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Research Article

Antimicrobial Resistance Profiling of *E. coli* Isolated from Various Sources of Broiler Farms in District Quetta, Balochistan

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Abstract | The rapid growth of the chicken business in Pakistan has brought about a large-scale outbreak of infectious illnesses, which posed difficulties for the sector. The present study was conducted to determine the prevalence of *Escherichia coli* (*E. coli*) in various broiler farms located in the Quetta district, and to evaluate the susceptibility of the *E. coli* isolates to different antimicrobial agents. A total of 150 samples from a variety of sources (cecum, feed, bedding, water, air, and flies; n = 25 each) were collected from five distinct broiler farms (n=30/farm) in the district of Quetta. Samples were streaked on selective (Xylose Lysine Deoxycholate agar) and differential medium (MacConkey's agar) plates and incubated at 37°C for 24 hours. After isolation, organisms were further identified through Gram staining, and different biochemical tests. Among the total samples, 92(61.33%) showed positive growth of *E. coli* whereas 58(38.6%) samples were negative for *E. coli*. Source-wise distribution results showed that *E. coli* was mostly isolated from water samples (88%) followed by cecal samples (80%), air samples (72%), feed samples (48%), bedding samples (40%), and flies samples (40%). The prevalence of *E. coli* in different types/sources of samples was found statistically different (P=0.0012). Likewise, the farm-wise data exhibited that the prevalence of *E. coli* in different broiler farms was significantly varied (P= 0.0011). Some antibiotics like amoxicillin-clavulanic acid, ampicillin, sulfamethoxazole-trimethoprim, streptomycin, and tetracycline were found 100% resistant while, imipenem, chloramphenicol, tobramycin and ciprofloxacin showed 100% susceptibility to *E. coli* isolates. These results showed that the prevalence of antibiotic-resistant *E. coli* is very high in Quetta district thus, strict biosecurity and control of non-judicial use of antibiotics in poultry production is warranted.

Keywords | Prevalence, Broilers, Farms, *Escherichia coli,* Antimicrobial, Resistance.

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INTRODUCTION

Escherichia coli is a facultative anaerobe that is prevalent in the gastrointestinal tracts of poultry, animals, and humans. It is a major contributor to foodborne illnesses and is a member of the *Enterobacteriaceae* family (Sorour et al., 2022). Avian pathogenic *E. coli* (APEC) that causes localized or systemic infection outside of the avian gut is known as extraintestinal pathogenic *E. coli* (ExPEC). Colibacillosis is the name given to the infection brought on by ExPEC. This pathogen may cause egg peritonitis, arthritis perihepatitis, omphalitis, pericarditis, cellulitis, osteomyelitis, airsacculitis, coli granuloma, and salpingitis in broiler chickens aged 4-6 weeks. All these diseases are

collectively known as colibacillosis (Shah et al., 2019)

Colibacillosis is a widespread bacterial disease that has an economic impact on the poultry industry by reducing the productivity of infected birds, increasing mortality, condemning infected carcasses at slaughter, and increasing the cost of prophylaxis and treatment (Lutful, 2010). The most prevalent isolates of *E. coli* found in poultry belong to the O78, O1, and O2 serogroups, as well as the O15 and O55 serogroups to some extent. Pathogenic *E. coli* strains are those that possess one or more virulence factors. Avian colibacillosis is frequently linked to *E. coli* strains like serotype O78 in domestic poultry (Rahman et al., 2004).

In veterinary medicine, antibiotics are thought to be the most important factor in the selection, spread, and emergence of antibiotic-resistant microorganisms. The microflora of exposed individuals (animals) or populations as well as pathogenic bacteria may develop resistance by antibiotic use (Mantilla et al., 2022). The use of antimicrobial agents has been determined to be one of the most important variables in the emergence, selection, and spread of antimicrobial-resistant bacteria and antimicrobial resistant agent has been considered as an emerging worldwide problem in both veterinary medicine and humans (Mancuso et al., 2021).

Antibiotic overuse is thought to be the primary cause of antibiotic resistance via gene mutations or horizontal gene transfer (Moreno et al., 2008; Hughes & Andersson, 2015). Multidrug resistance among APEC strains is positively correlated with certain virulence genes, which are frequently found in avian colibacillosis strains (Johnson et al., 2012). Multidrug-resistant (MDR) bacteria typically possess multiple drug-resistant genes (Nikaido, 2009). Significant morbidity and mortality have occurred in humans, animals, and birds as a result of the rapid emergence of multidrug-resistant *E. coli* strains (De Been et al., 2014).

Chicken colibacillosis is common in Pakistan (Usman et al., 2023) and this disease is communicable to human beings (Khoo et al., 2010). To our knowledge, no comprehensive studies have been carried out on the prevalence and antibiotic susceptibility of *E. coli* isolated from broiler farms in Quetta, Balochistan, which may shed light on how *E. coli* spreads throughout the poultry supply chain and farm. It will provide researchers and veterinarians with the necessary direction to comprehend the phenomenon of transmission in our local environment and select the most effective drugs for *E. coli* infections. As a result, the purpose of the current study was to investigate the antibiotic susceptibility profile of *E. coli* isolates as well as the presence of *E. coli* in various poultry farm samples (feed, bedding, water, air, flies, and cecal samples) collected from Quetta, Balochistan.

MATERIAL AND METHODS

Ethical approval

This study was approved by the Ethics Committee of the Department of Veterinary Microbiology, Sindh Agriculture University Tandojam, and CASVAB, University of Balochistan, Quetta, Balochistan. The study was carried out from April 2022 to March 2023 in the Quetta district, Balochistan province, Pakistan. The study was carried out in line with International Ethical Rules for Animal Use in Research and were approved by institutional board of Sindh Agriculture University Tandojam (Approval # No. DAS/94/2023).

Collection of samples

A total of 150 samples from various sources (cecum, feed, bedding, water, air, and flies; 05 each/farm) of poultry farms were collected in the district of Quetta. For this purpose, five broiler farms were randomly selected and n= 30 samples per farm were collected under strict sterile conditions. All samples were transported to the laboratory through a cold chain for further processing at the Center for Advanced Studies in Vaccinology and Biotechnology, University of Balochistan, Quetta.

Processing of samples

Samples collected from distinct broiler farms in the district of Quetta were streaked on selective (Xylose Lysine Deoxycholate agar) and differential medium (MacConkey's agar) plates and incubated at 37°C for 24 hours. Cecal contents (1 g) were serially diluted (ten-fold) into 0.9% normal saline, then 1 ml of a resultant suspension of each sample was used to culture on media plates. Water, samples were filtered through 0.45 µm pore size membrane filters, then filters were placed on the surface of the media. Incubated the media plates for 24 hours at 37°C (Adewale, & Toyin, 2017). One gram of each feed samples was homogenized into 9ml of 0.9% normal saline, serial dilution carried out to 10^{-5} dilution then one ml of the solution was inoculated on the surface of MacConkey's agar using the spread plate method, and incubated for 24 hours at 37^{0}C (Osaro et al., 2017).

Ten to fifteen grams of each bedding sample were dissolved in 1 mL of phosphate buffer solution (PBS) to make a serial dilution 1:10 solution. After that the solution was shaken for 15 minutes then one ml of the solution was spread on the surface of MacConkey's agar using the spread plate method and incubated the media plates for 24 hours at 37°C (Rommel et al., 2013). Flies samples were crushed from the outside of the bag and then homogenized at 230 rpm. After homogenization, 100ul of 10^{-1} ad 10^{-2} dilutions in PBS were streaked in two-fold onto the surface of MacConkey's agar using the spread plate method (Blaak et al.,

2015).

Air, samples were first filtered through 0.45 µm pore size membrane filters, then filters were placed on the surface of the MacConkey's agar. Incubated the media plates for 24 hours at 37°C. After, isolation organisms were further identified through gram staining, different biochemical tests, and investigated for antibiotic susceptibility pattern (Sandeep et al., 2012).

Antibiotic Sensitivity Test

This test was used to check the susceptibility of *E. coli* against different antibiotics. Muller Hinton agar (Oxoid, UK) was prepared according to manufacturer instructions, autoclaved at 121°C for 15 minutes and cool down at 45°C before pouring into petri plates. Suspension of bacterial cells was prepared by using saline and a vortex mixer to compare the turbidity of bacterial suspension with 0.5 McFarland standards (1.5 x 10^8 colony forming unit CFU/ml), against a white card with a heavy contrast black line. A sterile cotton swab was taken and dipped into an inoculum tube, excess liquid of cotton swab was removed to the wall of the tube. Inoculated the culture on the surface of Mueller-Hinton agar and covered the whole surface of plates by applying thrice rotated at 60 degrees. Antibiotics discs were applied on the surface of the culture plates. At least four discs were used in a 100mm plate. All plates were placed invertedly into incubator at 37°C for 24 hours. After incubation plates were examined for zones of inhibition. *Staphylococcus aureus* ATCC 25923 was used as a reference strain (Kibret & Abera, 2011)

Antibiotics used were: Amoxicillin (25 µg), amoxicillin/clavulanic acid (10 µg), ceftazidime (30 µg), cefotaxime (30 µg), imipenem (10 µg), streptomycin (10 µg), gentamicin (10µg), kanamycin (30 µg), tobramycin (30 µg), tetracycline (30 µg), chloramphenicol (30 µg), nalidixic acid (30 µg), ofloxacin (5 µg), ciprofloxacin (5 µg), trimethoprim/sulfamethoxazole (25 µg).

Statistical analysis

Microsoft Excel (Microsoft Inc., USA) was used to enter the data into a database for calculation. JMP statistical Package Software (version 5.0.1.a SAS Institute Inc., Cary, N.C.) was used to statistically analyze the prevalence of *E. coli* in various samples and the degree of antimicrobial resistance. One-way ANOVA was applied to determine the statistical differences between various group means. Level of significance was adjusted at $P < 0.05$.

RESULTS

Overall prevalence of *E. coli* in different broiler farms of Quetta district

A total of 150 samples from a variety of sources were collected from five distinct broiler farms in the district of Quetta. Among the total samples, 92 (61.33%) showed positive growth of *E. coli* whereas 58 (38.6%) samples were negative for *E. coli* as shown in Figure 1.

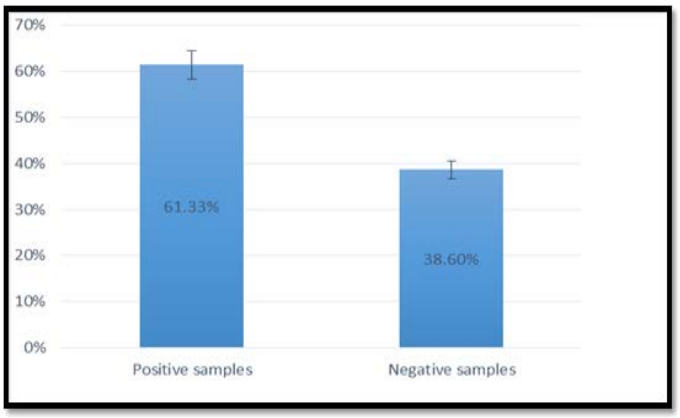

Figure 1: Prevalence of *E. coli* in broiler farms of Quetta district

Prevalence of *E. coli* in various samples of poultry farms in the Quetta district

The present study result showed that the prevalence of *E. coli* in various samples was significantly different ($P<0.05$) in all broiler farms included in the study. It was observed that all (100%) cecal and water samples of three farms (out of five) were observed contaminated with *E. coli.* Similarly, all air samples of a farm were found contaminated with *E. coli.* On overall basis, broiler farms exhibited 66.6% to 83.3% contamination of *E.coli* as shown in Table 1.

Prevalence of *E. coli* in different Sources of broiler farms of Quetta district.

As shown in Table 2, *E. coli* was mostly isolated from water samples (88%) followed by cecal samples (80%), Air samples (72%), feed samples (48%), bedding samples (40%), and flies samples (40%). The prevalence of *E. coli* in different types/sources of samples was found statistically different (P-value=0.0012).

Prevalence of *E. coli* in different broiler farms of Quetta district.

Farm-wise distribution, results showed that Farm 3 (83.33%) exhibited highest contamination of *E. coli* followed by Farm 1 & 2 (66.6% each), Farm 4 (53.33%), and Farm 5 (36.6%) as shown in Table 3. The statistical analysis revealed that the prevalence of *E. coli* in different broilers farms was significantly varied (P=0.0011).

Antibiotic susceptibility pattern of *Escherichia coli*

Escherichia coli showed sensitive to ceftazidime (23mm), imipenem (31mm), chloramphenicol (30mm), kanamycin (24mm), tobramycin (25mm), ciprofloxacin (30mm),

Table 1: Distribution of *E.coli* in various broilers farms in the Quetta district.

Sample Sources	No. of positive samples/Total samples (Percentage)				
	Farm 1	Farm 2	Farm 3	Farm 4	Farm 5
Cecum	5/5 (100%)	5/5(100%)	5/5(100%)	4/5(80%)	1/5(20%)
Feed	2/5 (40%)	3/5(60%)	3/5(60%)	1/5 (20%)	3/5(60%)
Bedding	3/5 (60%)	2/5(40%)	3/5(60%)	1/5 (20%)	1/5(20%)
Water	5/5(100%)	4/5(80%)	5/5(100%)	5/5(100%)	3/5(60%)
Air	4/5 (80%)	4/5(80%)	5/5(100%)	3/5(60%)	2/5(40%)
Flies	1/5 (20%)	2/5(40%)	4/5(80%)	2/5(40%)	1/5(20%)
p-value	0.0314	0.0071	0.0646	0.0057	0.0000

Table 2: Source-wise distribution of *E. coli* in different broiler farms of Quetta district.

Type of samples	Total samples	Positive samples	Percentage	P-value
Air sample	25	18	72%	0.0012
Feed sample	25	12	48%	
Bedding sample	25	10	40%	
Water sample	25	22	88%	
Cecal sample	25	20	80%	
Flies' sample	25	10	40%	
Total	150	92	61.33%	

Table 3: Farm-wise distribution of *E. coli* in Quetta district.

Farm identity	Total samples	Positive samples	Percentage	P-value
Farm 1	30	20	66.6%	0.0011
Farm 2	30	20	66.6%	
Farm 3	30	25	83.33%	
Farm 4	30	16	53.33%	
Farm 5	30	11	36.6%	
Total	150	92	61.33%	

Table 4: Antibiotic Susceptibility Pattern of *Escherichia coli* isolated from different farms of Quetta district (n= 92 isolates).

Classes	Antibiotics	Abbreviation	Disk potency	R (%)	S (%)
Penicillin	Ampicillin	AP	25µg	100	0
	Amoxicillin- clavulanic acid	AMP	10µg	100	0
Cephalosporin	Cefotaxime	CTX	30µg	60	40
	Ceftazidime	CTZ	30µg	20	80
Aminoglycoside	Gentamycin	GEM	10µg	80	20
	Imipenem	IMP	10µg	0	100
	Streptomycin	S	10µg	100	0
	Chloramphenicol	CHL	30µg	0	100
	Kanamycin	KAN	30µg	30	70
	Tobramycin	TOB	30µg	0	100
Quinolones	Ciprofloxacin	CIP	5µg	0	100
	Nalidixic acid	NAL	30µg	70	30
	Ofloxacin	OXF	5µg	30	70
Tetracycline	Tetracycline	TET	30µg	100	0
Sulfonamides	Sulfamethoxazole-Trimethoprim	SXT	25µg	100	0

R: Resistant; S: Susceptible

and ofloxacin (25 mm), while showed resistance against cefotaxime (16mm), gentamycin (20 mm) Amoxicillin-clavulanic acid (00mm), ampicillin (00mm), streptomycin (00mm), nalidixic acid (20 mm) sulfamethoxazole trimethoprim (00mm), and tetracycline (00mm). The results further demonstrated that all (100%) *E. coli* isolates were found resistant to ampicillin, amoxicillin- clavulanic acid, streptomycin, tetracycline and sulfamethoxazole-trimethoprim. While, imipenem, chloramphenicol, tobramycin and ciprofloxacin showed 100% susceptibility to *E.coli* isolates as shown in Table- 4.

DISCUSSION

Escherichia coli is a natural gut microbiota in birds (De Carli et al., 2015). Avian pathogenic *E. coli* (APEC) penetrates several organs of birds, causing specific or systemic diseases known as Extraintestinal pathogenic *E. coli* (EPEC) (Ibrahim et al., 2019). Colibacillosis is defined as pericarditis, air sacculitis, perihepatitis, peritonitis, and other extraintestinal disorders (Matter et al., 2011; Matin et al., 2017). *Escherichia coli* is one of the most significant contributors to economic losses as a result of infections in poultry production farms, as well as causing mortality and condemning corpses in slaughterhouses (Ewers et al., 2004). In terms of prevalence, colibacillosis is relatively common in Pakistan, affecting both livestock and poultry. Due to various factors such as poor hygiene, inadequate sanitation, and overcrowding, the transmission and spread of *E. coli* can occur more easily in commercial broiler farming (Sorour et al., 2022). The incidence of colibacillosis can vary depending on factors like flock size, management practices, and environmental conditions. It is important to note that colibacillosis outbreaks may occur seasonally, particularly during periods of stress and environmental changes. Mortality rates associated with colibacillosis can also vary. The disease can lead to dehydration, septicemia, and secondary infections, which can have detrimental effects on the affected birds' health and survival (Saeed et al., 2023).

The present study was conducted to determine the prevalence of *Escherichia coli* in various broiler farms located in the Quetta district, and to evaluate the susceptibility of the *E. coli* isolates to different antimicrobial agents. A total of 150 samples from a variety of sources were collected from five distinct broiler farms in the district of Quetta. Among the total samples, 92(61.33%) showed positive growth of *E. coli* whereas 58(38.6%) samples were negative for *E. coli*. Blaak et al. (2015) also reported a high prevalence (65%; 46/71) of ESBL-producing *E. coli* in broiler farms (n = 3) adopted in their Dutch study. Azam et al. (2019) also studied high rate of APEC isolates were recovered 75 (89.2%) from colibacillosis-affected broilers in the Faisalabad region of Pakistan due to the accusation of five virulent VAGs genes.

According to source-wise distribution, the present study results showed that *E. coli* mostly isolated in water samples (88%) followed by cecal samples (80%), air samples (72%), feed samples (48%), bedding samples (40%), and flies samples (40%). Blaak et al. (2015) in their study detected *E. coli* from run-off water (81%), followed by other farm animals (79%), dust (60%), surface water adjacent to farms (57%), soil (55%), on flies (15%), and in barn air (6%).

In the present study farm-wise distribution results showed that Farm 3 (83.33%) was highly contaminated with *E. coli* followed by Farm 2, Farm 1, Farm 4, and Farm 5 with *E.coli* recovery percentage of 66.66%, 66.66%, 53.33% and 36.6% respectively. According to Matin et al. (2017), the farm-wise prevalence of colibacillosis was 0.7% in Bangladesh Agricultural University (BAU) broiler farm, 0.4% in CP broiler farm, 1.2% in Abu Tarek broiler farm and in Nahar broiler farm, and 0.8% in Sotota layer farm. The prevalence of colibacillosis in all farms was found to be statistically significant (p=0.0054).

The present study result shows that *Escherichia coli* is a gram-negative, rod-shaped bacilli which were appeared singly or in pairs, with approximately 1-3 µm × 0.4-0.7 µm in size similar results were founded by Rahman et al., (2017). According to the current study, the colonies of *Escherichia coli* were appearing with a metallic sheen and a dark center after prolonged incubation on the surface of EMB agar similar results were founded by Rahman et al., (2017).

The current study result showed that biochemical tests used for the identification of *Escherichia coli* such as catalase, oxidase, IMVIC, Urease, TSI, and motility tests were similar to the study of Sandeep et al. (2012).

Antibiotics have been used for treatment and prevention of disease as well as growth promotion in livestock and poultry production (Allen et al., 2013). The use of antibiotics has potentially increased the prevalence of resistance determinants in animal microbiomes (Pal et al., 2016). According to the present study *Escherichia coli* was highly resistant against Amoxicillin-clavulanic acid (100%), ampicillin (100%), streptomycin (100%), sulfamethoxazole-trimethoprim (100%), tetracycline (100%), followed by gentamycin (80%), nalidixic acid (70%), and cefotaxime (20%), while showed sensitivity to ceftazidime, imipenem, chloramphenicol, kanamycin, tobramycin, ciprofloxacin, and Ofloxacin. Similar results were reported by Rekaz et al. (2019) with highest resistance against sulfamethoxazole-trimethoprim, florfenicol, amoxicillin, doxycycline and spectinomycin in percentage; 95.5, 93.7, 93.3, 92.2 and

92.2% due to accusation of resistance in the most predominant genes *Int1*, *tet A, bla TEM, Sul1*, and *Sul2* respectively. Another study by Masudur et al. (2020) reported similar results of antibiotic resistance to ampicillin, tetracycline, streptomycin, trimethoprim-sulfamethoxazole and gentamicin due to resistance in gene *tetA, sul*1, *aadA*1, *ereA* from broiler chickens but in contrast, the same study described resistance against chloramphenicol, and erythromycin in *E. coli* isolates. Islam et al. (2021) also reported that all *E. coli* isolates were 100% resistant against ampicillin, streptomycin, tetracycline, and erythromycin, which are much similar to the current study.

The current study have some limitations like, the present findings may be specific to the local context of District Quetta, Balochistan, and may not be directly applicable to other regions or poultry farming systems. As local variations in antimicrobial usage practices, farm management, and environmental conditions can influence resistance profiles (Christy et al., 2018). This study might solely rely on conventional antimicrobial susceptibility testing methods, lacking molecular analysis techniques such as whole-genome sequencing. Consequently, it may not provide detailed insights into specific resistance mechanisms or genetic determinants.

CONCLUSIONS

The results from the present study showed that the prevalence of *Escherichia coli* was very high in the different broiler farms of the Quetta district. The prevalence of *E. coli* was expressively highest in the water samples followed by cecal samples, while it was lowest in the bedding and flies samples. Some antimicrobial agents such as amoxicillin-clavulanic acid, ampicillin, streptomycin, sulfamethoxazole-trimethoprim, and tetracycline were found completely resistant against the *E. coli* isolates which is alarming and suggests the strict biosecurity and emergent discontinuation of in-feed antibiotics in poultry production.

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CONFLICT OF INTEREST

The author declared no conflict of interest.

AUTHORS CONTRIBUTION

HZ performed the experiments for data collection, AAK hypothesized the study design and supervised the project, ZA and MKT helped in sample collection and analysis, SA and UZ wrote the paper and submitted for publication.

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