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Antibiotic-Resistant *Escherichia coli* Isolated from Duck Cloacal and Tap Water Samples at Live Bird Markets in Bangladesh

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Abstract

Antibiotic resistance is a growing concern all over the world. The current study sought to identify antimicrobial resistance (AMR) patterns and antibiotic-resistant genes in *Escherichia coli* (*E. coli*) isolated from seemingly healthy ducks and neighboring tap water sources at three separate live bird markets (LBMs) in Chattogram, Bangladesh. A total of ninety cloacal swab samples of Khaki Campbell ducks and fifteen water samples from nearby tap water sources were collected from three LBMs. Several cultural and molecular tests were conducted to determine *E. coli* contamination. The disk diffusion technique was used to evaluate the antibiotic sensitivity of *E. coli* isolates to 12 different antibiotics. For each isolate, a Multiple Antibiotic Resistance (MAR) index was calculated. The resistance genes were detected using a polymerase chain reaction (PCR) assay. The overall prevalence of *E. coli* in feces and tap water samples was 64.4% (58/90, 95% CI 54.1-73.6) and 100% (15/15, 95% CI 76.1-100), respectively. Both fecal and water isolates showed 100% resistance to ampicillin, tetracycline, and nalidixic acid. Resistance to other antibiotics was also found to be high. Multidrug- resistance (MDR) was unveiled in all fecal (58/58) and water (15/15) isolates. MAR index ranged from 0.33 to 0.67 in all recovered isolates. Both fecal and water *E. coli* isolates harbored *bla*TEM, *tet*A, *sul*1, and *sul*2 genes. The resistance genes in MDR *E. coli* in live bird markets might transmit from ducks to humans and they, therefore local authorities should consider this issue a major public health risk.

Introduction

Antibiotics are one of the most effective weapons against life-threatening bacteria. Untreatable illnesses, nevertheless, are killing people all over the world because of the emergence and spread of antibiotic resistance (ABR) bacteria (Siddiky *et al.*, 2021). ABR is now a worldwide issue affecting both animal and human health. Antibiotic resistance is becoming an alarming and tough issue since it makes infection management impossible with these resistant medications. It has been common practice to use antibiotics in the poultry industry for different purposes such as disease treatment, feed additives and growth promoters (Dube and Mbanga, 2018). Antibiotic usage regularly exposes the gut flora to antibiotics, resulting in resistance through a variety of

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mechanisms like gene transfer, induction, mutation, etc (van den Bogaard *et al*., 2001; Yang *et al*., 2010).

The poultry industry is considered a propitious area for economic growth and generates employment opportunities that help to deplete the poverty in most developing countries (Hamid *et al.*, 2016). Chicken and duck are the most commercially valuable animals, providing eggs and meat that contribute significantly to a nutritious diet rich in nutrients for people of all ages (Layman and Rodriguez, 2009). Antibiotic prophylaxis at the flock level has been widely utilized in Bangladesh to prevent and control the spread of infectious illnesses in duck farms. Therefore, the commercial and native duck could act as a prospective carrier of resistant bacteria and play a significant role in resistant gene dissemination (Zhong *et al.*, 2009).

The causative agent of avian colibacillosis is *E. coli*, which causes high mortality and lower output in poultry, resulting in massive economic losses in the poultry industry across the world (Abd El Tawab *et al.*, 2015). The FDA stated that *E. coli* resistance to antimicrobial agents used in human and veterinary treatment is steadily rising worldwide (FDA, 2014). Because of particular toxicity, illnesses, medication allergies, and the growth of MDR strains of bacteria, a common bacterial bug like *E. coil* in poultry can harm human health. The genes encoding ABR in bacteria may easily spread horizontally and vertically to other bacteria, allowing them to enter the human food chain (Sarker *et al.*, 2019a).

In general, people buy freshly slaughtered or live poultry from wet markets or LBMs mostly in Asian countries (Li *et al.*, 2017). The live birds in LBMs come from different sources and could be a reservoir of ABR genes. Water sources like tap water in LBMs are also a reservoir of ABR genes in *E. coli* (Sarker *et al.*, 2019a). Furthermore, when customers come into direct touch with live or slaughtered ducks or tap water to wash their hands, the possibility of the ABR bacterium being transferred to the human food chain increases. It poses a serious health concern, particularly in low- and middle-income countries such as Bangladesh. The study of ABR in duck in Bangladesh is very scanty. As a result, the goal of this study was to investigate the prevalence of *E. coli* and ABR patterns in *E. coli* isolates obtained from ducks and tap water at LBMs in Chattaogram, Bangladesh, as well as to detect some associated resistance genes.

Materials and Methods

Sample collection

Duck cloacal swabs and tap water samples were collected from three different live bird markets (LBM) namely Jhautola (latitude 22°23'27.3"N and longitude 91°50'59.5"E), Pahartali (latitude 22°21'44.4"N and longitude 91°50'55.9"E) and Reazuddin Bazar (latitude 22°22'13.1"N and longitude 91°53'00.7"E), Chattaogram, Bangladesh in January to March 2017. A total of 90 fresh fecal swab samples (30 each market) and 15 tap water samples (five per market, each 40 mL) were collected in a sterile Falcon tube containing buffered peptone water (BPW) (Oxoid, UK), stored in an icebox and transferred to the laboratory in an unbroken freeze chain.

E. coli isolation and identification

For pre-enrichment, each BPW-containing sample incubated at 37 °C for overnight. Pre-enriched broth was plated onto MacConkey agar (Oxoid, UK) from BPW after pre-enrichment. At 37°C, plates were incubated for 18-24 hours. *E. coli* was suspected after large pink colonies on MacConkey agar were streaked over Eosin Methylene Blue (EMB; Oxoid, UK) agar and inoculation plates were incubated at 37 °C for 24 hours. Typical colonies that appeared green with metallic sheen were considered *E. coli*. The detected *E. coli* isolates were verified by PCR at the species level, using primers ECO-1 and ECO-2 to target 16S rRNA genes from a prior published work (Table 1).

Table 1. List of primers used in PCR for the detection of *E. coli* and antibiotic resistant genes

Target gene	Primer sequence (5´-3´)	Amplicon size (bp)	Annealing temperature(°C)	References
16S rRNA	F: GACCTCGGTTTAGTT CACAGA R: CACACGCTGACGCTGACCA	585	58	Seidavi *et al*., 2010
*bla*TEM	F: TACGATACGGGAGGGCTTAC R: TTCCTGTTTTTGCTCACCCA	716	53	Batchelor *et al*., 2005
blaCTX-M	F: CGATGTGCAGTACCAGTAA R: TTAGTGACCAGAATCAGCGG	585	55	Belaaouaj *et al*., 1994
*tet*A	F: GCTACATCCTGCTTGCCTTC R: CATAGATCGCCGTGAAGAGG	210	55	Karczmarczyk *et al*., 2011
*tet*B	F: TTGGTTAGGGGCAAGTTTTG R: GTAATGGGCCAATAACACCG	659	55	Lanz *et al*., 2003
*tet*C	F: CTTGAGAGCCTTCAACCCAG R: ATGGTCGTCATCTACCTGCC	418	55	Lanz *et al*., 2003
sul 1	F: CGGCGTGGGCTACCTGAACG R: GCCGATCGCGTGAAGTTCCG	433	68	Ng *et al*., 2001
sul 2	F: CGGCATCGTCAACATAACCT R: TGTGCGGATGAAGTCAGCTC	721	66	Sunde, 2005

The PCR conditions were initial denaturation (96 °C for 60 s), 35 cycles of denaturation (96 °C for 15 s), annealing (58 °C for 60 s), extension (72°C at 45 s), and a final extension (72 °C for 60 s) (Seidavi *et al.* 2010). On a 1.5% agarose gel, PCR products were stained with ethidium bromide and seen under UV light. For further research, positive *E. coli* isolates were preserved in Brain Heart Infusion broth (BHI; Oxoid, UK) with 15% glycerol at −80 °C.

Antibiotic susceptibility testing

In compliance with the Clinical and Laboratory Standards Institute's guidelines and recommendations, the disk diffusion technique was employed to assess antibiotic susceptibility (CLSI, 2015). Antibiotic susceptibility profiles of *E. coli* isolates were determined using commonly used antibiotics in human and veterinary medicine, namely ampicillin (10 µg), tetracycline (30 µg), nalidixic acid (30 µg), sulfamethoxazole- trimethoprim (25 µg), chloramphenicol (30 µg), erythromycin (30 µg), gentamicin (10 µg), ciprofloxacin (5 µg), azithromycin (15 µg), ceftriaxone (30 µg), cefotaxime (30 µg), and imipenem (10 µg) (Oxoid, UK) on Mueller-Hinton agar (MHA; Oxoid, UK). To interpret the obtained results, the CLSI (2015) recommendations were used. Isolates showing resistance to at least three distinct antibiotic classes were classified as MDR (Zhu *et al.,* 2017). The established technique was used to compute and interpret the MAR index (Paul *et al.,* 1997).

DNA extraction

With minor modifications, the boiling technique was used to extract DNA (Sánchez *et al.,* 2010). Using the vortex, 2-3 fresh colonies were homogenized in a 1.5 mL sterile Eppendorf tube containing 200 µL of deionized water. The mixture was then heated at 99 °C for 15 minutes before being centrifuged at 10000 × *g* for 2 minutes. Finally, the supernatant was collected and utilized as a DNA template, which was kept at -20°C until analysis.

Detection of antibiotic resistant genes

PCR was conducted in a final volume of 25 µL mixture including 12.5 µL 2X GoTaq master mix (Promega, USA), 1 µL DNA template, 0.5 µL of each forward and reverse primer, and 10.5 µL deionized water to identify antibiotic resistance genes. The presence of the *bla*TEM, *bla*CTX-M, *tet*A, *tet*B, *tet*C, *sul*1 and *sul*2 genes was determined using previously described PCR amplification protocols (Batchelor *et al.,* 2005; Belaaouaj *et al.,* 1994; Karczmarczyk *et al.,* 2011; Lanz *et al.,* 2003; Ng *et al.,* 2001; Sunde, 2005). A Thermo-cycler (Applied Biosystems, USA) was used to perform the PCR. The amplified PCR products were then electrophoresed in a 1.5 percent agarose gel stained with ethidium bromide (Sigma-Aldrich, USA), and the gels were viewed and photographed using a transilluminator under UV light (BDA digital, Biometra GmbH, Germany).

Results

Prevalence of *E. coli*

The overall prevalence of *E. coli* was 64.4% (95% CI 54.1-73.6) in feces and 100% (95% CI 76.1-100) in tap water samples. The prevalence of *E. coli* was 70% (95% CI 52-83.4), 60% (95% CI 42.3-75.4), and 63.3% (95% CI 45.5-78.2) in duck feces isolated from three LBMs namely Jhautola, Pahartali, and Reazuddin Bazar, respectively. *E. coli* was detected in 100% (95% CI 51.1-100) of tap water samples from each LBM.

Antibiotic resistance

Ampicillin, tetracycline and nalidixic acid resistance was detected in all 58 *E. coli* isolates from feces. Resistance to sulfamethoxazole-trimethoprim and ciprofloxacin was 84.5% and 69%, respectively. Imipenem was shown to be effective against 89.7% of *E. coli* isolates, followed by azithromycin (81%), gentamicin (69%), and ceftriaxone (65.5%). On the contrary, all isolates from water samples were resistant to ampicillin, tetracycline, and nalidixic acid. Imipenem resistance was not found in any of the isolates. Antibiogram profiles of feces and water isolates to different antibiotics are illustrated in Figure 1 and Figure 2.

Antibiotic-resistant genes

A total of seven antibiotic-resistant genes have been amplified, namely, *bla*TEM, *bla*CTX-M (beta-lactam resistant gene), *sul*1, *sul*2 (sulfur drug resistant gene), and *tet*A, *tet*B, *tet*C (tetracycline resistant gene). In case of fecal isolates, *bla*TEM (72.4%) and *tet*A (67.2%) were the most prevalent type. The *bla*TEM and *tet*A resistance genes were amplified from 80% and 73.3% of the water isolates, respectively. The PCR amplification of the *tet*B and *tet*C did not yield any amplicon from both fecal and water isolates. The occurrence percentage of targeted resistant genes among *E. coli* isolates is presented in Table 4.

Discussion

Antibiotic resistance is a burning issue and global threat that has a great impact both on human and animal health (Čižman, 2003; McEwen *et al.,* 2019). In general, bacteria can become resistant in different ways, but the indiscriminate use of antibiotics in food animals (cattle, goat, sheep, chicken, and duck) may lead to multidrug resistance. Multidrug resistance is one of the biggest public health challenges in the last years throughout the world. In Bangladesh, antibiotic-resistant *E. coli* has been found in different environmental and biological resources both of human

and animal origin (Jain *et al.*, 2021; Sarker *et al.*, 2019b).

The present study showed the overall prevalence of *E. coli* in duck feces and nearby water sources. *E. coli* was found in 64.4% and 100% of duck feces and nearby water from three LBMs in Chattogram, respectively. Singh *et al.* (2013) found almost similar results with *E. coli* detected in 66.67% and 75.0% of duck feces in both Nepal and Bangladesh, respectively. Recently, Dube and Mbanga (2018) reported that the prevalence of avian fecal *E. coli* in the duck was 56.0% which supports our results. On the other hand, some experts claimed that the incidence of *E. coli* in ducks is higher than our findings (Adzitey *et al.*, 2013; Kissinga *et al.*, 2018).

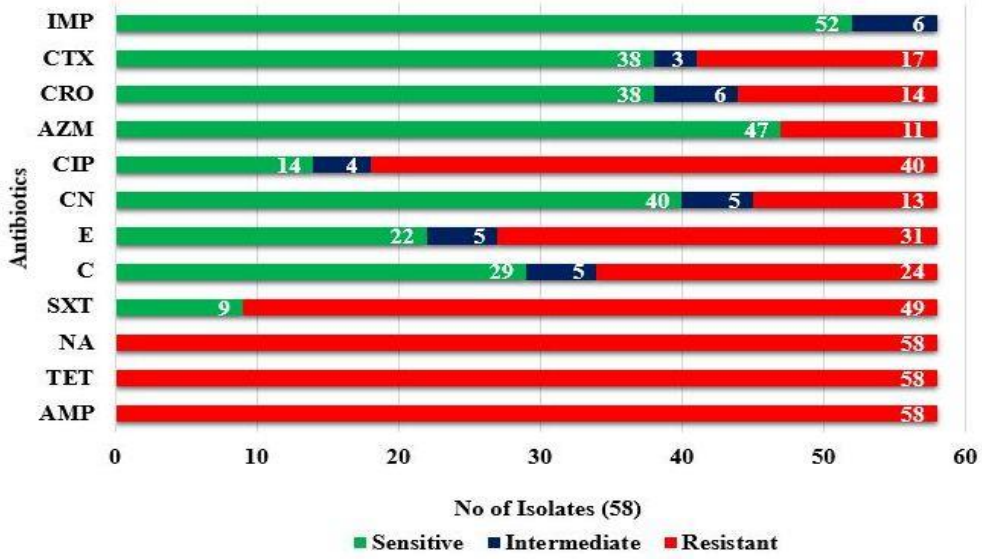

Figure 1. Antibiotic susceptibility profile of fecal *E. coli* isolates (n=58). AMP: Ampicillin, TET: Tetracycline, NA: Nalidixic acid, SXT: Sulfamethoxazole- trimethoprim, C: Chloramphenicol, E: Erythromycin, CN: Gentamicin, CIP: Ciprofloxacin, AZM: Azithromycin, CRO: Ceftriaxone, CTX: Cefotaxime, IMP: Imipenem.

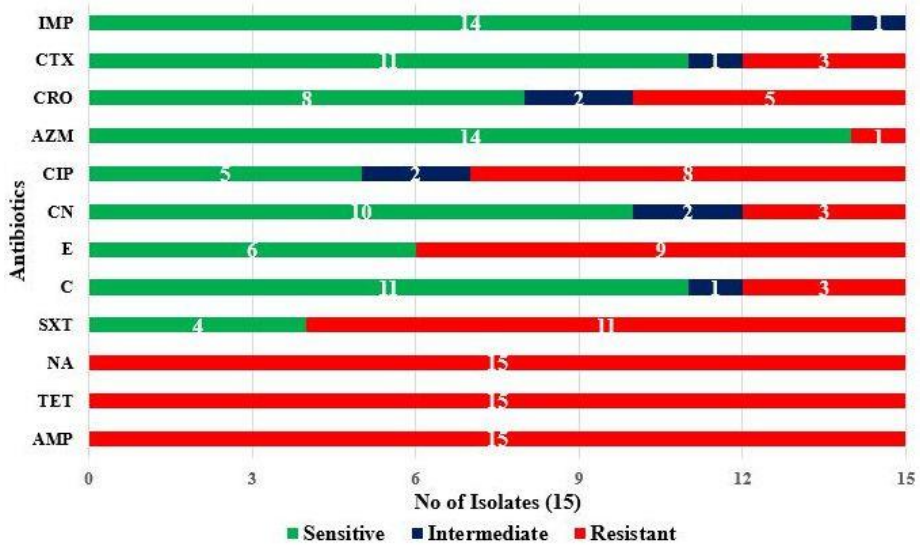

Figure 2. Antibiotic susceptibility profile of *E. coli* isolates from tap water sources (n=15).

All *E. coli* isolates from duck feces and tap water were MDR. The MDR profile of individual isolates from feces and water samples was demonstrated in supplementary Figure 3, respectively. The MAR index value for both fecal and water isolates ranged from 0.33 to 0.67 (Figure 4). The MAR indexes of individual *E. coli* isolates from both sources are represented in supplementary Table 2 and Table 3.

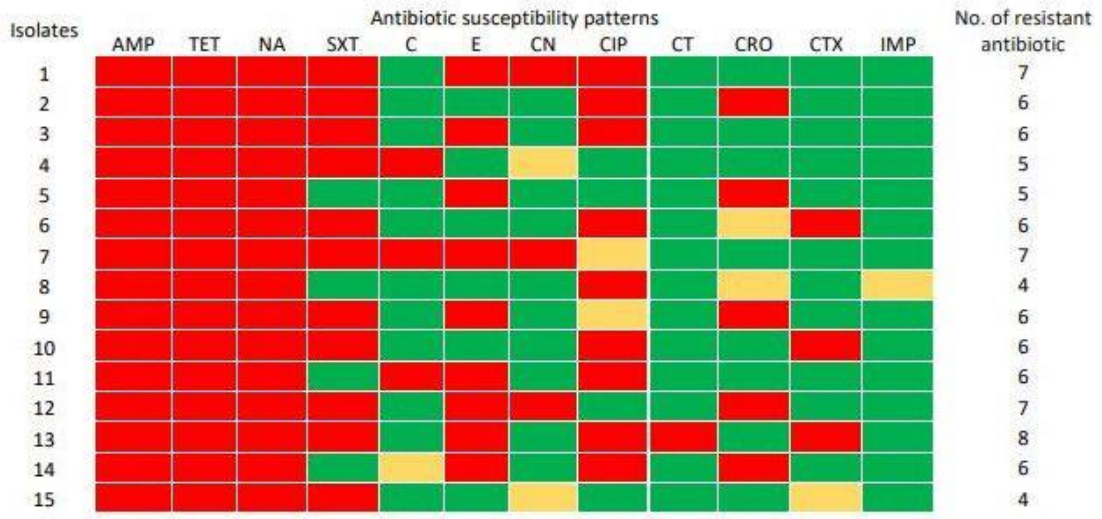

Figure 3. Heat map of antibiogram of *E. coli* isolates from tap water (n=15). R: Resistant, ABO: Antibiotic, Red cell: Resistant, Yellow cell: Intermediate, Green cell: Sensitive. All 15 isolates were MDR.

Table 2. MAR index value of fecal *E. coli* isolates (n=58)

Isolate No.	No. of resistant antibiotic	MAR index	Isolate No.	No. of resistant antibiotic	MAR index
1	8	0.67	30	7	0.58
2	8	0.67	31	7	0.58
3	6	0.5	32	5	0.42
4	7	0.58	33	8	0.67
5	8	0.67	34	7	0.58
6	6	0.5	35	8	0.67
7	6	0.5	36	5	0.42
8	7	0.58	37	8	0.67
9	5	0.42	38	7	0.58
10	7	0.58	39	6	0.5
11	8	0.67	40	6	0.5
12	7	0.58	41	7	0.58
13	6	0.5	42	6	0.5
14	5	0.42	43	7	0.58
15	6	0.5	44	7	0.58
16	8	0.67	45	6	0.5
17	5	0.42	46	5	0.42
18	8	0.67	47	7	0.58
19	6	0.5	48	6	0.5
20	6	0.5	49	6	0.5
21	6	0.5	50	7	0.58
22	8	0.67	51	4	0.33
23	5	0.42	52	7	0.58
24	7	0.58	53	6	0.5
25	5	0.42	54	5	0.42
26	8	0.67	55	6	0.5
27	7	0.58	56	6	0.5
28	5	0.42	57	5	0.42
29	7	0.58	58	5	0.42

Table 3. MAR index value of *E. coli* isolates from tap water (n=15)

Isolate No	No. of resistant antibiotic	MAR index
1	7	0.58
2	6	0.5
3	6	0.5
4	5	0.42
5	5	0.42
6	6	0.5
7	7	0.58
8	4	0.33
9	6	0.5
10	5	0.42
11	6	0.5
12	7	0.58
13	8	0.67
14	6	0.5
15	4	0.33

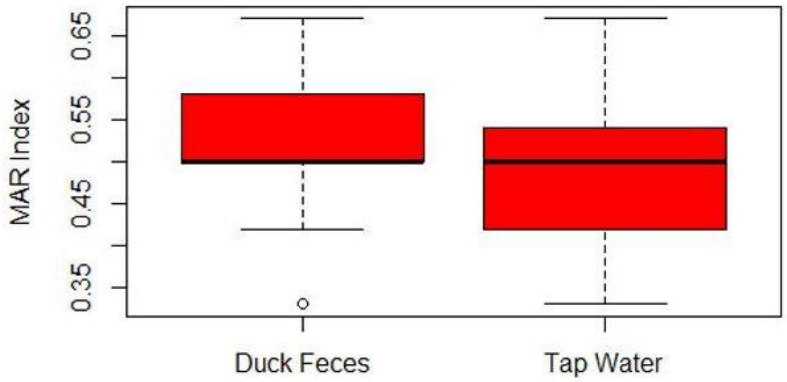

Figure 4. MAR Index of *E. coli* isolates from duck feces and tap water samples (n=15)

Table 4. Percentage occurrence of targeted resistant genes

Antibiotics	Resistant genes	% of resistant genes in feces	% of resistant genes in water
Ampicillin	*bla*TEM	72.4 (42/58)	80 (12/15)
Ceftriaxone	*bla*CTX-M	14.3 (2/14)	0 (0/5)
Tetracycline	*tet*A	67.2 (39/58)	73.3 (11/15)
	*tet*B	0	0
	*tet*C	0	0
Sulphamethoxazole-Trimethoprim	*sul*1	46.9 (23/49)	36.3 (4/11)
	*sul*2	55.1 (27/49)	54.5 (6/11)

In the current study, *E. coli* isolates from duck feces were shown to be resistant (100%, n=58/58) to ampicillin, tetracycline, and nalidixic acid. According to published statistics, *E. coli* isolates in various nations (Tanzania, Slovakia, and Malaysia) were more resistant to ampicillin than to other antibiotics (Adzitey *et al.,* 2013; Kissinga *et al.,* 2018; Tao *et al.,* 2010). The major explanation might be the overuse of ampicillin in human medicine, which could lead to pollution of the environment through human waste, which scavenges by ducks as feed. Also, the use of low therapeutic doses of antibiotic drugs as a growth stimulant in animal feed results in the development of resistance strains (Beninati *et al.,* 2015). Furthermore, a few studies in Bangladesh found that 100% of *E. coli* isolates in poultry were resistant to ampicillin and tetracycline (Azad *et al.,* 2019; Sarker *et al.,* 2019a) and even nalidixic acid resistance was prevalent (91.89%) (Sarker *et al.,* 2019a). Nevertheless, these results are exceedingly soaring but not shocking as the continuing use of these antibiotics in veterinary practice for a long time. Antibiotics such as tetracycline and sulfamethoxazole are commonly used to treat chickens and to promote growth performance in Bangladesh (Siddiky *et al.,* 2021 Due to a lack of adequate oversight, poultry

farmers can use these antibiotics without worry from veterinarians. The *E. coli* resistance to ciprofloxacin, which was 69% in this study, is a worrisome problem. Importantly, *E. coli* strains from ducks were susceptible to ciprofloxacin in Bangladesh as reported a decade ago (Singh *et al.,* 2013). This severe consequence in Bangladesh is due to the widespread use of ciprofloxacin in poultry for therapeutic purposes.

Antibiotic resistance is a worldwide problem because it may complicate the treatment of bacterial infections in both food animals and humans. The current study showed that 89.7%, 81.0%, 69.0%, and 65.5% of the *E. coli* isolates were susceptible to imipenem, azithromycin, gentamicin, and ceftriaxone, respectively, while Sarker *et al.* (2019a) depicted that 56.76% were susceptible to gentamicin and 56.76% to ceftriaxone in poultry. *E. coli* isolates in tap water indicated fecal contamination in the environment. All of the isolates were MDR in tap water. Vendors use tap water to drink the poultry in LBMs. It is assumed that resistant *E. coli* can transfer via drinking water to ducks. Notably, all *E. coli* isolates from both sources were MDR (100%) in this study. Some studies reported that isolates of *E. coli* were 100% MDR not only in Bangladesh but also in other countries such as Countries like America (Azad *et al.,* 2019; Rahman *et al.,* 2011). The indiscriminate use of antibiotic agents in food animals and humans leads to the development of MDR and this will tend to increase in the next years (van den Bogaard *et al.,* 2001). However, the results of MDR patterns will help veterinarian choose the right antibiotics for poultry diseases. In this investigation, 100% of *E. coli* isolates from both sources had a MAR index of 0.33 or higher, which is concerning. A MAR index of 0.4 or higher indicates human fecal contamination, whereas a MAR index of 0.2 or higher indicates high-risk contamination sources with regular antibiotic usage (Mishra *et al.,* 2013; Thenmozhi *et al.,* 2014).

Various resistance genes have been identified in *E. coli* resistant isolates; namely *bla*TEM, *bla*CTX-M, *tet*A, *tet*B, *tet*C, *sul*1 and *sul*2. The *bla*TEM (72.4%) and *tet*A (67.2%) genes were the most prevalent type in fecal isolates. The occurrence of *bla*TEM, *tet*A, and *sul*2 resistant genes in *E. coli* isolates in poultry was reported in the last few years (Adelowo *et al.,* 2014; Sarker *et al.,* 2019a) which corroborates our findings as those resistance genes prevail in our country. Though, it is quite variable of different resistance genes of *E. coli* from poultry in comparison with the respective study. It is possible to deduce that bacterial resistance is influenced by medication selections and regional differences.

Conclusion

The occurrence of MDR *E. coli* in ducks and water supplies in live bird markets is still very concerning and may lead to public health problems. Antibiotics had been utilized extensively in duck farms, as evidenced by the presence of a MAR index of more than 0.2 in all of the *E. coli* isolates. The results of this study unveiled the necessity of AMR surveillance throughout the country to monitor the indiscriminate use of antimicrobials. The findings of this study would help to implement the national AMR surveillance strategy in LBMs to ensure food safety and minimize the spread of resistant bacteria to the environment through good market management. Moreover, extensive studies are required to ascertain the sources and risk factors associated with AMR in ducks and water sources in live bird markets.

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Conflict of interest

None to declare

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References

Abd El Tawab A, Ammar A, Nasef S & Reda R. 2015. Prevalence of *E. coli* in diseased chickens with its antibiogram pattern. Benha Veterinary Medical Journal, 28(2): 224–230. DOI: 10.21608/bvmj.2015.32507

Adelowo OO, Fagade OE & Agersø Y. 2014. Antibiotic resistance and resistance genes in *Escherichia coli* from poultry farms, southwest Nigeria. Journal of Infection in Developing Countries, 8(9): 1103–1112. DOI: 10.3855/jidc.4222

Adzitey F, Ali GRR, Huda N & Ting SL. 2013. Antibiotic resistance and plasmid profile of *Escherichia coli* isolated from ducks in Penang, Malaysia. International Food Research Journal, 20(3): 1473–1478.

Azad M, Rahman MM, Amin R, Begum M, Fries R, Husna A, Khairalla AS, Badruzzaman AT, El Zowalaty ME, Lampang KN & Ashour HM. 2019. Susceptibility and Multidrug Resistance Patterns of *Escherichia coli* Isolated from Cloacal Swabs of Live Broiler Chickens in Bangladesh. Pathogens (Basel, Switzerland), 8(3): 118. DOI:

10.3390/pathogens8030118

Batchelor M, Hopkins K, Threlfall E J, Clifton-Hadley FA, Stallwood AD, Davies RH & Liebana E. 2005. blaCTX-M genes in clinical *Salmonella* isolates recovered from humans in England and Wales from 1992 to 2003. Antimicrobial Agents and Chemotherapy, 49(4): 1319–1322. DOI: 10.1128/AAC.49.4.1319-1322.2005

Belaaouaj A, Lapoumeroulie C, Caniça MM, Vedel G, Névot P, Krishnamoorthy R & Paul G. 1994. Nucleotide sequences of the genes coding for the TEM-like β-lactamases IRT-1 and IRT-2 (formerly called TRI-1 and TRI-2). FEMS Microbiology Letters, 120(1–2): 75–80. DOI: 10.1111/j.1574-6968.1994.tb07010.x

Beninati C, Reich F, Muscolino D, Giarratana F, Panebianco A, Klein G & Atanassova V. 2015. ESBL-producing bacteria and MRSA isolated from poultry and turkey products imported from Italy. Czech Journal of Food Sciences, 33(2): 97–102. DOI: 10.17221/428/2014-CJFS

Čižman M. 2003. The use and resistance to antibiotics in the community. International Journal of Antimicrobial Agents, 21(4): 297–307. DOI: 10.1016/S0924-8579(02)00394-1

CLSI. 2015. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-fifth informational supplement, Document M100-S25. Clinical and Laboratory Standards Institute, Wayne, PA.

Dube N & Mbanga J. 2018. Molecular characterization and antibiotic resistance patterns of avian fecal *Escherichia coli* from turkeys, geese, and ducks. Veterinary World, 11(6): 859–867. DOI: 10.14202/vetworld.2018.859-867

FDA. 2014. National antimicrobial resistance monitoring system. http://www.fda.gov/AnimalVeterinary/SafetyHealth/AntimicrobialResistance/NationalAntimicrobialResistanceMonitoring System/default.htm [accessed on 10 November (2020)]

Hamid MA, Rahman MA, Ahmed S & Hossain KM. 2016. Status of poultry industry in Bangladesh and the role of private sector for its development. Asian Journal of Poultry Science, 11(1): 1–13. DOI: 10.3923/ajpsaj.2017.1.13

Jain P, Bepari AK, Sen PK, Rafe T, Imtiaz R, Hossain M & Reza HM. 2021. High prevalence of multiple antibiotic resistance in clinical *E. coli* isolates from Bangladesh and prediction of molecular resistance determinants using WGS of an XDR isolate. Scientific Reports, 11(1): 22859. DOI: 10.1038/s41598-021-02251-w

Karczmarczyk M, Abbott Y, Walsh C, Leonard N & Fanning S. 2011. Characterization of multidrug-resistant *Escherichia coli* isolates from animals presenting at a university veterinary hospital. Applied and Environmental Microbiology, 77(20): 7104–7112. DOI: 10.1128/AEM.00599-11

Kissinga HD, Mwombeki F, Said K, Katakweba AAS, Nonga HE & Muhairwa AP. 2018. Antibiotic susceptibilities of indicator bacteria *Escherichia coli* and *Enterococci* spp. isolated from ducks in Morogoro Municipality, Tanzania. BMC Research Notes, 11(1): 4–9. DOI: 10.1186/s13104-018-3201-4

Lanz R, Kuhnert P & Boerlin P. 2003. Antimicrobial resistance and resistance gene determinants in clinical *Escherichia coli* from different animal species in Switzerland. Veterinary Microbiology, 91(1): 73–84. DOI: 10.1016/S0378-1135(02)00263-8

Layman DK & Rodriguez NR. 2009. Egg protein as a source of power, strength, and energy. Nutrition Today, 44(1): 43–48. DOI: 10.1097/NT.0b013e3181959cb2

Li B, Ma L, Li Y, Jia H, Wei J, Shao D, Liu, K, Shi Y, Qiu Y & Ma Z. 2017. Antimicrobial resistance of Campylobacter species isolated from broilers in live bird markets in shanghai, China. Foodborne Pathogens and Disease, 14(2): 96–102. DOI: 10.1089/fpd.2016.2186

McEwen SA, Aarestrup FM & Jordan D. 2019. Monitoring of Antimicrobial Resistance in Animals: Principles and Practices. Antimicrobial Resistance in Bacteria of Animal Origin, 388: 397–413. DOI: 10.1128/9781555817534.ch23

Mishra M, Patel AK & Behera N. 2013. Prevalence of multidrug resistant *E. coli* in the river Mahanadi of Sambalpur. Current Research in Microbiology and Biotechnology, 1(5): 239-244.

Ng LK, Martin I, Alfa M & Mulvey M. 2001. Multiplex PCR for the detection of tetracycline resistant genes. Molecular and Cellular Probes, 15(4): 209–215. DOI: 10.1006/mcpr.2001.0363

Paul S, Bezbaruah RL, Roy MK & Ghosh AC. 1997. Multiple antibiotic resistance (MAR) index and its reversion in Pseudomonas aeruginosa. Letters in Applied Microbiology, 24(3): 169-71. DOI: 10.1046/j.1472-765x.1997.00364.x

Rahman MM, Bashar T, Rahman M, Rabbi FA & Noor R. 2011. Enterotoxin profiling and antibiogram of *Escherichia coli* isolated from poultry feces in Dhaka district of Bangladesh. Stamford Journal of Microbiology, 1(1): 51–57. DOI: 10.3329/sjm.v1i1.9134

Sánchez S, Martínez R, García A, Benítez JM, Blanco J, Blanco JE, Dahbi G, López C, Mora A & Alonso JM. 2010. Variation in the prevalence of non-O157 Shiga toxin-producing *Escherichia coli* in four sheep flocks during a 12-month longitudinal study. Small Ruminant Research, 93(2-3): 144-148. DOI: 10.1016/j.smallrumres.2010.05.014

Sarker MS, Mannan MS, Ali MY, Bayzid M, Ahad A

& Bupasha ZB. 2019a. Antibiotic resistance of *Escherichia coli* isolated from broilers sold at live bird markets in Chattogram, Bangladesh. Journal of Advanced Veterinary and Animal Research, 6(3): 272–277. DOI: 10.5455/javar.2019.f344

Sarker MS, Ahad A, Ghosh, SK, Mannan MS, Sen A, Islam S & Bayzid M, Bupasha ZB. 2019b. Antibiotic-resistant *Escherichia coli* in deer and nearby water sources at Safari parks in Bangladesh. Veterinary World, 12(10): 1578–1583. DOI: 10.14202/vetworld.2019.1578-1583

Seidavi A, Mirhosseini SZ, Shivazad M, Chamani M, Sadeghi AA & Pourseify R. 2010. Detection and investigation of *Escherichia coli* in contents of duodenum, jejunum, ileum and cecum of broilers at different ages by PCR. Asia Pacific Journal of Molecular Biology and Biotechnology, 18: 321-326.

Siddiky NA, Sarker MS, Khan MSR, Begum R, Kabir ME, Karim MR, Rahman MT, Mahmud A & Samad MA. 2021. Virulence and antimicrobial resistance profiles of *salmonella* enterica serovars isolated from chicken at wet markets in Dhaka, Bangladesh. Microorganisms, 9: 952. DOI: 10.3390%2Fmicroorganisms9050952

Singh A, Khan MSR, Saha S, Hassan J & Roy U. 2013. Isolation and detection of antibiotic sensitivity pattern of *escherichia coli* from ducks in Bangladesh and Nepal. Microbes and Health, 1(1): 6–8. DOI: 10.3329/mh.v1i1.13704

Sunde M. 2005. Prevalence and characterization of class 1 and class 2 integrons in *Escherichia coli* isolated from meat and meat products of Norwegian origin. Journal of Antimicrobial Chemotherapy, 56(6): 1019–1024. DOI: 10.1093/jac/dki377

Tao R, Ying GG, Su HC, Zhou HW & Sidhu JPS. 2010. Detection of antibiotic resistance and tetracycline resistance genes in *Enterobacteriaceae* isolated from the Pearl rivers in South China. Environmental Pollution, 158(6): 2101–2109. DOI: 10.1016/j.envpol.2010.03.004

Thenmozhi S, Rajeswari P, Kumar BS, Saipriyanga V, Kalpana M. 2014. Multi-drug resistant patterns of biofilm forming Aeromonas hydrophila from urine samples. International Journal of Pharmaceutical Sciences and Research, 5(7): 2908. DOI: 10.13040/IJPSR.0975-8232.5(7). 2908-18

van den Bogaard AE, London N, Driessen C & Stobberingh EE. 2001. Antibiotic resistance of faecal *Escherichia coli* in poultry, poultry farmers and poultry slaughterers. Journal of Antimicrobial Chemotherapy, 47(6): 763–771. DOI: 10.1093/jac/47.6.763

Yang B, Qu D, Zhang X, Shen J, Cui S, Shi Y & Wang D. 2010. Prevalence and characterization of *Salmonella* serovars in retail meats of marketplace in Shaanxi, China. International Journal of Food Microbiology, 141(1–2): 63–72. DOI: 10.1016/j.ijfoodmicro.2010.04.015

Zhong CY, Cheng AC, Wang MS, Zhu DK, Luo QH, De Zhong C, *et al*. 2009. Antibiotic susceptibility of riemerella anatipestifer field isolates. Avian Diseases, 53(4): 601–607. DOI: 10.1637/8552-120408-ResNote.1

Zhu Y, Lai H, Zou L, Yin S, Wang C, Han X, Xia X, Hu K, He L, Zhou K & Chen S. 2017. Antimicrobial resistance and resistance genes in *Salmonella* strains isolated from broiler chickens along the slaughtering process in China. International journal of food microbiology, 259: 43-51. DOI: 10.1016/j.ijfoodmicro.2017.07.023