

*Full Length Research Paper*

# **Prevalence and Antibiotic resistance profile of Avian Pathogenic *Escherichia coli* (APEC) strains isolated from poultry feeds in Abidjan District, Côte d'Ivoire**

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The aim of this study was to isolate avian pathogenic *Escherichia coli* (APEC) strains from poultry feeds for assessing their susceptibility patterns to antibiotic agents. From November 2018 to March 2019, one hundred eighteen poultry feeds samples were collected in farms of Abidjan District and *E. coli* strains were isolated on TBX (Tryptone Bile Glucuronic) agar medium, followed by biochemical identification and APEC virulence genes detection via polymerase chain reaction (PCR) analysis. Among these samples, 44 (37.29%) were positive to *E. coli*. Municipalities of Anyama, Bingerville and Port-Bouët provided most contaminated poultry feeds with respectively 100, 54.04 and 30.76% of prevalence rate. Moreover, increased serum survival (*iss*) and iron-acquisition system (*iucD*) genes were respectively detected in 10 (15.87%) and 15 (23.81%) tested strains while seven *E. coli* isolates were positive for both genes. Antibiotic susceptibility tests by the disk diffusion method in Mueller-Hinton agar medium showed high resistance level to tetracycline (100%) and nalidixic acid (61.90%) while moderate resistance rates was observed with amoxicillin +clavulanic acid (28.57%) and ciprofloxacin (16%). Moreover, all the tested strains were susceptible to gentamicin. This study indicate the necessity to control the quality of poultry feeds in Côte d'Ivoire and especially to research alternative methods to reduce extensive antibiotics use in this sector in Côte d'Ivoire.

**Key words:** Avian pathogenic *Escherichia coli* (APEC), poultry feeds, antibiotic, poultry farm, Côte d'Ivoire.

## **INTRODUCTION**

In Côte d'Ivoire, poultry production systems have significant effect on national economy (Koné and Danho, 2008). Indeed, a recent report by Interprofessional Ivorian Poultry sector organization in 2017 showed that total income in 2015 was estimated at about 412.8 millions US

Dollar (IPRAVI, 2017). This sector accounts for 4.5% of agricultural GDP and 2% of total GDP. In addition, Côte d'Ivoire's government intends to increase this performance by reaching 60000 tons of poultry meat and more than 1.678 billion of eggs / year in 2020 to fully

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cover population needs of animal proteins (IPRAVI, 2017). However, poultry production sector is affected by many factors including several illnesses which lead to a significant decrease of chicken meat and eggs production. For example, in 2012, the loss of production was estimated to 39.45% corresponding to more than 240 000 USD. Generally, these diseases are due to various microorganisms including virus, fungi, parasites and bacteria agents (Zhao et al., 2005). Among poultry diseases due to bacteria, colibacillosis is the primary cause of morbidity, mortality, and condemnation of carcasses in the poultry industry worldwide. It is, thus, an economically devastating disease for poultry industry in many parts of the world (Zhao et al., 2005). Strains causing these systemic diseases in poultry are termed avian pathogenic *Escherichia coli* (APEC) (Zhuang et al., 2014; Schouler et al., 2018). *E. coli* is, normally, one of the common microbial flora of gastrointestinal tract of poultry but may become pathogenic because of specific virulence attributes that have been associated with a systemic disease, colibacillosis (Jawetz et al., 1984; Levine, 1987; Yang et al., 2004). Colibacillosis of poultry is characterized in its acute form by septicemia resulting in death (Calnek et al., 1997). At days, approaches to prevent and control this pathology in the poultry industry include improved hygienic methods, good practices of micro-environment management, vaccination and use of antimicrobial agents. However, many reports have described increased multidrug resistance of *E. coli* to commonly used antimicrobial agents for treatment (Yang et al., 2004, Zhao et al., 2005). One of the potential source of *E. coli* contamination is chicken feeds. The latter has a significant impact on poultry health and its zootechnical performances. Unfortunately, data on microbiologic qualities of these feeds in Côte d'Ivoire remain unavailable. Moreover, antibiotics are extensively used as growth promoters in poultry production or to control infectious diseases. This misuse of antibiotics is considered the most vital selection force to antimicrobial resistance of bacteria (Okeke et al., 1999; Moreno et al., 2000; Ouattara et al., 2013). Moreover, the resistant *E. coli* could be passed from poultry to people via handling of feeds or direct contact with infected chicken. In addition, acquired resistance to antimicrobial agents creates an extensive trouble in case of management of intra and extra intestinal infections caused by *E. coli*, which are a major source of illness, death, and increased healthcare costs both in poultry and in human (Gupta et al., 2001). Therefore, the present study was designed to isolate avian pathogenic *E. coli* strains from poultry feeds for assessing their susceptibility and resistance patterns to some selected antimicrobials in Côte d'Ivoire.

## MATERIALS AND METHODS

### Study area and sampling

This study was conducted in five municipalities including Yopougon,

Songon, Bingerville, Port-Bouët and Anyama because of their high poultry production capacity in the District of Abidjan, Côte d'Ivoire. The geographical location of each municipality is shown in Figure 1. A preliminary survey was conducted in the Abidjan District studying the major pathologies such as affecting poultry production systems (Doumbia, 2018). According to all information recorded, we hypothesized that foods used for animal could be contamination source of poultry by pathogenic microbial flora such as Avian pathogenic *Escherichia coli* (APEC), and reported in breeder chickens in the District of Abidjan. Therefore, a total of 118 dehydrated samples of industrial and farmers formulated feeds were collected from August 2018 to March 2019. Modern breeding farms including at least 1000 chicken heads, based on the previous survey (Doumbia, 2018; Goualié et al., 2020), were selected for this study.

In each farm, three to four samples were collected according to the number of building and feeders by farmers in the farm. Approximately 200 to 300 g of samples were directly taken from feeders and put in sterile boxes. After collection, all the samples were labeled and rapidly transported to the laboratory in a cooler containing ice.

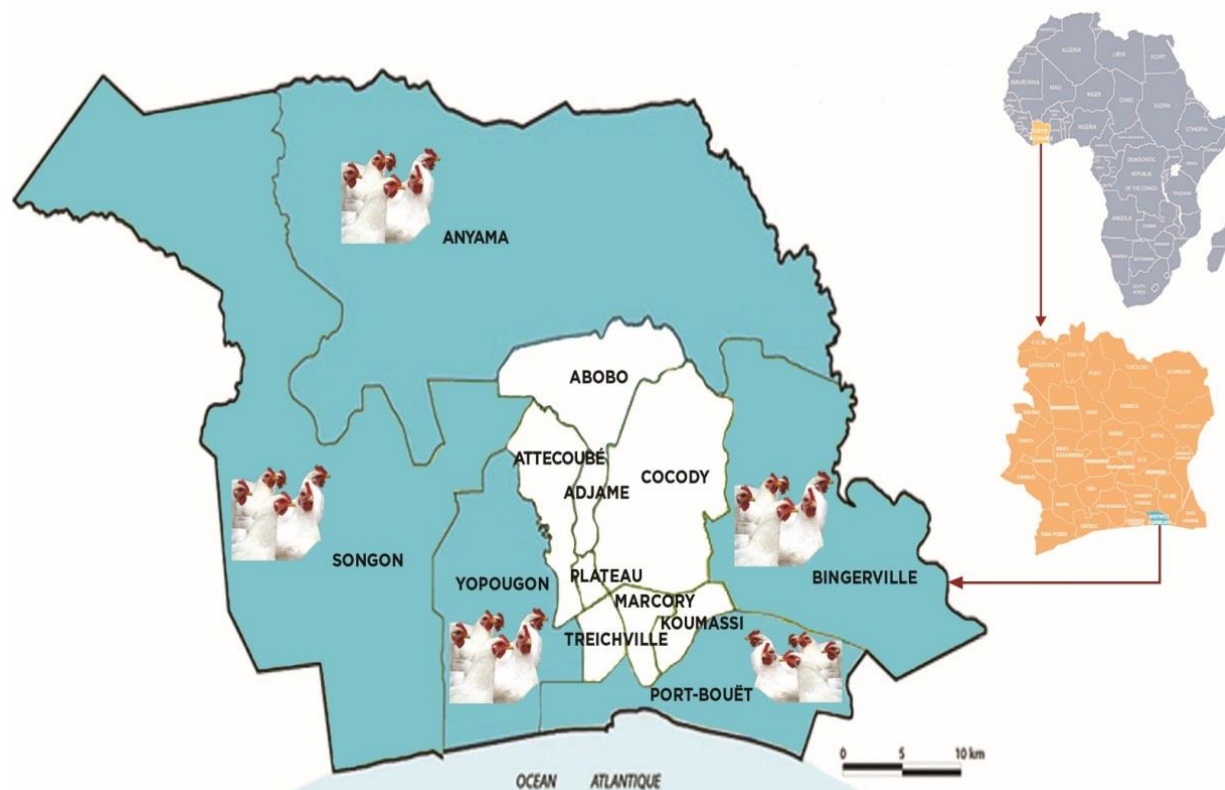
### Bacteriological analysis

Isolation of *E. coli* was performed by culture on TBX agar (Conda, Madrid, Spain) preceded by enrichment in buffered peptone water (BioRad, La Marnes-la-Coquette, France). Briefly, 10 g of foods sample were transferred to 90 ml of buffered peptone water. After manual homogenization, and overnight incubation was carried out for 24 h at 37°C and 100 µl of the enriched broth were spread on a solid surface of TBX agar. Then, all plates were incubated at 37°C for 24 h. After incubation, one typical *E. coli* colony (a blue colony on TBX agar) was selected from each plate and identified according to Buchanan and Gibbons (1974) following a series of biochemical tests included gram staining, tests for oxidase, methyl red, Voges-Proskauer reactions, indole, citrate, catalase, urea hydrolysis, gelatin hydrolysis, lactose fermentation, nitrate reduction, casein hydrolysis and sugar fermentation. All the process was conducted under sterile conditions by using Bunsen burner.

### APEC strains molecular identification

Molecular identification of APEC in *E. coli* isolates was performed by detection of two virulence genes (Ewers et al., 2005). The investigations were based on the detection of virulence-associated genes including *iucD* and *iss* respectively coding for iron-acquisition system (aerobactin) and a protein for increased serum survival. Indeed, *iss* and *iucD* genes have generally been recognized to be associated with virulence factors of *E. coli* isolated in colibacillosis cases in poultry farms (Nakazato et al., 2009; Ashraf et al., 2020).

The simplex polymerase chain reaction (PCR) was performed in final volume of 50 µl mix containing 0.6 µl of each dNTP (10 mM), 3 µl of MgCl<sub>2</sub> (25 mM), 10 µl of Buffer 5X DNA Taq polymerase, 0.2 µl of Taq polymerase (Promega, WI USA), 1.4 µl of each primer (100 µM). Amplification reactions were carried out using thermal cycler (Gene Amp PCR system type 9700, Applied Biosystems, Villebon-sur-yvette, France) with the following program: an initial denaturation at 94°C for 5 min followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 58°C for 30 s and polymerization at 72°C for 90 s. A final extension was performed at 72°C for 5 min. The amplification generated 309 bp and 714 bp DNA fragments corresponding, respectively, to *iss* and *iucD* genes. Table 1 shows primer sequences used in this study. For visualization of PCR products, 15 µl samples of the reaction mixtures were analyzed by gel electrophoresis in a 1% agarose, dissolved in 1 X TBE (8.9 M Tris, 8.9 M boric acid, 0.2 M EDTA), for 90 min at 90 V. The



**Figure 1.** Abidjan District map indicating study areas.

**Table 1.** *iss* and *iucD* primer sequences used in PCR.

Gene	Primer sequence	Amplicon size (bp)
<i>iss</i>	F 5' ATCACATAGGATTCTGCCG 3'	309
	R 5' CAGCGGAGTATAGATGCCA 3'	
<i>iucD</i>	F 5' ACAAAAAGTTCTATCGCTTCC 3'	714
	R 5' CCTGATCCAGATGATGCTC 3'	

Primer source: Ewers et al. (2005).

were stained with safe SYBR green and photographed under UV exposure.

#### Antibiotics sensitivity test

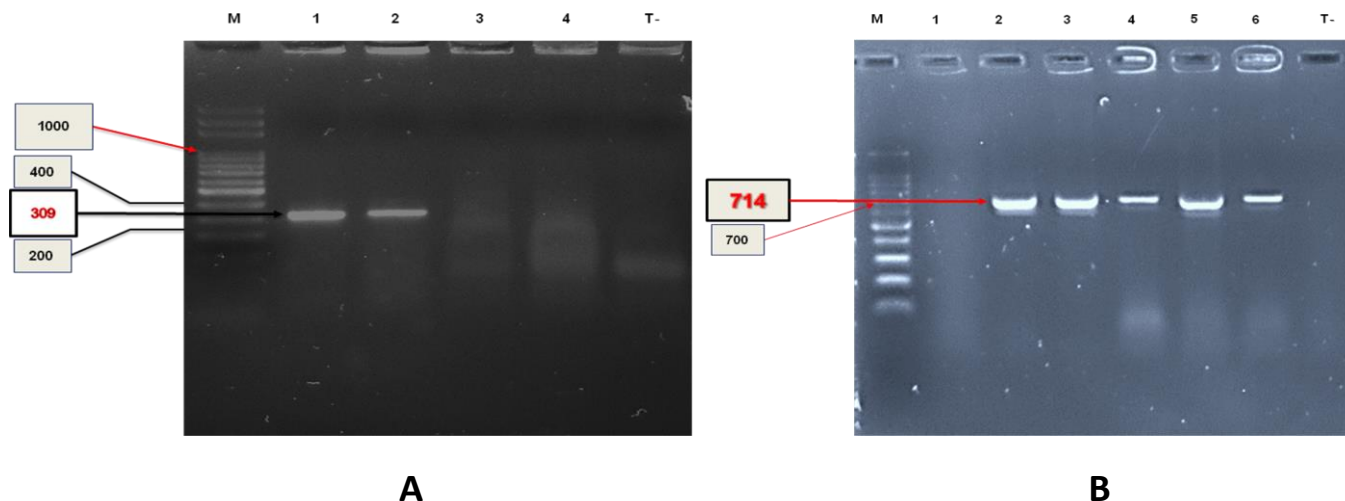
Antibiotic sensitivity was determined by the disk diffusion method on Mueller-Hinton agar medium (BioRad, France) according to the guidelines of the "Comité de l'Antibiogramme de la Société Française de Microbiologie" (CASFM, 2018). Standard paper disks containing antibiotics widely used in the poultry industry in Côte d'Ivoire including tetracyclin (30 µg), ciprofloxacin (5 µg), nalidixic acid (30 µg), combination of amoxicillin and clavulanic acid (20/10 µg), gentamicin (10 µg) were laid on the medium. All commercial antibiotic disks were purchased from BioRad (France). The plates were aerobically incubated for 24 h at 37°C. Inhibition zones were measured and analyzed according to the CASFM (2018). *E. coli*

ATCC 25922 and *Staphylococcus aureus* ATCC 25923 were used as reference strains.

## RESULTS

### Prevalence of APEC

A total of 118 chicken feeds samples were collected and analyzed for *E. coli* isolation. Among them, 44 (37.29%) were positive for APEC consisting of a total 63 strains. Moreover, most contaminated samples were collected from Anyama, Bingerville and Port-Bouët areas with prevalence of 100%, 54.04 and 30.76% respectively. Among the 63 isolated strains, 10 (15.87%) and



**Figure 2.** Electrophoretic profile obtained after amplification by PCR. (A) using *iss* virulence gene primers, (B) using *iucD* virulence gene primers. M: DNA Ladder, 1 (A) and 2 (B): positive control, T- Negative control.

**Table 2.** Antibiotic resistance of *APEC* isolates.

Antibiotics used	Resistance percentage	Number of drugs families
CIP	16	1
NAL	61.9	1
AMC	28.57	1
GMN	0	1
TET	100	1
CIP/NAL	16	1
NAL/TET	61.9	2
CIP/NAL/TET	4.76	2
NAL/AMC/TET	28	3
NAL/CIP/AMC/TET	9.52	3

CIP (Ciprofloxacin), NAL (Nalidixic acid), AMC (combination of Amoxicillin and clavulanic acid), GMN (Gentamicin), TET (Tetracyclin).

15 (23.81%) *E. coli* strains were *iss* and *iucD* genes respectively corresponding to the DNA bands sizes (Figure 2). Among these 25 isolates, seven (11.11%) strains were positive for both *iss* and *iucD*.

#### Antimicrobial susceptibility profiles of tested strains

Antimicrobial susceptibility was tested for all the 25 *E. coli* isolates which include one or two virulence genes and antimicrobial resistance profiles were shown in Table 2. All of these strains were resistant to one or more antimicrobial agents. The highest rate of antimicrobial resistance was detected with tetracyclin (100%) and nalidixic acid (61.90%). Comparatively, resistance levels to ciprofloxacin and combination of amoxicillin and

clavulanic acid were low with 16 and 28.57% respectively. Moreover, cross resistance were observed for both nalidixic acid and ciprofloxacin belonging both to fluoroquinolones family with 16% (3/25) rate. Multidrug resistance (MDR) concerning three drugs families was detected in 28% (7/25) of the tested *APEC* strains. However, all tested strains (100%) were sensitive to gentamicin.

#### DISCUSSION

The aim of this study was to isolate and identify *APEC* strains from poultry feeds for assessing their resistance patterns to selected and locally used antimicrobials. The results indicate high prevalence of *E. coli* in analyzed

samples. Our findings are in accordance to previously reported works. Indeed, other studies conducted in Algeria and in Chad indicated high prevalence of *E. coli* in poultry and swine feeds (Aimeur et al., 2014; Bodering et al., 2018). However, it is reported a low feeds contamination by ANSES (2018) in France with prevalence ranged from 0.1 to 2%. In general, a high prevalence of bacteria in feeds could be associated to poor quality of raw materials, handling of feed by producers and farmers, on-farm storage and poor practices in buildings and breeding equipment (ANSES, 2018). *E. coli* is one of the commensals microbial floras of poultry gastrointestinal tract but some serotypes are pathogenic (Jawetz et al., 1984; Levine, 1987) such as APEC associated with avian colibacillosis which is implicated in recognized economical losses in poultry production systems worldwide.

The identification of these pathogens is based on the detection of specific markers involved in their pathogenicity. Indeed, investigations have indicated that the distribution of various virulence factors are useful markers for the detection and characterization of APEC, and could, therefore, be used in the diagnosis of colibacillosis in poultry (Jansen et al., 2001).

In fact, the majority of APEC strains have been characterized by possession of *fimC*, *iucD*, *irp2*, *iss* and *tsh* virulence genes. Moreover, previous studies reported that episomal *iss*, the increased serum survival gene, was identified as significantly more associated with the APEC strains than with fecal isolates from healthy birds (Pfaff-McDonough et al., 2000; Rodriguez-Siek et al., 2005, Johnson et al., 2008a; b). Moreover, iron acquisition systems such as *iucD* have been recognized to be associated with bacterial virulence especially in those bacterial pathogens causing septicemia (Nakazato et al., 2009). In our study, seven strains were identified to harbor both of the studied virulence-associated genes. Thus, these feed could be a potential source of colibacillosis in the farms because of the key role of both *iucD* and *iss* in avian *E. coli* pathogenesis (Pfaff-McDonough et al., 2000).

Indeed, it has been reported that the presence of several virulence genes in an isolate would increase the pathogenicity of the strains as there is a real interaction between APEC virulence factors (Ashraf et al., 2020).

In general, *E. coli* are considered as indicator of faecal contamination in food and about 10 to 15% of intestinal coliforms are opportunistic and could be induced various diseases in poultry as well as in human (Barnes and Gross, 1997). Thus, presence of *E. coli* non-APEC observed in this study indicates poor conditions of poultry feeds production and existence of over health risk for the visited farms.

On the other hand, the absence of *E. coli* found in samples from Yopougon and Songon municipalities may be due to good hygiene practices in the sampled poultry farms. In this study, high percentage of antimicrobial

resistance was observed for tetracyclin and nalidixic acid in our studied isolates. Similar results were previously reported by Johnson et al. (2007) and by Akond et al. (2009). Moreover, results concerning increase of avian *E. coli* resistance to antibiotics were shown by many researchers such as Salehi and Farashi (2006) in Iran; Saidi et al. (2013) in Zimbabwe, Messaï et al. (2014) in Algeria; Garcia-graells et al. (2014) in Belgium; and Bodering et al. (2017) in Chad. These authors reported high resistance to tetracyclines ranged from 67 to 100% and to nalidixic acid with 23 to 100% resistance level.

The resistance observed to tetracycline could be due to mutations in porin structures or the decrease of their synthesis. Moreover, one or more modifications in porins could induce to drug resistance like beta-lactams, quinolones, chloramphenicol, sulfonamides, trimethoprim and tetracyclines (Fauchère and Avril, 2002).

Generally, the overuse or misuse of antibiotics is considered to be the key factor promoting the emergence, selection and dissemination of antibiotic-resistant microorganisms in both veterinary and human medicine (Neu, 1992; Witte, 1998; Ungemach et al., 2006). In Côte d'Ivoire, antibiotics such as tetracycline are intensively used in animals for therapy and control of bacterial infections (Ouattara et al., 2013) and as growth promoters. Unlike other antibiotics tested, no resistance was observed with gentamicin. This result is in agreement with those indicated by Doumbia (2018) during his study of poultry contamination risk factors by enteropathogenic microorganisms in the municipality of Bingerville. Its probably indicates that this drug is rarely used in poultry production in Côte d'Ivoire. However, the most relevant aspect of antimicrobial resistance remains multiresistance, which leads to therapeutic failure in cases of bacterial infection. In this study, multiresistance concerning fluoroquinolones, penicillins and tetracyclines families was found in 28% of APEC isolates. Occurrence of this multidrug resistance is directly related to the extensive use of several of these antimicrobial agents in poultry farming in the District of Abidjan. In addition, these results indicate a risk of therapeutic failure in the treatment of avian colibacillosis and other bacterial infections, since tetracyclines are to date one of the most used antibiotic families in the treatment of poultry illnesses. Hence, it becomes urgent to avoid the overuse and misuse of antibiotics and promote alternative methods to control and reduce bacterial related pathologies in poultry farming.

## CONCLUSION

The results of this study showed high contamination level of poultry feeds on farms in the District of Abidjan by APEC. However, *fimC*, *irp2* and *tsh* virulence genes also specific to APEC must be detected in these strains to better evaluate the sanitary risk due to these feeds. They

also showed high resistance to antimicrobials of the fluoroquinolone and tetracycline families. In short, this study highlights the need to control the microbiological quality of poultry feed, improve hygiene conditions during poultry feeds production and the urgent requirement to seek alternatives to avoid the overuse and misuse of antibiotics in the poultry sector of Côte d'Ivoire.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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