler flock (Table 1). Chick samples were collected and euthanized by cervical dislocation. Samples were kept in air-tight plastic containers and transported in an ice chest packed with coolants to the laboratory. Conventional bacterial isolation was performed within 24 hours upon arrival.

Table 2. Cultural, microscopic and biochemical profile of the bacterial isolated obtained from clinically infected flock

Bacterial Profile	Laboratory Result	Feature/s
Cultural hemolysis	gamma	gamma hemolytic
Microscopic	Gram-negative	rod-shaped
Biochemical		
Endospore staining	-	non-sporeformimg
Methyl red (MR)	+	
Voges-Proskauer (VP)	-	
Oxidase	-	
Catalase	+	
Hydrogen Sulfide (H2S)	-	
Indole	-	
Sulfur-indole-motility (SIM)	+	
Nitrate reduction	+	
Urease	-	
Citrate	-	
Starch hydrolysis	+	fermentative
Sugar Fermentation		
Lactose	+	
Glucose	+	with gas formation
Saccharose	+	

+ positive; - negative

Isolation and Conventional Identification of APEC

Sterile swabs from the heart, spleen, liver, and lungs of broilers were cultured on 5% sheep blood agar and MacConkey agar media (Eiken, Japan). A standard microbiological identification of the isolates was performed (Table 2) using morphology and cultural characteristics, gram staining, and biochemical tests such as indole, methyl-red, Voges-Proskauer, and Citrate Test (IMViC), oxidase test, carbohydrate fermentation test, and motility test. Isolated colonies were sub-cultured on eosin methylene blue agar (EMB) (Eiken, Japan). Isolated colonies of *E. coli* strains were stored in 20% glycerol with brain

and heart infusion broth (Eiken, Japan) at − 80 °C.

Molecular Confirmation of APEC Isolates

Approximately three distinct colonies of suspected APEC strains were randomly selected from the EMB agar plates and were suspended in 1 ml distilled water. Approximately 200ul of the boiled *E. coli* antigen was used for the extraction of genomic DNA using commercial kits (QIAamp® DNA Mini Kit, Qiagen, West Sussex, UK) according to the manufacturer's instructions. Amplification of minimal predictors of APEC (*iutA, hlyF, iss, iroN and ompT*) were performed using Sapphire Amp Fast PCR Master Mix (Takara Bio-Inc., Shiga, Japan) with gene-specific primers (Table 3) (Johnson *et al.,* 2008a) in a thermocycler following an initial denaturation at 94°C for 3 min, then 35 cycles of denaturation at 94°C for 10 s, annealing at 55°C for 10 s and extension at 72°C for 10 s. The final extension was carried out at 72°C for 10 min. Amplified products were analyzed by electrophoresis using 2% agarose gel with 1x TBE (Tris-Borate, EDTA). The PCR product was stained with Gel Red® (Biotium, Inc., California, USA) and was then visualized using a UV transilluminator (OmniDOC Gel Documentation System, UK). The expected amplicon sizes of the target genes are presented in Table 3.

Table 3. Conventional PCR primers used in this study.

Genes	Amplicon Size (bp)	Sequence	Description of Virulence Gene
iroN	553	F: 5'-AATCCGGCAAAGAGACGAACCGCCT-3' R: 5'-GTTCGGGCAACCCCTGCTTTGACTTT-3'	Salmochelin siderophore
iss	323	F: 5'-CAGCAACCCGAACCACTTGATG-3' R: 5'-AGCATTGCCAGAGCGGCAGAA-3'	Increased serum survival
ompT	496	F: 5'-TCATCCCGGAAGCCTCCCTCACTACTAT-3' R: 5'-TAGCGTTTGCTGCACTGGCTTCTGATAC-3'	Episomal outer membrane Protease Gene
iutA	302	F: 5'-GGCTGGACATCATGGGAACTGG-3' R: 5'-CGTCGGGAACGGGTAGAATCG-3'	Aerobactin siderophore
hlyF	450	F: 5'-GGCCACAGTCGTTTAGGGTGCTTACC-3' R: 5'-GGCGGTTTAGGCATTCCGATACTCAG-3'	Avian hemolysin

RESULTS

Farm History and Clinical Profile

Pertinent clinical information such as farm location, year of collection, farm size, age at the time of collection, and clinical signs are presented in Table 1. Most samples were collected in 2019 and most were from Region 3 where the majority and bulk of the overall poultry production in the Philippines is concentrated. Farms of varying sizes from 20,000 to 200,000 birds participated in the study covering the potential differences in practices and management vis-à-vis the farm type which is usually attributed to the farm size. Furthermore, hallmark clinical signs observed were lethargy, omphalitis, abdominal

distention, pasty droppings, diarrhea, and an increased in mortality rate.

Table 4. Percent positivity of *E. coli* samples tested using the five minimal APEC predictive genes.

Genes	Positive	Negative	% Positivity
iroN	15	3	83
iss	17	1	94
ompT	17	1	94
iutA	11	7	61
hlyF	16	2	89

Table 5. Conventional PCR assay results in the detection of five minimal predictor for APEC.

Farm Code	PCR					Interpretation
	iroN	***iss***	***ompT***	***iutA***	***hlyF***	
PHBR6B1 219B10	Positive	Positive	Positive	Positive	Positive	APEC
PHBR6B1 219B19	Positive	Positive	Positive	Positive	Positive	APEC
PHBR6B1 219B21	Positive	Positive	Positive	Positive	Positive	APEC
PHBR3P1 119R1	Negative	Negative	Negative	Negative	Negative	Non-APEC
PHBR3P1 119R2	Positive	Positive	Positive	Negative	Positive	Non-APEC
PHBR3P1 119R3	Positive	Positive	Positive	Positive	Positive	APEC
PHBR3P0 619R1	Negative	Positive	Positive	Positive	Positive	Non-APEC
PHBR3P0 619R2	Positive	Positive	Positive	Positive	Positive	APEC
PHBR3P0 619R3	Negative	Positive	Positive	Positive	Positive	Non-APEC
PHBR3P0 619R4	Positive	Positive	Positive	Positive	Positive	APEC
PHBR3P0 619R5	Positive	Positive	Positive	Positive	Positive	APEC
PHBR3P0 619R6	Positive	Positive	Positive	Negative	Positive	Non-APEC
PHBR3P0 819R1	Positive	Positive	Positive	Negative	Negative	Non-APEC
PHBR3B0 619R1	Positive	Positive	Positive	Positive	Positive	APEC
PHBR3B0 619R2	Positive	Positive	Positive	Negative	Positive	Non-APEC
PHBR3B0 619R3	Positive	Positive	Positive	Positive	Positive	APEC
PHBR3B0 619R4	Positive	Positive	Positive	Negative	Positive	Non-APEC
PHBR3B0 619R5	Positive	Positive	Positive	Negative	Positive	Non-APEC

Conventional Bacterial Isolation

All of the 18 broiler flocks investigated were positive for *E. coli* through conventional bacterial isolation. Isolated strains were gram-negative rods, motile, non-spore-forming, catalase positive, oxidase negative, MR positive, VP negative, fermentative, indole positive, citrate negative, urease negative, nitrate reduction positive, H_2S negative, lactose positive,

glucose positive, saccharose positive and gas former. All isolates were gamma-hemolytic (see Table 2).

Molecular Detection of APEC in the Philippines

Samples collected from the participating farms were tested for the five (5) minimal predictive pathogenic genes using the protocols of Johnson *et al.* (2008a) (Table 3). Samples positive for the following genes yielded the following PCR amplicons: 553 bp product for *iroN*; 323 bp product for *iss*; 496 bp product for *ompT*; 302 bp product for *iutA*; and 450 bp product for *hlyF* (Figure 1). Out of the 18 farms, nine (9) tested positive for all the aforementioned APEC virulence genes (50%) (Figure 2). The percent positivity of each gene of interest is as follows: *iroN* (83%), *iss* (94%), *ompT* (94%), *iutA* (61%), and *hlyF* (89%) (Figure 3, Table 4). Out of the nine non-APEC samples, seven isolates were positive in four out of five minimal predictors for APEC, one isolate was positive in three out of five minimal predictors and one isolate was negative in all of the minimal predictors for APEC (Figure 2). Most of the non-APEC samples had at least one negative gene of interest wherein most were observed in *iutA* gene. Incidentally, both the *iss* and *ompT* genes were all present in almost all of the *E. coli* samples except PHBR3P1119R1 wherein none of the virulence genes tested positive (Table 5).

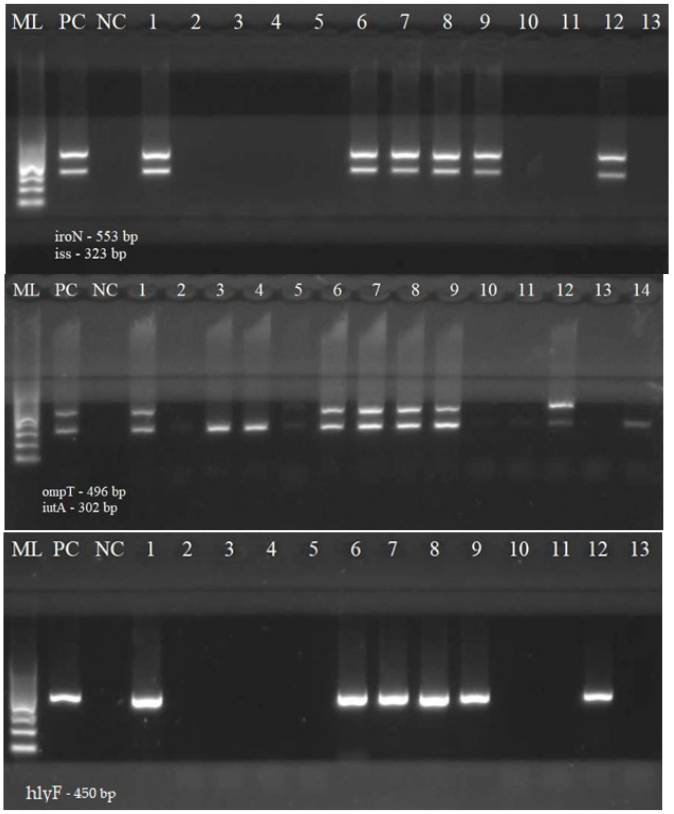

Figure 1. PCR products of the five minimal APEC predictive genes

[Positive PCR products of the different APEC predictive genes (*iroN, iss, ompT, iutA,* and *hlyF*) are presented. Each figure indicated the target base pair of the different APEC predictor genes. ML - Molecular Ladder. PC – Positive Control, NC – Negative Control, Lane 1-13 – field *E. coli* sample]

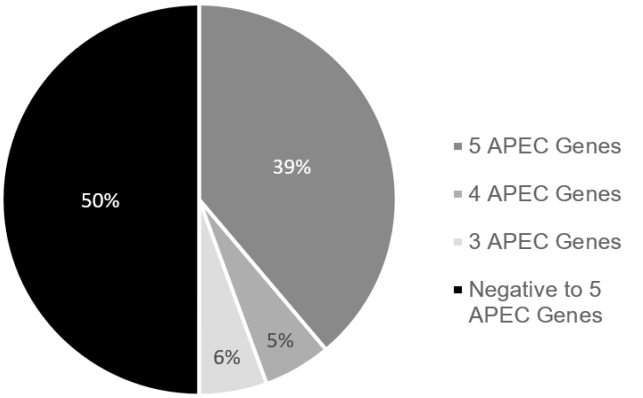

Figure 2. Distribution of positive APEC predictive genes in the field *E. coli* samples.

[Nine (9) field *E. coli* isolates were positive (50%) in all the five minimal APEC predictive genes. Seven isolates were positive in four of the five minimal predictors, one isolate was positive in three of the five minimal predictors, and one isolate was negative in all of the minimal predictors for APEC.]

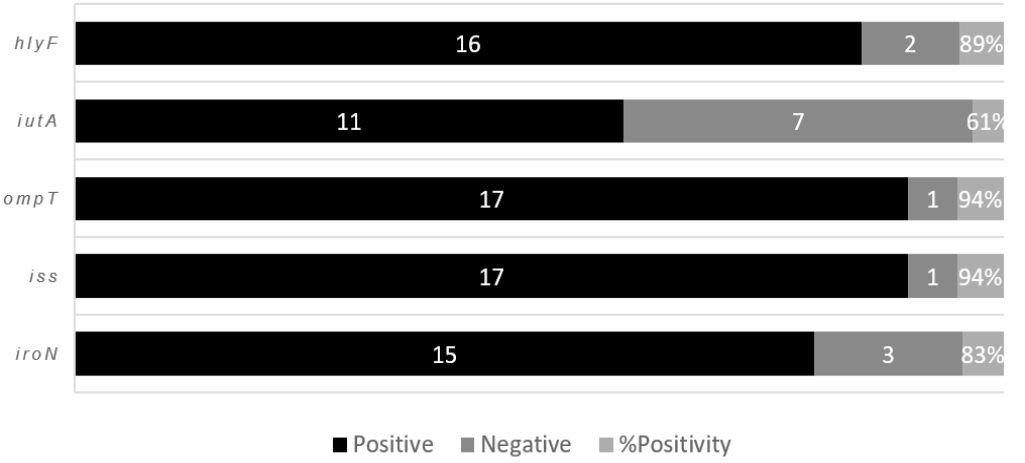

Figure 3. Percent positivity of the APEC predictive genes in the field *E. coli* samples

[The percent positivity of the five minimal APEC predictive genes are presented. Both the *iss* and *ompT* genes were present in almost all the APEC samples except PHBR3P1119R1.]

DISCUSSION

The findings from the current study revealed that broiler flocks, especially in high-producing regions across diverse farm sizes, consistently displayed clinical symptoms such as lethargy, omphalitis, and increased mortality rates. These indicators were linked to APEC infection, as bacterial isolation confirmed the presence of *E. coli* with distinct characteristics. Molecular analysis uncovered a substantial prevalence (50%) of APEC virulence genes, highlighting significant variations in the possession of genes among the samples. This underscores the importance of specific genes like *iss* and *ompT* in the majority of *E. coli* strains. It can be noted that many avian species have *E. coli* as normal microflora

in their lower digestive tracts, and the intestinal contents of birds often have 10^4-10^7 colony-forming units (CFU) per gram. However, specific strains of *E. coli* called APEC cause the disease colibacillosis and chronic respiratory disease in poultry (Ozawa *et al*., 2008). Colibacillosis is an infectious disease that has been associated with significant profit losses among commercial farms causing mortality of up to 20% and decreased farm meat yield with a 2% decline in live weight, regressed feed conversion by 2.7%, and a 43% increase in carcass condemnation at slaughter with a drastic reduction in hatching rates (Kathayat *et al*., 2021). In broilers, infection often occurs concomitantly with other immunocompromising diseases such as infectious bronchitis and mycoplasmosis, causing respiratory complications that may progress to peripheral organs through circulation (Gross, 1991). In such cases, the APEC organism can also reach the muscles and hence, be carried on meat, particularly in situations where there is a lack of food safety practices, and can then be transferred to humans via the food chain.

Infection with APEC organisms clinically is manifested by airsacculitis, enteritis, pericarditis, omphalitis, perihepatitis, peritonitis, and septicemia (Gross, 1991). The upper respiratory system especially the pharynx and trachea of chickens can be colonized by the organism, and depending on the extent of environmental contamination, it can also be isolated from the skin and feathers (Dho-Moulin and Fairbrother, 1999). This bacteria can infect chicks in the first hours after hatching, and APEC strains proliferate in the intestine. Throughout a bird's life, it may acquire many different strains. Numerous causes are hypothesized as to how these day-old chicks are infected, some eggs are inherently infected, and some are infected right after the eggs are laid through surface contamination. Vertical contamination occurs when APEC is transmitted from breeders to chicks *in ovo* or through infected shells. Horizontal APEC contamination mainly arises as a result of interaction with other sick birds and ingestion of contaminated feeds and water (Dho-Moulin and Fairbrother, 1999). It may also lead to respiratory tract infection that frequently results in septicemia (Ewers *et al*., 2004). APEC can affect the carcass quality and overall performance of the flock even in considered "developed" countries where strict hygiene and production parameters are set (Joseph *et al.,* 2023). In the study by Gretarsson *et al.* (2023) the most common cause of carcass condemnation was abscess/cellulitis in Norwegian aviary-housed layers (2.03%) and this was mostly caused by *E. coli.* Moreover, APEC and bacterial pathogens in humans have exhibited a shared virulence component that demonstrates comparable adaptations in the extraintestinal environment showing that APEC may cause disease in humans via horizontal gene transmission through the transfer of plasmids that retained the virulence genes (Mokady *et al.,* 2005).

Although APEC has a wide range of serological variability (Wang *et al*., 2010; Kim *et al*., 2020), all APEC strains belong to the phylogenetic group of extraintestinal pathogenic *E. coli* (ExPEC). APEC serogroups that are frequently isolated from birds with colibacillosis belong to O1, O2, and O78; however, these serogroups are also often isolated in healthy birds (Yaguchi *et al*., 2007). As of writing, studies and reports on APEC strains in poultry farms in the Philippines are limited. Most of the studies conducted in recent years were all concerned with the antibiotic resistance profile of the bacteria.

The clinical and farm profiles of the participating farms in this study were all listed in Table 1. Interestingly, most of the samples collected were from chicks ranging from 1 – 5 days old. Literature suggests that APEC organisms are credited to fifty percent (50%) of the "first-week mortalities" due to omphalitis and subsequent life-threatening septicemia and

these may or may not be exacerbated by other enteric bacteria (Christensen *et al*., 2021).

This study utilized the predictive model performed by Johnson *et al*. (2008a) in identifying APEC samples from non-APEC (AFEC). Using an extensive number of samples of confirmed APEC and AFEC, Johnson *et al*. (2008a) were able to determine the virulence genes needed to identify an APEC sample, through virulence genotyping, multiple correspondence analysis, and cluster analysis. Originally, 46 virulence genes are identified to classify *E. coli* as APEC. Testing for these 46 genes proved to be arduous and complicated hence, the need for a simplified testing procedure to distinguish APEC from non-APEC samples. It was revealed that distinct demarcation between APEC and non-APEC samples exists. APEC samples possess both plasmid-associated and chromosomal-associated traits, in contrast to non-APEC isolates. Plasmid-associated genes include episomal *iss*, *iroN*, episomal *ompT*, *eitAB, cvaABC, cbi, cma*, *iutA*, *hlyF*, and *etsAB* while chromosomal-associated genes include the chromosomal *ompT*, *ireA, fyuA, papACEFG,* and *vat*. Aside from these genes, some plasmid-associated genes such as *sitA, iutA*, *hlyF*, episomal *ompT*, etsAB, episomal *iss*, *iroN*, and cvaABC and chromosomal genes, including *fyuA, ireA,* the *pap operon* genes, *vat*, capsular biosynthesis genes (*K1* and *K2* capsule types), and other PAI markers (*malX, ibeA, and gimB*) are also conserved and can be found in almost all APEC samples. From these results, a pentaplex PCR-based testing was produced using the best five highly conserved APEC genes with 85.4% accuracy in discriminating APEC from non-APEC samples (Johnson *et al*., 2008a).

The five corresponding genes used for the identification of APEC possess virulence factors that belong to the protectins, *iron* acquisition systems, and toxins factors (Nolan *et al.*, 2020). Protectins are molecules secreted by the bacteria that counteract the host immune complement response. This includes the bacterial capsule, lipopolysaccharides (LPS), and outer membrane proteins. Previously mentioned *ompT* and *iss* genes are also classified in this virulence group wherein both are exposed on the outer membranes of the bacteria. In addition, *iss* gene or increased serum survival gene is a lipoprotein observed in the outer membrane of APEC. It inhibits serum neutralization, capsule synthesis, and the complement immune pathway (Johnson *et al.*, 2008b, Nolan *et al.,* 2020, Christensen *et al*., 2021). Another virulence factor contributing to the pathogenic effects of APEC is the *iroN* acquisition systems. The genes *iutA* and *iroN* are essential in mediating the uptake of beneficial *iroN* particles in host cells during colonization. Finally, *hlyF* genes encode for a putative avian toxin hemolysin that cleaves and destroys the host tissues specifically erythrocytes. (Nolan *et al.*, 2020; Kathayat *et al*., 2021). The presence of these genes denotes the heightened virulence and pathogenicity of an APEC species (Johnson *et al*., 2008a, de Oliveira *et al*., 2015 and Jørgensen *et al*., 2019).

Aside from the serious effects of APEC on animal health, the issue of public health in particular with the ability of poultry products to become a reservoir for ExPEC, humans pose a great risk on account of the great similarities observed in APEC and human ExPEC. Literature suggests that experimental *in vitro* and *in vivo* infection studies of both species are similar predominantly owing to the akin genomics, phylogeny, plasmid content, AMR patterns, virulence factors, and serogroups (Nolan *et al.*, 2020). Likewise, the fact that the two organisms have the same antimicrobial resistance and plasmid patterns means that plasmid-associated multi-drug resistance (MDR) and virulent genes in one species can be passed freely and easily through horizontal transmission principally through conjugation. Poultry products still comprise a great bulk of human daily food consumption, and this

necessitates that the people involved in the food supply chain such as farm handlers, dressing plant workers, and veterinarians strictly comply with various food hygiene protocols to at least prevent the introduction of APEC in the human food chain. Moreover, its phylogenetic background being similar to human extraintestinal pathogenic *E. coli* (ExPEC) including their shared distinct virulence factors and identical serotypes showed that APEC is transferred easily to humans via the horizontal gene transfer (Boyd and Brussow, 2002) and this likelihood is increased in poor hygienic conditions.

Due to the increasing demand for poultry meat, production practices became more intensive with the aid of modern techniques in breeding, nutrition, and the administration of antibiotics to achieve optimum size and growth (Wessels *et al.*, 2021). Consequently, the intensive use of antibiotics led to its indiscriminate use which resulted in the emergence of multidrug-resistant (MDR) bacteria. This MDR strain of bacteria is a persistent problem that caused a negative impact on medical advancement in many parts of the world (Fuhrmeister and Jones, 2019). The APEC isolates, as confirmed in this study to be found in poultry operations in the Philippines, are potential carriers of MDR genes as well. Hence, it is imperative that the antibiotic sensitivity profile of the APEC isolates be studied as a surveillance initiative and use the data as a mitigation measure and for policy recommendation. .

CONCLUSION

In conclusion, APEC is known to be a serious and life-threatening disease in broilers and a public health concern. This study evaluated the biochemical, clinical, and virulence genetic profile of APEC in commercial broilers in the Philippines. The data generated from this investigation will be helpful in the development of diagnostic kits and the formulation of appropriate preventive and control protocols against APEC in the Philippines.

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