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Antibiotic susceptibility profile and resistance patterns of *Escherichia coli* strain to colistin isolated from Chicken meat (Broiler, Layer &Sonali) at Sylhet division of Bangladesh

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Abstract:
Background: *Antimicrobial resistance is the most urgent threat to human and animal health. Escherichia coli (E. coli) is one of the normal inhabitants of the gastrointestinal tract of both humans and animals but few strains of E. coli have become highly adapted to cause diarrhea and a range of extra-intestinal diseases. Therefore, the present study was conducted to determine the susceptibility and resistance patterns of E. coli to colistin isolated from chicken at Sylhet division of Bangladesh.*
Materials and Methods:*A total number of 300 meat samples of broiler, layer and Sonali (50 samples from each type from two districts) were randomly collected from two districts of the Sylhet division. Isolation and identification of E. coli were performed by using cultural and biochemical tests. The resistance and susceptibility patterns of isolated E. coli to colistin were determinedby the Kirby-Bauer disc diffusion method.*
Results: *In case of Sylhet district, the positive cases of E. coli in broiler, layer, and Sonali were 78%, 70%, and 64% respectively and in Moulvibazar district the positive cases were 70%, 72%, and 66% respectively. E. coli isolated from broiler, layer, and Sonali from both the districts showed 53%, 46%, and 49% respectively, susceptibility to colistin. In Sylhet district, E. coli isolated from chickens showed 30.19% resistance and 69.81% susceptibility to colistin whereas isolates of chickens of Moulavibazar district showed 28.85% resistance and 71.15% susceptibility to colistin. In case of chicken, overall susceptibility and resistance patterns of E. coli isolates in Sylhet division were recorded as 70.48% and 29.52% respectively.*
Conclusion:*Taken together, this finding of our study let us conclude that E. coli isolates of chickens showed resistance to antibiotic colistin, which is an alarming issue for animal and human health. Furthermore, the results of our finding might be useful for improving experimental antimicrobial interventions as well as exploring alternative antimicrobial agents.*
Key words*: E. coli, Antibiotic resistant, Colistin, Chickens, Bangladesh.*

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I. Introduction

In the 21st century, antimicrobial resistance (AMR) has appeared as one of the major public health problems which threatens effective treatment and prevention of bacterial, viral, fungal, and parasitic infections treating with common medicines [1]. AMR is primarily generated throughthe impulsive usage of antimicrobial agents, which is becoming an enhancing concern inhumans and animals ubiquitously. Owing to the hugeness of the threat, the World Health Organization (WHO) suggested universal surveillance outlines inhuman as well as animal populations[2].

Escherichia coli(*E. coli*) is a bacterial species that commonly inhabit the gastrointestinal tract of human, animals, and poultry and may become pathogenic to both [3]. The majority of the *E.coli* strains are usually nonpathogenic and often found as an indicator of fecal contamination in food. In nature, around 10-15% are opportunistic and pathogenic coliform bacteria [4].Nevertheless, in immunocompromised hosts, pathogenic *E. coli*

may cause a range of lesions that sometimes become severe, even fatal such as endocarditis, urinary tract infection, meningitis, septicemia, and epidemic diarrhea of adults and children [5] and cellulitis, omphalitis, coligranuloma, swollen head syndrome, colibacillosis as well as yolk sac infection in poultry [6].

Colistin is used both in veterinary and human medicine. Colistin is usually used in humans for the treatment of infections caused by extensively drug-resistant, multidrug-resistant (MDR), and pan drug-resistant bacteria [7]. In veterinary medicine, colistin is regarded the prime drug of option for the intervention of recurrent *E. coli*-associated digestive tract contaminations applied for food production animals. The drug is amongst the terminal-option antimicrobials for the intervention of ailments induced through MDR Gram-negative bacteria. The increasing frequency of colistin resistance is mostly ascribed to the usage of the drug in veterinary medicine. The development of colistin resistance has lately been elevated life-threatening apprehensions [8,9]. However, the worldwide use of antimicrobials for animal health and production purposes surpasses the use in humans [10].

The risk of AMR appears is high in various countries like Bangladesh where legislation, monitoring systems and regulatory surveillance on the use of antimicrobials, and the prevention and control measures of AMR, are very poor or insufficient. Furthermore, reserved antimicrobial agents such as colistin are extensively used for the prevention and/or treatment of diseases of food animals in Bangladesh. Moreover, the wide apply of antimicrobial agents in humans has aggravated the disseminated of antimicrobial resistance. There is a scarcity of evidence in the literature on AMR in poultry in Bangladesh [2]. The present study was carried out to isolate *E. coli* strains from the meat of chicken in Bangladesh, in order to discover the susceptibility and resistance patterns of isolated *E. coli* to colistin at the Sylhet division of Bangladesh.

II. Material And Methods

Ethics Statement: The current study was conducted at the Department of Pathology, Faculty of Veterinary, Animal and Biomedical Sciences, Sylhet Agricultural University, Sylhet, Bangladesh. In this study, the handling of animals was performed according to the current Bangladesh legislation (Animal Welfare Act 2019 and Cruelty to Animals Act of 1920). Furthermore, all of the procedures in animal experiments were approved by the Ethics Committee of the Sylhet Agricultural University, Bangladesh.

Sample Collection: Chickens (broiler, layer and Sonali) meat samples were collected from various retail shops of different markets of Sylhet and Moulvibazar district at Sylhet division of Bangladesh. A total of 300 meat samples, 150 from Sylhet district and 150 from Moulvibazar district, were randomly collected from individual brolier (50), layer (50), and Sonali (50) from different retail shops. After collecting, all of the samples were quickly shipped to the laboratory of Pathology, Sylhet Agricultural University, Sylhet, Bangladesh maintaining cold conditions for further processing.

Isolation and Identification of *E. coli*: Upon arrival, 5 grams of meat from each sample was taken by using sterile forceps. Then it was grinded with the help of mortar and pestle and a swab was taken by using a cotton bud. Then the bud was transferred immediately into the autoclaved nutrient broth and incubated at 37°C for 24 hours. After 24 hours, each tube was examined for the satisfactory growth of organisms and then subsequent streaking on MacConkey agar (Merck, Darmstadt, Germany) were performed and aerobically incubated at 37°C for 24 hours. Then, lactose-fermenting colonies were picked to re-streaked on eosin methylene blue (EMB) agar (Merck, Darmstadt, Germany) and kept in incubation at 37°C for 24 h. Colonies of *E. coli* were observed as green metallic sheen after the incubation period. Biochemical tests were then performed from these colonies which include growth on lysine iron agar (LIA) and triple sugar iron agar (TSI), the oxidative/fermentative metabolism of glucose, as well as the test of the abilities for indole fermentation, citrate utilization, tryptophan degradation, urease production, glucose degradation {methyl red (MR) test} and motility.

Antimicrobial Susceptibility Testing by Disc Diffusion Method: As per Clinical and Laboratory Standards Institute (CLSI) guidelines, antimicrobial susceptibility test of E. coli isolates was carried out on Muller-Hinton agar plates (Merck, Darmstadt, Germany) through Kirby-Bauer disc diffusion method [11]. In this study, the antimicrobial agent, colistin (10 µg) (Oxoid, England) was used. The colistin was selected since recently it is the most commonly used agent in food animals of Bangladesh and has its public health significance, as described by the CLSI. As a control, a standard strain of *E. coli* (ATCC25922; American Type Culture collection, Rockville, MD, USA) was used in all experiments. In accordance with CLSI guidelines, the sensitivity of *E. coli* isolates to colistin was determined and interpreted after aerobic incubation at 37°C for 18 to 24 hours. When the inhibition zones (in diameter) of the control *E. coli* (ATCC25922) strain were within the performance ranges, test results were only considered valid. Both resistant and intermediate resistant isolates were collectively referred to as non-susceptible [7].

III. Result

From 300 specimens, positive samples of *E. coli* were identified based on microbiological and biochemical tests except theVoges-Proskauer (VP) test (Table 1).The biochemical test results revealed that the causative agent wasglucose (+), indole(+). MR test (+), and catalase (+).

Table 1: Results of different biochemical tests

SL.NO	Name of the tests	*E. coli*
1	Glucose test	+
2	Indole	+
3	MR test	+
4	VP test	-
5	Catalase test	+

All the samples were examined bacteriologically. The overall prevalence of *E. coli* from meat sample of broiler, layer, and Sonali was collected from Sylhet and Moulvibazar districts were 70% (Table 2). Our study demonstrated that Sylhet district has the highest prevalence of 70.67% where Moulvibazar district shows the lowest prevalence that was 69.33%. But the results of these study were statistically nonsignificant (p<0.05).

Table 2: Prevalence of *E. coli* in meat samples of broiler, layer, and Sonali.

Type	Sylhet (n=150)	Moulavibazar (n=150)	*P*-value[a]	Prevalence	Overall meat type prevalence n=300
Broiler	39 (78.00%)	35 (70%)	0.284	74 (74.00%)	210 (70.00%)
Layer	35 (70.00%)	36 (72.00%	0.727	71 (71.00%)	
Sonali	32 (64.00%)	33 (66.00%)		65 (65.00%)	
Overall district wise prevalence	106 (70.67%)	104 (69.33%)			

Number of samples (n)= 50 for each species and each district, [a] *P* values were calculated using a two-way analysis of variance (ANOVA) without replication. *P* values > 0.05 were considered to be statistically nonsignificant.

Using the agar disc diff usion method, antimicrobial resistance patterns of the tested *E. coli* isolates against colistin were determined; the results are summarized in Table 3. The isolates in Sylhet district were 30.19% resistant and 69.81% sensitive whereas in Moulavibazar district were 28.85% resistant and 71.15% sensitive to colistin.

Table 3: Sensitivity and resistance patterns of *E. coli* to colistin isolated broiler, layer, and Sonalifrom two districts in Sylhet division.

Antibiotic	**Chickens**	**Sylhet**		**Chickens**	**Moulvibazar**	
		S	**R**		**S**	**R**
Colistin (10mg)	Broiler (n=39)	27 (69.23%)	12 (30.76%)	Broiler (n=35)	26 (74.29%)	9 (25.71%)
	Layer (n=35)	24 (68.57%)	11 (31.43%)	Layer (n=36)	22 (61.11%)	14 (38.89%)
	Sonali (n=32)	23 (71.86%)	9 (28.13%)	Sonali (n=33)	26 (78.79%)	7 (21.21%)
Overall	n= 106	74 (69.81%)	32 (30.19%)	104	74 (71.15%)	30 (28.85%)

n= Number of *E. coli* positive cases

On the other hand, Table 4 showed that all the isolates from chicken were 70.48% susceptible and 29.52% resistant to colistin.

Table 4: Overall susceptibility and resistance patterns of *E. coli* isolates to colistin.

Antibiotic	**Chickens**	**Total no. of positive cases(n)**	**Susceptible (%)**	**Resistance (%)**
Colistin	Broiler	74	53 (71.62)	21 (28.38)
	Layer	71	46 (64.79)	25 (35.21)
	Sonali	65	49 (75.38)	16 (24.62)

Overall	210	148 (70.48)	62 (29.52)

IV. Discussion

Commensal *E. coli* is a normal habitant which conserves the usual gut microbiota in poultry. Nevertheless, colibacillosis is largely produced through avian pathogenic strains (APEC), including localized as well as systemic infections such as cellulitis, omphalitis, respiratory coli-septicemia, swollen head syndrome, enteric coli-septicemia and diarrhea [8,12]. Moreover, it could have a zoonotic pathogenic potentiality like neonatal meningitis [13]. The contamination of E. coli in poultry farms can circulate both horizontally as well as vertically It can circulate instantly through contaminated dust, feces fomites as well as water. It can be inspired or ingested to induce infection, leading to ailment. Higher ammonia concentrations that can damage the skin or respiratory epithelium as well as create it easier for *E. coli* to insert the body, are amongst the almost substantial non-infectious predisposing variables. The prevalence and severity of E. coli contaminations are subsequently enhanced through these factors along with poor hygiene, short distances between houses, flocks of diverse ages on a farm, as well as short service intervals among flocks [9].

AMR is a worldwide serious issue for humans and animals. The extensive use of antimicrobial agents in livestock is one of the causes of antimicrobial resistance development in microorganisms. Due to the rapid rise of antimicrobial resistance in both the human and veterinary world, antimicrobials are gradually becoming nonproductive which is generating one of the major threats to both humans and animals. Common human microbiota like *Escherichia coli*, *Salmonella spp.*, *Klebsiella pneumonia*, *Streptococcus pneumoniae*, and *Staphylococcus aureus* were also reported as major flora among food borne pathogens, common antimicrobial-resistant bacteria and important human pathogens. Entrance of bacterial resistance to antimicrobial agents has chance a substantial as well as prevailing public health hazard particularly when there are insufficient or no accessible alternate efficient antimicrobial agents for the intervention of infections generated through these bacteria [2].

Owing to the haphazard usage of antibiotics as prophylactic treatment of infectious diseases and feed additives, AMR in chickens is a common problematic in various developing countries like Bangladesh [2]. According to our present study, meat samples were collected from healthy chicken (broiler, layer, and Sonali) meat and thus *E. coli* from these specimens can be considered as non-pathogenic. In our present study, 70% were found positive in chickens for *E. coli* following cultural and morphological methods. In Bangladesh, various studies reported that the prevalence of *E. coli* in broiler chicken were 58% [14] and 63.6% [15]. Similarly, the prevalence of *E. coli* in layer chicken was 54% [16] and 56.4% [15] which were very close to our present findings. Another study in Bangladesh showed that the prevalence of *E. coli* in indigenous chickens, layers, and broilers were 70%, 78.67%, and 82% respectively [17] which was in larger compared to our present findings. Nevertheless, the overall prevalence of *E. coli* in chicken meat was 70% which was supported by the findings of Jakaria[17] who reported a 70% prevalence of *E. coli* in chicken from Bangladesh. Bhattacharjee [18] demonstrated that 40.82% prevalence of *E. coli* in poultry from Bangladesh which was in smaller compared to our present findings.

In Bangladesh, another study also revealed that *E. coli* isolates exhibited susceptibility to colistin sulphate as 100% [19]. The rate of susceptibility was only 70.48% and resistance was 29.52% among our isolates, which was supported by the finding of Azad [2] who reported a 73.5% susceptibility rate to colistin in Bangladesh. This may be attributable to either the misuse of colistin sulphate, or the sharing of colistin resistance genes. The variation observed in prevalence among different studies could be attributed to the difference in study design, procedures of sampling, variability in sampled populations, the quantity of animals, diverse geographical origins of animals, season, sanitation, and treatment at the time of sample collection.

V. Conclusion

In conclusion, the comparatively high antimicrobial resistance levels as well as susceptibility of *E. coli* to colistin isolates in the present study can be ascribed to the unnecessary usage of antimicrobial agents in Bangladesh. Our results only emphasized Sylhet division of Bangladesh out of 8 known divisions. Overall, our findings may not totally speculate the susceptibility and resistance patterns of *E. coli* to colistin in the country. Furthermore,the results of our finding might be useful to make surveillance on antimicrobial resistance in chickens at the molecular level and to provide valuable guidelines on the use of reserve antibiotics in common food animals. So, antimicrobial susceptibility surveillance on regular basis is also essential for achieving the goal of prevention of antibiotic resistance. Additionally, more research specifically on alternatives to available antibiotics is immediately required for the compensation of the shortage of effective antimicrobials.

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Conflicts of Interest

Authors have declared that no competing interests exist

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