

Avian Pathogenic *Escherichia coli*

Subjects: Microbiology

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Avian pathogenic *E. coli* (APEC) are the major etiology of avian colibacillosis. Unlike other pathogenic *E. coli*, APEC causes extra-intestinal infections. Here we focus on the first time detection of APEC from layer farms in Bangladesh using molecular based approach targeting specific APEC associated virulence genes. In addition, their antibiotic resistance profile were also evaluated. Surprisingly all the isolates were found MDR in nature which is very alarming from public health point of view because of their zoonotic potentiality.

Keywords: APEC ; antibiotic resistance ; poultry ; air ; Bangladesh

1. Introduction

Avian pathogenic *Escherichia coli* (APEC) as a subgroup of *E. coli* having certain unique characteristics that allow them to produce extra-intestinal infection [1]. They are primary pathogens for many avian species of birds. APEC are characterized by presence of specific virulence genes such as *iss*, *kps*, *cvi*, *tss*, *papC*, *fimC*, *iucD*, and others [2,3]. They are the major causal agents of avian colibacillosis causes ominous effects in poultry industries globally and poses significant human health risks being multidrug resistant in nature.

Poultry farming is a well-developed and profitable agri-business in Bangladesh. It is estimated that the livestock and poultry sector contribute around 1.47% of the total GDP of the country [4]. Poultry products including eggs and meat chiefly fulfil the daily protein requirements of Bangladesh's population. However, from time to time, the advancement of poultry production is seriously hampered by various infectious diseases [5]. Among the infectious agents, avian pathogenic *Escherichia coli* (APEC) is thought to be prevalent in Bangladesh. Although *E. coli* is part of the normal bacterial microbiota of the intestinal tract, other mucosal surfaces of hosts, and the poultry farm environment, few of these strains are endowed with specific virulence factors that define the APEC phenotype [6]. Most APEC strains are phylogenetically associated with extra-intestinal location [7,8]. Principally, they are associated with respiratory tract and systemic infections [2]. In addition, APEC respiratory infections are secondary to other respiratory tract infections, including infectious bronchitis virus (avian coronavirus), Newcastle disease virus, and *Mycoplasma gallisepticum* [9].

Avian colibacillosis, caused by APEC, is a complex syndrome that has an ominous impact on the poultry sector worldwide [10]. Avian colibacillosis is characterized by multiple organ lesions such as air sacculitis, pericarditis, peritonitis, salpingitis, synovitis, osteomyelitis, yolk sac infections, etc. [11,12]. Thus, APEC is a major cause of extensive economic loss in the poultry industry due to high morbidity and mortality [7]. Both the broiler and layer farms are affected by APEC. Several of the characteristic virulence associated genes (VAGs) of APEC are *iss*, *kps*, *cvi*, *tss*, *papC*, *fimC*, *iucD*, etc. [2,3]. Virulence factors (invasins, adhesins, iron acquisition systems, toxins, and protectins) coded by multifarious VAGs facilitate the infection-causing abilities of *E. coli* strains [10]. These virulence factors may become useful for pathogenic strains of *E. coli* by enabling invasion, colonization, and adherence and protecting *E. coli* from host defenses [13-15]. Among them, *papC* (pyelonefritis associated to pili C) is associated with the production of adhesion-related factors to enable the adhesion of *E. coli* and is responsible for occurring infections [10,16], *fimC* (Type 1 fimbriae C) is connected with the adhesion and colonization of *E. coli* on epithelial cells [17], and *iucD* (iron-uptake systems of *E. coli* D) demonstrates the difference between APEC and non-APEC isolates in terms of the iron acquisition system [18]. The virulence of any particular isolate of *E. coli* correlates with the number and combination of these virulence-associated genes [19]. These virulence factors may be found as single genes or as associations of different gene combinations in both healthy and clinical isolates [20].

Along with APEC, uropathogenic *E. coli* (UPEC) and neonatal meningitis *E. coli* (NMEC) are also considered as extra-intestinal pathogenic *E. coli* (ExPEC). In humans, ExPEC may cause urinary tract infections (UTIs), neonatal meningitis, and septicemia [21,22]. Phylogenic similarities among APEC, UPEC, and NMEC strains—transmissible plasmids, virulence genes, and other genetic characteristics—indicate that APEC are zoonotic in nature [22,23]. In addition, APEC

are present in the intestines and meat of healthy poultry, possessing genetic similarities with human ExPEC, indicating a possible transmission of animal APEC to humans [23]. Some experimental studies have expounded the transmission pattern between avian ExPEC and human ExPEC with a negative impact on public health [24].

Antimicrobial resistance (AMR) is serious global public health challenge of the 21st century. The G20 partners have recognized AMR as a major “growing threat to public health and economic growth”. It causes an estimated 700,000 deaths each year across the world [25]. Drug-resistant APEC strains can contaminate the food supply from farm to fork through eggs, meat, and other contaminants and thus pose a severe threat to the consumer's health [26]. The indiscriminate use of antibiotics in poultry production may have contributed to drug resistance in APEC. From the poultry farm, drug-resistant strains are deposited into soil, wastewater, air, and the environment [27].

2. Model

Total 99 samples comprising internal organs (trachea, intestine, liver, lung and egg yolk material; n=36), feces (n=32) and air (n=31) were collected from 32-layer farms. Isolation was performed by culturing samples on eosin-methylene blue agar plates followed by morphological study by Gram staining, basic sugar fermentation test, methyl red test, Voges-Proskauer test, and indole test. The molecular detection of APEC was done by PCR and the antibiogram study was done by disk diffusion method.

3. Data

3.1. Prevalence of *E. coli* isolates

Among 99 samples, 82 (82.83%) were positive for *E. coli* by PCR targeting *E. coli* 16S rRNA gene. The highest prevalence was found in feces (100%) and lowest in air samples (67.74%). Statistical analysis revealed that feces carried significantly higher percentage of *E. coli* among samples investigated (Chi-square test, 95% CI, p=0.003).

3.2. APEC-associated virulence genes

Among the 82 *E. coli*, 36 (36.36%) were found to be positive for APEC-associated virulence genes. Significantly higher prevalence of APEC associated virulence genes were in internal organs compared to air (16.13%) and feces (21.87%) (Chi-square test, 95% CI, p= 0.001).

Among the 36 *E. coli* isolates carrying APEC-associated virulence genes, 7 were positive for three virulence genes (*fimC*, *iucD*, and *papC*); 18 were positive for two virulence genes (in different combinations), 11 were carrying single virulence gene. The most prevalent combination was *fimC-iucD* (in 11 isolates), however, *fimC-papC* was in three isolates. Statistical analysis indicated that *fimC* (97.22%) was significantly more prevalent than *iucD* (58.33%) and *papC* (33.33%) in 36 *E. coli* (Chi-square test, 95% CI, p=0.003).

3.3. Antibiogram profile of *E. coli* isolates carrying APEC-associated virulence genes

Antibiogram study showed that all 36 *E. coli* isolates carrying APEC-associated virulence genes were resistant to ampicillin and tetracycline (100%), followed by to chloramphenicol and erythromycin (97.2%), to enrofloxacin (55.5%), to norfloxacin and ciprofloxacin (50.0%), to streptomycin (19.4%), to colistin (11.1%), to gentamicin (8.3%) (Figure 1). All APEC isolates were MDR in nature.

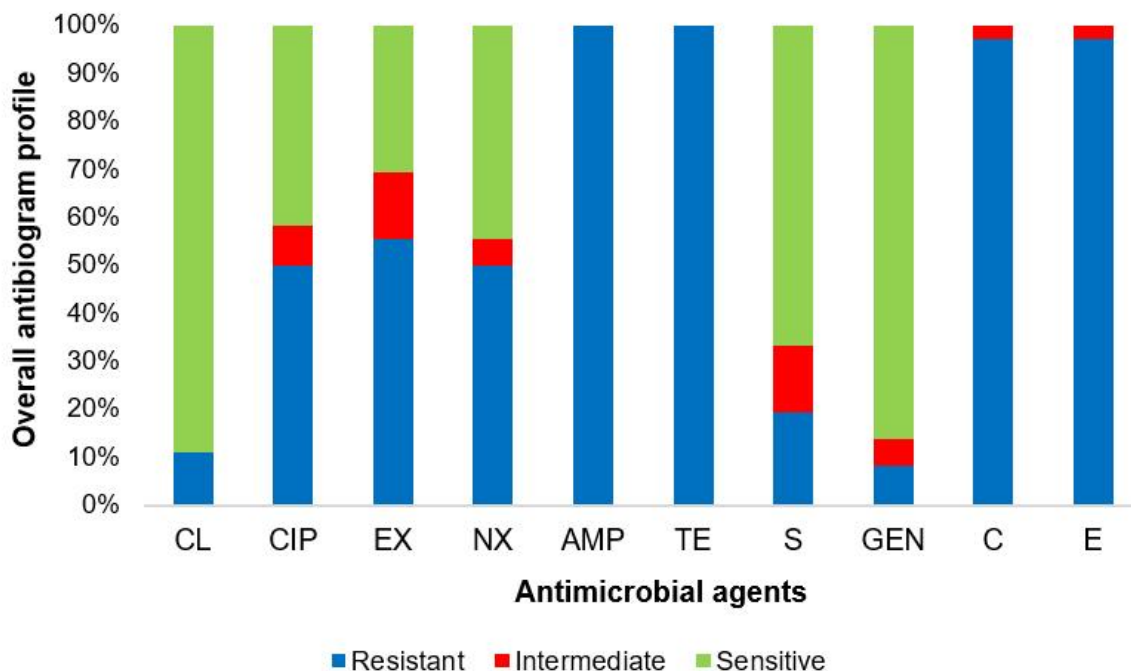


Figure 1. Overall antibiogram profile of the isolated APEC. CL, Colistin; CIP, Ciprofloxacin; EX, Enrofloxacin; NX, Norfloxacin; AMP, Ampicillin; TE, Tetracycline; S, Streptomycin; GEN, Gentamicin; C, Chloramphenicol; E, Erythromycin

A total of 16 antibiotic resistance patterns were observed among the APEC isolates. Among antibiotypes, resistance pattern 1 (AMP, TE, C, E) was the most prevalent (30.5%) followed by pattern 9 (CIP, EX, NX, AMP, TE, C, E) in 16.7% isolates and pattern 12 (CIP, EX, NX, AMP, TE, S, C, E) in 11.1% isolates. An isolate (Io-Lu2) showed resistance to nine antibiotics (7 classes of antimicrobials) of ten tested.

3.4. Pairwise correlation between resistance antimicrobials

Statistical analysis revealed that norfloxacin and ciprofloxacin resistance showed a strongest significant correlation (Spearman's rank correlation coefficient, $\rho = 0.8315$ with $p\text{-value} = 0.0000$); followed by significant correlation between enrofloxacin and ciprofloxacin resistance ($\rho = 0.6625$ with $p\text{-value} = 0.0000$), and norfloxacin and enrofloxacin resistance ($\rho = 0.6203$ with $p\text{-value} = 0.0001$).

4. Discussion

APEC associated avian colibacillosis has significant impact on poultry industry across the globe. APEC are also important from the public health point of view [24]. Previous studies have indicated avian colibacillosis as a prominent disease of commercial chicken [18, 22]. Globally, poultry industries are being confronted by enormous economic losses due to the dramatic impact of the disease [28]. To control colibacillosis, multiple antimicrobials have been used indiscriminately especially in middle- and low-income countries including Bangladesh contributing to the development and spread of AMR. The subsequent selection of MDR strains generated serious challenges in terms of public health. Sustainable development goals (SDGs) are affected by AMR especially in targeting hunger, poverty, malnutrition, health and economic growth [25]. Thus, investigations of APEC strains with regard to virulence genes and AMR profiles may help to curtail their hazardous effects. Therefore, present study was designed to determine the prevalence of APEC and the associated virulence genes in *E. coli* in layer farms in Bangladesh, as well as their antibiotic resistance profile.

The evidences exhibited from our study denote that APEC strains harbored by commercial chickens and its environment can be transmitted from bird to bird and farm to farm through a variety of ways, including air and feces. Moreover, APEC strains are zoonotic in nature and their presence in air and feces within a farm where human are associated, is a one health issue; hence, poses great risk for human health. In addition, the evidences of antimicrobial resistant APEC exposed severe threat in the treatment, treatment cost, ultimately economic losses for poultry sector and health burden in context of humans. However, the most alarming finding from our study is that all APEC isolates were MDR in class. The indiscriminate use of antimicrobials is linked to high prevalence of MDR strains in *E. coli* and in other enterobacteria [29].

5. Conclusion

In addition to internal organs, fecal samples and air samples were also found to carry APEC. Interestingly all the APEC isolates were MDR in nature. Our current findings demonstrate that layer birds are potential reservoirs of antibiotic resistant APEC posing high public health risks to people who are exposed with them directly or indirectly. However, considering the zoonotic significance, we propose routine screening of APEC targeting their virulence genes for the early detection of avian colibacillosis, hence to protect human health. Furthermore, prevention of antimicrobial misuse, application of effective biosecurity measures, adaptation of the one health approach are exigent measures for reducing AMR related hazards.

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