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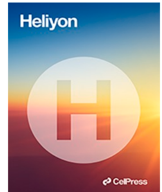

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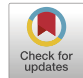

Molecular detection of avian pathogenic *Escherichia coli* (APEC) in broiler meat from retail meat shop

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ABSTRACT

Avian pathogenic *Escherichia coli* (APEC) is a major bacterial pathogen responsible for the most widespread form of colibacillosis, resulting in substantial economic losses within the poultry sector and posing a potential public health risk. From July to September 2021, our study investigated the antibiotic resistance pattern of *Escherichia coli* (*E. coli*) and the presence of virulence-associated genes (*iucD*, *iutA*, *iss*, and *ompT*) linked to APEC using 105 broiler meat samples comprising liver, thigh, and breast muscle, in Chitwan, Nepal. *E. coli* was isolated and identified by culturing samples on MacConkey's agar, Eosin-methylene blue (EMB) agar and performing different biochemical tests. Antibiotic resistance patterns of *E. coli* were determined by the Kirby-Bauer disc diffusion method. Following the isolation of *E. coli*, the molecular detection of APEC was performed using conventional polymerase chain reaction (PCR). Out of the 105 samples analyzed, 61 (58.1 %) tested positive for *E. coli*. In antibiotic susceptibility test (AST), gentamicin and tetracycline exhibited the highest resistance rates, with 90.2 % and 67.2 %, respectively and 29.5 % of the *E. coli* isolates displayed multidrug-drug resistance. Out of 61 confirmed *E. coli* isolates, *iutA* was detected in 47 (77.0 %) samples, *iucD* in 46 (75.4 %), *iss* in 53 (86.8 %), and *ompT* in 39 (63.9 %) samples. This study reports the occurrence of MDR *E. coli* in meat samples, together with virulence genes associated with APEC which poses a public health threat. Continuous surveillance is vital for monitoring APEC transmission within poultry farms, coupled with efforts to raise awareness of food safety among consumers of broiler meat.

1. Introduction

Escherichia coli (*E. coli*), a gram-negative and non-spore forming bacterium, is widely distributed and typically prevails as the predominant species in the gut microbiota of humans, animals, and birds [1]. In addition to the commensal strains, there exists a diverse range of pathogenic *E. coli* strains which can infect the intestines or other parts of the body. Among these strains, avian pathogenic *E. coli* (APEC) specifically causes extraintestinal infections in poultry [2]. Colibacillosis, a condition characterized by both localized and systemic infections, can be induced by APEC in various avian species, including commercial chickens, ducks, turkeys, and

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other poultry species [3,4]. This syndromic disease is characterized by the presence of fibrinous lesions around the visceral organs [5]. Colibacillosis can present in various forms, ranging from acute septicemic cases to subacute ones, including pericarditis, air-sacculitis, arthritis, perihepatitis, peritonitis, and salpingitis progressing to septicemia, and death [5,6]. Scientific evidence suggests that colibacillosis can result in substantial mortality, with rates as high as 20 %. It also induces morbidity, leading to a decrease in meat production with an approximate live weight loss of 2 % and a decline of 2.7 % in feed conversion ratio [2]. Poultry affected by colibacillosis may experience up to a 20 % reduction in egg production, lower hatch rates, and an elevated incidence of carcass condemnation at slaughter, reaching up to 43 % [7–9].

APEC strains mostly have several virulence factors and serotype due to which they are very diverse in nature. These components include lipopolysaccharide and capsule complexes, as well as adhesins, toxins, colicin plasmid, iron acquisition systems, F hemolysin, serum resistance proteins, and temperature-sensitive hemagglutinin [2,10]. An aerobactin siderophore receptor gene, *iutA*, contribute to iron uptake [11]. Research findings indicate that the prevalence of aerobactin iron-uptake genes in APEC isolates may exceed 80 % [8,12]. It specifies a protein that is part of the outer membrane and is involved in the high-affinity binding of Fe3-aerobactin [13]. In certain APEC strains, this gene can be located on plasmids or encoded on the chromosome [14]. The aerobactin system in APEC plays a crucial role in both the persistence of the bacteria and the development of lesions in infected chickens. The expression of aerobactin is a major factor in the pathogenesis of APEC. Key genes involved in aerobactin synthesis, such as *iucA*, *iucB*, *iucC*, and *iucD*, contribute to its production. Additionally, the gene *iutA* is responsible for the expression of a specific outer membrane receptor protein that enables the uptake of ferric aerobactin [15]. Genes *iutA* and *iucD* are controlled by iron availability via the Fur protein, with *iucD* also influenced by H-NS (Histone-like Nucleoid Structuring Protein) and Fis (Factor for Inversion Stimulation). Gene *iss* is upregulated at body temperature and oxidative stress, regulated by H-NS and IHF (Integration Host Factor), while *ompT* responds to osmotic stress and quorum sensing, modulated by temperature and pH [2,16,17].

Increasing evidence suggests that extraintestinal pathogenic *E. coli* (ExPEC) strains, capable of causing infections in both animals and humans, exhibit significant genetic similarities and share similar mechanisms of pathogenesis [18]. Whole-genome sequencing has shown that human ExPEC strains share genetic characteristics with avian isolates. Some *E. coli* strains can acquire new genes through horizontal gene transfer, which can make them more virulent and capable of causing a wider range of diseases in both animals and humans [18,19]. APEC has the potential to act as a reservoir for virulence genes and antibiotic-resistant genes that can be transferred to human ExPEC strains. This implies a potential route of zoonotic transmission, where APEC from poultry could transfer genetic elements to ExPEC strains in humans [20].

Numerous studies have reported that APEC strains globally exhibit antimicrobial resistance [21,22]. The global rise in antimicrobial-resistant *E. coli* infections is a major concern due to its impact on the effective treatment of infections [23]. This situation leads to substantial morbidity, mortality, increased medical expenses, and production losses within the poultry industry [2]. In an effort to address the issue of ExPEC infections, antibiotics are commonly used with the assumption that they can inhibit bacterial growth and treat microbial infections [24]. Studies have provided evidence of antibiotic usage in chicken broilers, primarily for growth promotion and disease control. However, the widespread application of antimicrobials in food-producing animals has led to various negative consequences. These include disruptions to the intestinal microbiota, the presence of antibiotic residues in food products, environmental repercussions, and the emergence of antimicrobial resistance among microorganisms [25,26]. The coexistence of antimicrobial resistance genes and virulence genes, frequently found on plasmids, enhances the potential for horizontal gene transfer between bacteria [27]. This emphasizes the necessity for continuous monitoring and intervention. The presence of drug-resistant microbes poses significant challenges in treating zoonotic diseases and increases the burden of chronic illnesses. Transmission to humans can occur through direct contact with animals, exposure to animal manure, consumption of undercooked meat, and contact with meat surfaces, posing a serious threat to public health [28]. The APEC isolates, especially those classified under ST95 and ST131 or serogroups O1, O2, and O18, share genetic similarities and common virulence genes with human uropathogenic *E. coli* (UPEC) and neonatal meningitis *E. coli* (NMEC). These isolates possess the ability to cause urinary tract infections and meningitis in humans [2].

Colibacillosis is a widespread and endemic disease affecting commercial chickens in Nepal. Research indicates that the prevalence of colibacillosis among commercial chickens varies from 10 % to 60 % in different regions of the country [22]. Several factors contribute to the high risk of colibacillosis in commercial chickens in Nepal, including open housing with deep litter management, inadequate managemental practices, compromised water quality, and insufficient biosecurity measures [5]. Like many other developing nations, Nepal encounters challenges associated with the lack of advanced technology for hygienic raw food processing. Moreover, there is a notable absence of adequate surveillance systems for monitoring antimicrobial drug resistance. The spread of drug-resistant bacterial strains is made worse by the unchecked and unrestrained use of antibiotics in food animals for disease prevention and treatment [29]. Given that these resistance strains may be spread to people through the consumption of contaminated meat, this scenario represents a serious threat to the general public's health. The uncontrolled utilization of antimicrobials for preventing and/or treating diseases in food animals heightens the likelihood of the emergence and spread of antimicrobial-resistant bacterial strains [30,31]. The combination of the *iss, iucD, ompT, hlyF, iroN*, and *iutA* genes was shown to be the most often related with APEC in a prior study conducted in Nepal [22]. However, there have been limited studies conducted in Nepal regarding the molecular characterization of APEC, with the existing works primarily focusing on prevalence studies. Thus, this study was conducted in Chitwan, Nepal aimed to perform bacteriological and antimicrobial resistance patterns exhibited by *E. coli* and molecular characterization of APEC strains isolated from broiler chicken meat.

2. Materials and methods

2.1. Sample collection

A cross-sectional study was conducted in the Bharatpur metropolitan city of the Chitwan district, located in the Bagmati province of Nepal, from July 2021 to September 2021. A total of 105 broiler meat samples were collected using the snowball sampling method from various locations within the city. The 25 g of broiler meat samples were aseptically placed in autoclavable plastic bags and were transported to the Center for Biotechnology at the University of Agriculture and Forestry (AFU) for laboratory analysis. The broiler meat samples were obtained from the liver, breast, and thigh. The plastic bags containing the samples were stored in an icebox at a temperature of 4 °C until they were transported to the laboratory.

2.2. Bacterial isolation and identification

Each plastic bag with meat was placed on the mortar and then uniformly pressed all over with the pestle. After grinding the meat aseptically transferred the sample into a sterile 250 mL autoclavable plastic container containing 225 mL of buffered peptone water (M614, HiMedia, India). This container was then incubated at 37 °C for 24 h. To isolate *E. coli*, an inoculum from incubated peptone water was streaked onto MacConkey agar (M081, HiMedia, India) and incubated aerobically at 37 °C for 24 h. *E. coli* colonies appeared as convex-shaped, dark pink colonies on the agar plates. From the primary culture on MacConkey agar, a single pink colony was selected and sub-cultured onto an Eosin-methylene blue (EMB) (M317, HiMedia, India) plate, which was incubated aerobically for 24 h. The colonies grown on the EMB agar exhibited precipitating colony with a distinct metallic green sheen. Typical colonies (greenish metallic sheen) were identified as presumptive *E. coli*. These colonies were selected and subcultured on nutrient agar (HiMedia, India). Presumptive isolates were confirmed by using various biochemical tests, including the indole test, methyl red test, voges-proskauer test, simon's citrate test, triple sugar iron (TSI) agar test, as well as the catalase and oxidase tests (Table 1). *E. coli* isolates were stored at −80 °C in brain heart infusion (BHI) broth (Oxoid Ltd. UK) containing 25 % glycerol until further used.

2.3. Antimicrobial susceptibility testing (AST)

The antimicrobial susceptibility test was conducted using the Kirby-Bauer disk diffusion method, as initially described by Bauer et al. (1966). Amikacin, gentamicin, ciprofloxacin, tetracyclines, and ampicillin (HiMedia, India) were used for the AST test (Table 2). These antibiotics were selected because these are commonly used antibiotics in poultry sector of Nepal [22] and are readily available throughout the country [29,32,33]. The *E. coli* ATCC 25922 strain was used as a negative control to ensure the correct performance of the test. The zone of complete inhibition was measured in millimeter using Antibiotic Zone Scale (HiMedia, India). Following the guidelines provided by the Clinical and Laboratory Standards Institute classified as resistant (R), susceptible intermediate (I), and susceptible (S) uses the resistance breakpoints listed (Table 2) [34].

2.4. DNA extraction

The rapid boiling method was employed for DNA extraction. In this method, 1 mL of luria bertani broth (M124, HiMedia, India) was added to an eppendorf tube. Approximately 4–5 colonies of each isolate were picked and dissolved in the eppendorf tube, followed by incubation at 37 °C. The overnight bacterial culture was then cooled at 4 °C for 10 min and centrifuged at 13,000 rpm for 5 min. The supernatant was carefully discarded, and the pellet was resuspended in 500 μl of nuclease-free water (AM932, Ambion, USA). Subsequently, the sample was boiled for 15 min in a water bath at 100 °C and immediately cooled at −20 °C (freezer) for 10 min. After centrifugation at 13,000 rpm for 5 min, the supernatant containing genomic DNA was transferred to a new tube and used for subsequent PCR amplification.

Table 1
Biochemical characterization of *E. coli*.

Test or substrate	Result		*E. coli* reaction
	Positive	Negative	
Triple sugar iron (TSI) agar test	Yellow slant, yellow butt, gas positive	Red butt	Yellow slant, yellow butt, gas positive
H_2S (on TSI)	Blackening	No blackening	Negative
Indole test	Red color at surface	Yellow color at surface	Positive
Methyl Red test	Diffuse red color	Diffuse yellow color	Positive
Voges-Proskauer test	Pink to red color	No color change	Negative
Catalase test	Presence of bubbles	No bubbles	Positive
Oxidase test	Dark purple color	No color change	Negative
Simmon's citrate test	Growth; blue color	No growth; No color	Negative

Table 2
Antibiotics used for in antibiotic susceptibility test of *E. coli* and their resistance breakpoints in broiler meat from retail meat shop of Chitwan, Nepal.

Antimicrobial Classes	Disc potency (mcg)	Diameter of Zone of inhibition (mm)		
		Resistant	Intermediate	Susceptible
Aminoglycosides				
Amikacin (AK)	30	≤15	15–16	≥17
Gentamicin (GEN)	10	≤12	13–14	≥16
Fluroquinolones				
Ciprofloxacin (CIP)	5	≤21	22–25	≥26
Tetracycline				
Tetracycline (T)	30	≤11	12–14	≥15
Penicillin				
Ampicillin (AMP)	10	≤13	14–16	≥17

2.5. Quantification of DNA

The DNA extracted from the *E. coli* culture was measured using a UV–VIS Spectrophotometer (Q5000, Quawell, USA) at wavelengths of 260 nm and 280 nm for the determination of the concentration and purity of the DNA sample. To quantify the DNA, a 1 μl aliquot of the diluted DNA sample was loaded into the UV–VISIS Spectrophotometer. The Q5000 V6.0.2 software integrated with the spectrophotometer facilitated the calculation of DNA concentration and purity, were displayed on the computer monitor, enabling analysis and interpretation.

2.6. Polymerase chain reaction (PCR)

The genome of targeted primers were amplified using the PCR thermal cycler (T100TM, Bio-Rad, USA). The thermal cycling conditions consisted of an initial pre-denaturation step at 94 °C for 4 min, followed by denaturation at 94 °C for 30 s. The PCR protocol included 35 cycles, with annealing temperatures at 60 °C for 1 min and extension temperatures at 68 °C for 2 min. Following the cycling, a final elongation step was carried out at 72 °C for 7 min. The resulting PCR product was stored at 4 °C. For the PCR reaction mixture, 7 μL of nuclease-free water, 10 μL of mastermix (13001014, Invitrogen, USA), 10 pmoles of forward and reverse primer (Sigma-Aldrich, Germany), 200 ng of DNA template with the total reaction volume of 20 μL (Table 3). All components were thoroughly mixed in PCR tubes, with the DNA template added last. The PCR tubes were then placed in the thermocycler and preheated at 94 °C. Once the thermocycler reached 105 °C, the PCR program was initiated. Conventional PCR was performed to amplify four virulence-associated *E. coli* genes, *iutA*, *iucD*, *iss* and *ompT* [22].

2.7. Agarose gel electrophoresis

The gel (1.5 %) was prepared by boiling 0.6 g of agarose (CSL-AG100, Cleaver Scientific, USA) powder in 40 mL of 0.5× TBE (Tris-Borate-EDTA, 10 ×) buffer (T4415, Sigma, USA) in a microwave until the agarose was fully dissolved. The gel cooled to around 60 °C, it was carefully poured into a dual comb caster and allowed to sit undisturbed for 1 h. Following this, the gel was transferred into an electrophoresis tank (Maxicell EC360M, Electrophoretic Gel System, Florida, USA), and the PCR products were loaded onto the gel. Prior to loading, a tracking dye was added to the PCR products. For each row of wells on the gel plate, 10 μL of PCR products mixed with 2 μL of loading dye called bromophenol blue were loaded to aid in visualizing their movement on the agarose gel. Additionally, 1 μL of a DNA molecular weight marker (100 bp DNA Ladder, New England Biolabs) was loaded to allow for comparison of the molecular weights of the PCR amplicons. The samples were electrophoresed simultaneously by applying a voltage of 89 V to the gel for 50 min. This voltage caused the negatively charged DNA molecules to migrate towards the positively charged electrode. After electrophoresis, the gel was placed in a shaker (Lab-Line Instruments Model 3518 L.E.D. Orbit Shaker, Philippines) and agitated at 100 rpm for 25 min while submerged in distilled water. To stain the DNA, 15 μL of ethidium bromide was added, allowing for visualization of the DNA as a single, compact band of the expected size under ultraviolet (UV) light. After staining, the gel was shaken for an additional 10 min at

Table 3
Primer sets for detection of target virulence genes of avian pathogenic *E. coli* (APEC) isolated from broiler meat from retail meat shop of Chitwan, Nepal.

Virulent genes	Primer sequence (5′—3′)	Size (bp)
*iutA*_F	GGCTGGACATCATGGGAACTGG	302
*iutA*_R	CGTCGGGAACGGGTAGAATCG	
*iucD*_F	ACAAAAAGTTCTATCGCTTCC	714
*iucD*_R	CCTGTCCAGTGATGATGCTC	
*iss*_F	CAGCAACCCGAACCACTTGATG	323
*iss*_R	AGCATTGCCAGAGCGGCAGAA	
*ompT*_F	TCATCCCGGAAGCCTCCCTCACTACTAT	496
*ompT*_R	TAGCGTTTGCTGCACTGGCTTCTGATAC	

100 rpm in distilled water to remove excess ethidium bromide. The integrity of the DNA was assessed by visualizing it under UV light using a gel documentation system (Clear View UV Transilluminator, Cleaver Scientific Ltd., Country) and photographed using a Canon PC 1815 7.4 V camera.

2.8. Data analysis

Data entry and analysis were carried out using Microsoft Excel 2019. To calculate the prevalence rate for each category, the number of positive tests was divided by the total number of samples examined. Multi-drug resistance (MDR) was described as the resistance to more than two antibiotic classes among all the antibiotics examined. Multiple antibiotic resistance (MAR) index was determined and analyzed by employing the formula: a divided by b, where 'a' stands for the number of antibiotics to which an isolate displayed resistance, and 'b' represents the total number of antibiotics used in AST. The cutoff values provided in the manufacturer's brochure (HiMedia, India) were utilized, following the guidelines set by the Clinical and Laboratory Standards Institute [34], to determine the patterns of antimicrobial resistance (AMR), including resistance, intermediate, and sensitivity.

3. Results

The microbiological and biochemical analysis of the 105 fresh retail broiler meat samples reviled 61 (58.1 %) samples tested positive for *E. coli*. Gentamicin resistance was found to be 90.2 %, followed by tetracycline (67.2 %), ampicillin (54.1 %), ciprofloxacin (6.6 %), and amikacin (1.6 %) (Fig. 1).

In our study, 29.5 % (18/61) *E. coli* isolates were MDR (resistant to at least 3 classes of antibiotics)(Table 4). Out of 61 *E. coli* isolates, 21.3 % were found to be resistant to at least three different antibiotics. The study found that 4.5 % (4 out of 61) of the isolates were resistant to at least four classes of the five antibiotics classes tested. Only one of the 61 isolates (1.6 % of the total) tested resistant to all type of antibiotic classes tested. Among the isolates, 50.8 % (31/61) had a MAR index of 0.4, with 21.3 % and 6.5 % demonstrating MAR indices of 0.6 and 0.8, respectively (Fig. 2).

Conventional PCR and gel electrophoresis were used to amplify and visualize the presence of virulence-associated genes *iutA*, *iucD*, *iss* and *ompT* in the *E. coli* isolates. From a total of 61 confirmed avian pathogenic *E. coli* isolates, *iutA* was found in 47 (77.0 %), *iucD* was found in 46 (75.4 %), *iss* was found in 53 (86.8 %) and *ompT* was found in 39 (63.9 %) samples.

4. Discussion

E. coli can be found in the environment around poultry as well as in the typical intestinal microflora. Nevertheless, the strains have the ability to behave as APEC that have particular virulence characteristics and the capacity to induce avian colibacillosis, which can cause significant financial loss.

Our study revealed a higher occurrence of *E. coli* compared to previous research conducted in Nepal [35,36]. Similarly, a study conducted in Iceland show a high percentage of various meats were contaminated with *E. coli* ranging from 73 % to 100 % [37]. The increased occurrence of *E. coli* in our study may be attributed to various factors, including prolonged exposure to contaminated water, inadequate sanitation practices, high levels of post-slaughter meat contamination, storage at room temperature, and poultry respiratory disorders leading to infections [38]. Cross-contamination is a significant risk during the processes of defeathering and freezing, while contamination resulting from human error is more likely to occur during evisceration, carcass cleaning, and processing [39,40]. A study by Matias et al. (2010) highlights the impact of control measures implemented by slaughterhouses on the microbiological

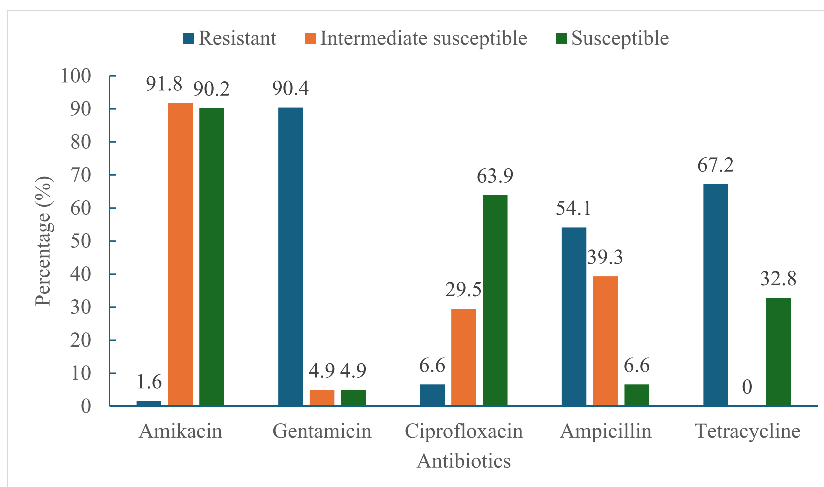

Figure 1. Antibiotic resistance pattern of *E. coli* isolates in broiler meat from retail meat shop of Chitwan, Nepal.

Table 4
Multiple antibiotic resistance (MAR) index and multi drug resistance (MDR) pattern of *E. coli* isolates in broiler meat from retail meat shop of Chitwan, Nepal.

Number of antibiotics used	Resistance pattern	MAR index	Number of isolates	Percentage (%)
1	GEN	0.2	12	19.6
2	GEN + TET	0.4	23	50.8
	GEN + CIP		1	
	GEN + CIP		7	
3	GEN + AMP + TET	0.6	9	21.3
	GEN + CIP + TET		4	
4	GEN + CIP + AMP + TET	0.8	4	6.5
5	GEN + AK + CIP + AMP + TET	1	1	1.6

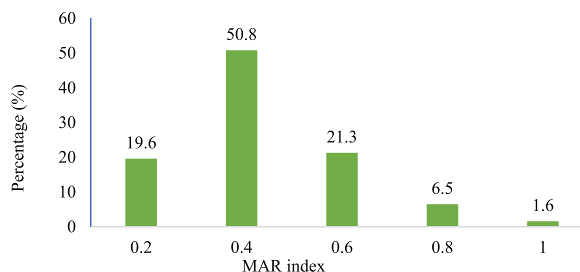

Fig. 2. Multiple antibiotic resistance (MAR) index of *E. coli* isolates in broiler meat from retail meat shop of Chitwan, Nepal.

quality of chicken carcasses, as evidenced by the variations in contamination levels throughout the processing stages [41].

Antimicrobials have been extensively employed in the poultry industry to combat bacterial infections associated with various diseases. However, the excessive and routine use of high doses of antimicrobials in poultry, particularly in countries like Nepal, often occurs without appropriate consultation or oversight from veterinary professionals. This widespread antimicrobial use in livestock agriculture has been correlated with the emergence of antimicrobial resistance [42]. The chicken industry's industrialization has led to a substantial increase in antibiotic usage for disease control and growth promotion [22]. In Nepal, the total utilization of antimicrobial medications in food animals witnessed a significant 53.6 % rise between 2008 and 2012, with an average annual growth rate of 11.4 % [29]. Furthermore, projections indicate a projected 129 % increase in the use of antimicrobials in poultry within the Asia-Pacific region by 2030, and a 67 % higher rate of antimicrobial usage in global food animal production by the same year [26].

Consistent with the findings of Zhao et al. (2005) [43] and Mohamed et al. (2014) [44], our study revealed that 29.5 % *E. coli* isolates from broiler meat exhibited resistance to multiple antibiotics ($\geq$3). None of the antibiotic classes investigated were effective against the pathogenic *E. coli* isolates. In our study, gentamicin resistance was found in 90.2 % *E. coli* isolates, followed by tetracycline (67.2 %), ampicillin (54.1 %) which is comparable to the findings of Mohamed et al. (2014), where 100 % of *E. coli* isolates were resistant to gentamicin [44]. This contrasts with previous reports from Bangladesh by Hasan et al. (2011) and Hassan (2014), which indicated a lower rate of resistance to gentamicin [45,46]. These findings emphasize the importance of conducting thorough laboratory analysis prior to the use of gentamicin in order to prevent the development of resistance due to its misuse and overuse. Regarding amikacin, our study found a resistance rate of 1.6 %, consistent with the findings of a previous study by Radwan et al. (2014) that reported a 2.4 % resistance rate in APEC [47]. However, our findings differ from a study by Subedi et al. (2018), which reported a resistance rate of 16 % to amikacin [22]. Ahmed et al. (2013) also found a higher rate of resistance to this aminoglycoside [48]. For ciprofloxacin, our study found a resistance rate of 6.6 %, consistent with the findings of Talebiyan et al. (2014) [49]. However, other studies by El-Sayed et al. (2015) and Sharada et al. (2008) reported resistance rates of 20 % and 16 % to ciprofloxacin, respectively, which were higher than the rate observed in our study [50,51].

The antibiotic resistance patterns observed in this study indicate a significant problem with antibiotic-resistant *E. coli* bacteria among broiler chickens in the Chitwan district. One isolate displayed resistance to all tested antimicrobials, and 29.5 % of the isolates exhibited multi-resistance to three or more antibiotic classes, with the most common profile being resistance to gentamicin and tetracycline. The misuse of antimicrobials is a potential concern in Nepal, where these medications are easily accessible without prescriptions from veterinarians. In overall 100 % of the isolates had a multiple antibiotic resistance (MAR) index greater than 0.2, indicating a substantial risk of contamination and the extensive use of antibiotics for disease management [52]. This provides solid evidence that widespread use of various antibiotics in poultry feed and for disease control is commonplace in the Chitwan districts and other parts of Nepal. The worrying rise in the prevalence of poultry diseases multidrug resistance limits available treatment options [53]. This type of antibiotic resistance is replacing drug-sensitive bacteria in antibiotic-rich environments [54]. To address antibiotic abuse, it is crucial to adopt a multi-faceted approach in the poultry farm that includes the use of existing substitutes such as probiotics, prebiotics, phage therapy, antimicrobial peptides, and essential oils. Continued research and development in these areas will enhance their efficacy and provide sustainable alternatives to antibiotics, reducing the reliance on traditional antibiotics and mitigating the risk of antibiotic resistance. Additionally, awareness and education about antimicrobial resistance (AMR) and its consequences among

poultry farmers, veterinary technicians, and the general public play a critical role in reducing antibiotic misuse and promoting sustainable practices [55,56].

Pathogenicity of *E. coli* is linked to a diverse set of virulence genes. Conventional PCR analysis was used in this investigation to confirm the existence of four virulence genes, namely genes *iutA*, *iucD*, *iss* and *ompT*. The *iutA* gene encodes the aerobactin siderophore ferric receptor protein, facilitates iron acquisition by mediating the uptake of siderophores and *iucD* involve in iron acquisition mechanisms. The genes *iss* and *ompT* encoded for protectins. Virulence factors (invasins, adhesins, iron acquisition systems, toxins, and protectins) coded by multifarious virulence associated genes facilitate the infection-causing abilities of *E. coli* strains. In this study, we compared the occurrence of four genes among APEC strains and found that the *iutA* gene was the most common, with a frequency of 77.1 %. This finding is consistent with studies conducted in Egypt [48], Brazil [57], and the United States [58], which reported frequencies of 78 %, 81.5 %, and 80.7 %, respectively. However, our data showed a slightly lower occurrence of the *iucD* gene at 75.0 % compared to the findings of Kwon et al. (2008), who reported an occurrence of 83 % [59]. Similarly in our study, the occurrence of *iss* and *ompT genes* was 86.8 % and 63.9 % respectively which is lower than the study of Subedi et al. (2018), where occurrence of both genes was 100 %. Variations in diagnostic methods, regional differences, and sample sizes may contribute to these differences in outcomes. The existence of *iucD* enhances the viability of APEC by being linked to iron, a vital element for the survival of *E. coli*. The identification of virulence genes in the samples indicates that *iutA*, *iucD*, *iss* and *ompT* are crucial virulence genes for APEC strains.

The high prevalence of APEC in poultry meat, along with the alarming levels of antibiotic resistance, underscores the urgent need for improved management practices on farms. Strategies such as enhanced biosecurity measures, better sanitation, and stricter control over antibiotic usage are crucial. Implementing alternatives to antibiotics, such as probiotics, prebiotics, phage therapy, antimicrobial peptides, and essential oils, can help reduce reliance on traditional antibiotics and mitigate the risk of resistance. From a public health perspective, the study highlights the necessity for robust policies to regulate antibiotic use in poultry farming. Educating poultry farmers, veterinary technicians, and the general public about antimicrobial resistance (AMR) is crucial. Policies should focus on ensuring that antibiotics are used judiciously and only under veterinary supervision to prevent misuse. Furthermore, regular monitoring and surveillance of antibiotic resistance patterns in poultry should be mandated to inform and update treatment guidelines.

This study had some limitations that should be considered. Firstly, the study only included samples from Bharatpur, Chitwan, Nepal, which means that the findings may not be representative of the entire district or country. It is important to conduct studies in different locations to obtain a more comprehensive understanding. Secondly, the study focused on investigating only four virulence genes of APEC. To gain a more comprehensive understanding of virulence factors, future studies are encouraged to investigate a larger number of genes. Moreover, the current study's sample collection was confined to chicken meat, thus, it is imperative for subsequent research to expand sampling sources, encompassing various environments such as the farm, and clinically infected birds. This broader sampling approach will provide a more representative depiction of the prevalence and distribution of virulence genes across different contexts relevant to avian colibacillosis. Furthermore, to ensure robust findings, it is recommended for the further studies to incorporate a larger sample size, and to conduct an extensive analysis of the risk factors associated with the occurrence of APEC.

5. Conclusions

We found that broiler chicken meat is contaminated with *E. coli* having 29.5 % (18/61) multidrug resistant from Chitwan, Nepal. Detection of genes *iutA* (77.0 %), *iucD* (75.4 %), *iss* (86.8 %) and *ompT* (63.9 %) in *E. coli* isolates suggest at least 63.9 % of them were APEC. To mitigate the risk of transmission of multidrug resistant APEC from colibacillosis affected poultry and enhance public health, it is crucial to regularly screen and monitor the virulence genes present in broiler meat. A comprehensive approach is essential for preventing and controlling avian colibacillosis not only in Chitwan, Nepal but also in other regions of the country. Controlling multidrug resistant APEC will ensure contamination as well as multidrug resistant free boiler meat for consumers. Educating poultry farmers, veterinary technicians, and the public about antimicrobial resistance is crucial for reducing antibiotic misuse.

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Data availability statement

Data will be made available on request.

CRediT authorship contribution statement

Ganesh Ranabhat: Writing – original draft, Software, Resources, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Deepak Subedi:** Writing – review & editing. **Jasmina Karki:** Visualization, Methodology. **Roshan Paudel:** Investigation, Data curation. **Himal Luitel:** Supervision, Resources. **Rebanta Kumar Bhattarai:** Writing – review & editing, Validation, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to

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