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Journal

Microbial Ecology: an international journal, 83(4)

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Publication Date

2022-05-01

DOI

10.1007/s00248-021-01803-x

Peer reviewed

Microbial Ecology (2022) 83:942–950
https://doi.org/10.1007/s00248-021-01803-x

ENVIRONMENTAL MICROBIOLOGY

Detection of bla_{TEM}, bla_{CTX-M}, bla_{CMY}, and bla_{SHV} Genes Among Extended-Spectrum Beta-Lactamase-Producing *Escherichia coli* Isolated from Migratory Birds Travelling to Bangladesh

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Received: 25 April 2021 / Accepted: 18 June 2021 / Published online: 27 July 2021

Abstract

Extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* cause severe health hazards. Migratory birds are reservoirs and transmitters of many pathogens including ESBL-producing *E. coli*. To examine migratory birds as potential carriers of ESBL-producing *E. coli* and *E. coli*-carrying antibiotic resistance genes, 55 PCR-positive *E. coli* isolates were screened using the disk diffusion method, double-disk synergy test, and further polymerase chain reaction (PCR) tests. Genes encoding resistance to tetracycline [*tetA*, 100% (35/35); *tetB*, 31.43% (11/35)], fluoroquinolone [*qnrA*, 35.71% (10/28); *qnrB*, 25% (7/28)], and streptomycin [*aadA1*, 90.24% (37/41)] were detected in the isolated *E. coli*. Of the 55 *E. coli* isolates, 21 (38.18%) were ESBL producers, and all of them were multidrug resistant. All the ESBL-producing *E. coli* isolates harbored at least two or more beta-lactamase genes, of which bla_{TEM}, bla_{CMY}, bla_{CTX-M}, and bla_{SHV} were detected in 95.24%, 90.48%, 85.71%, and 42.86% of isolates, respectively. All the beta-lactamase genes were present in four of the ESBL-producing *E. coli* isolates. Furthermore, 95.24% of ESBL-producing *E. coli* isolates were positive for one or more antibiotic resistance genes. To the best of our knowledge, this is the first study to detect *E. coli*-carrying antibiotic resistance genes including beta-lactamase bla_{CMY} and bla_{SHV} originating from migratory birds in Bangladesh. These results suggest that migratory birds are potential carriers of ESBL-producing *E. coli* along with other clinically important antibiotic resistance genes which may have detrimental impacts on human health.

Keywords Migratory birds · Antibiotic resistance genes · ESBL · bla_{CMY} · bla_{SHV} · Public health

Introduction

Antimicrobial resistance (AMR) is a major global health concern of the twenty-first century affecting all the components of health including animals, humans, and environments [1]. Selective pressure resulting from the overuse of antibiotics is a major driver for the development of resistance [2]. Migratory birds during the course of their travels acquire and transmit antibiotic-resistant bacteria and/or AMR genes across environments [3, 4].

Escherichia coli, commensal bacteria, are regarded as excellent indicative species to reveal the transmission and dissemination of AMR via water contaminated by fecal materials [5, 6]. In addition, *E. coli* are abundant in different water bodies where migratory birds reside and are involved in retaining AMR in those locations [7]. Thus, waterfowl including migratory birds have the potential to be sentinels of AMR *E. coli* in the environment.

Cephalosporins comprise a widely used group of beta-lactam antibiotics in humans. The dissemination of extended-spectrum beta-lactamase (ESBL)-producing *E. coli* in humans and animals has increasingly emerged around the globe. *E. coli* increasingly encounters beta-lactam antibiotics and has developed resistance, causing common community-related infections [8]. The production of beta-lactamase, a degradative enzyme, usually mediates beta-lactam resistance in *E. coli* pathogens [9]. The existence of ESBL resistance within *E. coli* has emerged as a global

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crisis in antibiotic choices [10]. Different classes of antimicrobials, e.g., tetracyclines, aminoglycosides, fluoroquinolones, and trimethoprim–sulfamethoxazole, have been commonly become ineffective in beta-lactamase-producing *E. coli* resulting in increased morbidity–mortality, prolonging hospitalization, increased treatment cost, and deterioration of healthcare systems [10, 11]. Two plasmid-borne classes of beta-lactam enzyme, ESBL and AmpC beta-lactamases (*AmpC*), impact the health sector negatively throughout the world, causing resistance to beta-lactam antibiotics [12]. Additionally, CTX-M, TEM, and SHV genes comprise the key forms of ESBL and are present in a wide range of clinically important pathogens globally [13].

Bangladesh, having suitable water bodies and comfortable weather, attracts a plethora of migratory birds mostly during the winter migration every year [14]. Migratory birds can contaminate these water bodies by transmitting pathogens via their fecal materials [15]. In Bangladesh, most of the habitats of migratory birds directly or indirectly interact with humans. Rural people are intimately associated with the water bodies which form the habitats of migratory birds and use them for activities such as fishing, bathing, and rearing ducks. In Bangladesh, most rural people rear ducks, chickens, and cattle and/or goats in their courtyards. In addition, they tend to use the contaminated water from these water bodies for agricultural purposes. Therefore, pathways exist for transmission of the pathogens of migratory birds to human and other animals. Thus, by contamination of water bodies, migratory birds pose a risk to both human and animal health.

Beta-lactamase-producing *E. coli* is often used to trace the evolution of multi-resistant bacteria in the environment and in migratory birds [5]. Several previous studies have indicated that migratory birds are carriers and reservoirs of ESBL-producing *E. coli* to humans and animals [4, 16]. In addition, they are suggested as carriers and reservoirs of antibiotic resistance genes globally [17, 18]. However, in Bangladesh, there is scarce data on the prevalence of ESBL-producing *E. coli*-carrying antibiotic resistance genes, and until now, the antibiotic resistance gene-carrying capabilities of migratory birds have been neglected. In the present study, we screened fecal materials of birds migrating via Bangladesh for ESBL-producing *E. coli*, to gain a better insight into the role of migratory birds as carriers of resistant bacteria, especially ESBL-producing *E. coli*.

Materials and Methods

Ethical Statement

The methodologies and related protocols used in this study were approved by the institutional ethical committee (AWEEC/BAU/2019(14)).

Selection of E. coli Isolates

E. coli strains used in this study were obtained in our previous study on migratory birds [19]. Original detection and isolation of these *E. coli* strains were done following culture on eosin methylene blue agar (HiMedia, India) plates, grams staining, biochemical tests (catalase test, coagulase test, sugar fermentation tests, methyl red test, Voges–Proskauer test, and indole test), and PCR targeting *malB* gene [19].

Detection of ESBL-Producing E. coli and Antimicrobial Susceptibility Testing

Antimicrobial resistance profiles of the isolated *E. coli* strains were determined by the Kirby–Bauer disk diffusion test [20]. Different classes of antibiotics, namely, penicillins (ampicillin, 25 µg), tetracyclines (tetracycline, 30 µg), fluoroquinolones (ciprofloxacin, 5 µg), aminoglycosides (gentamicin, 10 µg; streptomycin, 10 µg), macrolides (erythromycin, 15 µg), carbapenems (meropenem, 10 µg), polypeptides (colistin, 10 µg), and amphenicols (chloramphenicol, 10 µg) (HiMedia, India), were used in the sensitivity test. The results were interpreted in accordance with the guidelines of the Clinical and Laboratory Standards Institute [21]. ESBL-producing *E. coli* isolates were phenotypically screened by amoxicillin/clavulanate acid (amoxyclav) (30 µg), cefotaxime (30 µg), and ceftazidime (30 µg) disks (HiMedia, India) following the double-disk synergy test [21]. On Mueller–Hinton agar (HiMedia, India) plates inoculated with freshly growth bacterial culture (matched with 0.5 McFarland standard) (HiMedia, India), two third-generation cephalosporin-containing disks, namely, cefotaxime (30 µg) and ceftazidime (30 µg), were placed 30 mm apart (center to center) from an amoxicillin/clavulanic acid disk (amoxicillin, 20 µg, and clavulanic acid, 10 µg), followed by incubating in an incubator (SANYO, MCO175, Japan) for overnight at 37 °C and measuring the zone diameter of each disk. ESBL-producing *E. coli* were categorized as strains resistant to ceftazidime ($\leq$ 17 mm) and cefotaxime ($\leq$ 22 mm) and showed the enhancement of zone diameter ($\geq$ 5 mm) of any of these two disks toward clavulanic acid containing disk [21]. Furthermore, any isolates showing resistance against three or more different classes of antibiotic were recognized as multidrug resistant (MDR) [22].

Detection of Beta-Lactamase Genes and Antibiotic Resistance Genes of E. coli Isolates

A simplex PCR assay was performed for the detection of beta-lactamase genes (bla_{TEM}, bla_{CTX-M}, bla_{CMY}, and bla_{SHV}) [23–26]. In addition, the genes associated with resistance to

tetracyclines (*tetA*, *tetB*) [23], fluoroquinolones (*qnrA*, *qnrB*) [27, 28], aminoglycosides (*aadA1*) [23], macrolides (*ereA*) [24], and amphenicols (*catA1*) [24] were tested. Target genes and primers (Macrogen, Korea) associated with different beta-lactamase genes and antibiotic resistance genes are documented in Table 1.

For PCR, genomic DNA were extracted by boiling method as previously described [2]. Briefly, 1 ml of overnight freshly growth culture was centrifuged at 2,300 × g for 5 min in a centrifuge machine (KUBOTA 6500, Japan), and the supernatant was discarded. Then the pellet was suspended to 200 µl of phosphate buffer solution (PBS) (HiMedia, India) and boiled and cooled for 10 min in each step. After that, the suspension was centrifuged at 9,200 × g for 10 min, and the supernatant was collected as genomic DNA, followed by storing in Eppendorf tubes at − 20 °C for further use.

The PCR amplification was carried out with a 20 µl of final volume [master mix (2 ×) (Promega, Madison, WI, USA), 10 µL; nuclease-free water (Promega, Madison, WI, USA), 4 µL; each primer, 1 µL (0.2 pmol/ml); and genomic DNA (50 ng/ µL), 4 µL] in PCR thermal cycler (ASTEC, Japan). The thermal profile of PCR consisted initial denaturation, 95 °C for 5 min; 30 cycles of denaturation, 95 °C for 1 min; annealing variable temperature (Table 1) for 1 min; elongation, 72 °C for 1 min; and final extension, 72 °C for 10 min. PCR-positive controls consisted of *E. coli* genomic DNA which were previously positive for relevant genes. Non-template controls were used as PCR negative controls, where PBS was used instead of genomic DNA. The amplified PCR products were then analyzed with 1.5% agarose (Invitrogen, USA) in a gel electrophoresis apparatus (Nippon Genetics, Japan) and subsequently stained with ethidium bromide (0.5 µg/ml) (HiMedia, India). Finally, the ultra-violet trans-illuminator (Biometra, Germany) was used to capture the expected amplicon sizes. The sizes were compared with a 1 kb DNA ladder (Promega, Madison, WI, USA).

Statistical Analysis

Descriptive Analysis

Excel 2013 spreadsheet (Microsoft Office 2013, Microsoft, Los Angeles, CA, USA) was used to analyze data, and the Statistical Package for Social Science (SPSS) (IBM SPSS 25, IBM, Chicago, IL, USA) and GraphPad Prism version 8.4.3 (GraphPad Software, Inc.) were employed to perform statistical analysis.

The Pearson chi-square test for goodness-of-fit was performed to identify the variations among beta-lactamase genes of *E. coli* isolates. P-values less than 0.05 (p-value < 0.05) were deemed statistically significant. Following the Wilson/Brown hybrid method [29], binomial 95% confidence intervals were computed using GraphPad Prism.

Table 1 List of primers used in the current study to detect beta-lactam and antibiotic resistance genes of *Escherichia coli* isolated from fecal materials of migratory birds

Target genes	Primer sequence (5'–3')	Amplicon size (bp)	Annealing temperature (°C)	References
tetA	F: GGTTCACTCGAACGACGTCA R: CTGTCCGACAAGTTGCATGA	577	57	[23]
tetB	F: CCTCAGCTTCTCAACGCGTG R: GCACCTTGCTGATGACTCTT	634	56	[23]
qnrA	F: GGGTATGGATATTATTGATAAAG R: CTAATCCGGCAGCACTATTTA	670	55	[27]
qnrB	F: GATCGTGAAAGCCAGAAAGG R: ACGATGCCTGGTAGTTGTCC	469	53	[28]
aadA1	F: TATCAGAGGTAGTTGGCGTCAT R: GTTCCATAGCGTTAAGGTTTCATT	484	55	[23]
ereA	F: GCCGGTGCTCATGAACTTGAG R: CGACTCTATTCGATCAGAGGC	419	52	[24]
catA1	F: AGTTGCTCAATGTACCTATAACC R:TTGTAATTCATTAAGCATTCTGCC	547	55	[24]
bla_{TEM}	F: CATTTCCGTGTCGCCCTTAT R: TCCATAGTTGCCTGACTCCC	793	56	[23]
bla_{CTX-M}	F: ATGTGCAGYACCAGTAARGTKATGGC R: TGGGTRAARTARGTSACCAGAAYSAGCGG	592	55	[25]
bla_{CMY}	F: TGGCCAGAACTGACAGGCAAA R: TTTCTCCTGAACGTGGCTGGC	462	47	[26]
bla_{SHV}	F: TCGCCTGTGTATTATCTCCC R: CGCAGATAAATCACCACAATG	768	52	[24]

Bivariate Analysis

Using IBM SPSS statistics (version 25), a bivariate analysis was employed to evaluate the correlation between antibiotics resistance to ESBL-producing *E. coli* and to determine the associations between beta-lactamase genes of *E. coli* isolates. Any p-value less than 0.05 was considered statistically significant.

Results

Phenotypic Prevalence of ESBL-Producing E. coli

Among 55 PCR-positive *E. coli* isolates, 21 (38.18%; 95% CI, 26.52–51.39%) were confirmed as phenotypically ESBL-producing *E. coli* by double-disk synergy test.

Genotypic Prevalence of Antibiotic-Resistant E. coli

In the antibiogram, all the 55 *E. coli* isolates were found phenotypically resistant to ampicillin and erythromycin, 41 to streptomycin, 35 to tetracycline, 28 to ciprofloxacin, and 24 to chloramphenicol. By PCR, 90.24% (95% CI, 77.45–96.14%) of streptomycin-resistant isolates were found positive for the *aadA1* gene; 100% (95% CI, 90.11–100.00%) and 31.43% (95% CI, 18.55–47.98%) of tetracycline-resistant isolates, respectively, positive for *tetA* and *tetB* genes; 35.71% (95% CI, 20.71–54.17%) and 25% (95% CI, 12.68–43.36%) of ciprofloxacin-resistant isolates positive for *qnrA* and *qnrB* genes, respectively; and all the erythromycin- and chloramphenicol-resistant isolates were negative for *ereA* and *catA1* genes (Fig. 1).

Furthermore, of 21 ESBL-producing *E. coli* isolates, 20 isolates were positive for at least one antibiotic resistance gene. Among them, three isolates were positive for four resistance genes, followed by 11 for three genes and three for two or one gene. Notably, the *tetA* and *aadA1* genes were present in 17 ESBL-producing *E. coli* isolates (Table 2).

CIP, ciprofloxacin; *GEN*, gentamicin; *E*, erythromycin; *TE*, tetracycline; *MEM*, meropenem; *AMP*, ampicillin; *C*, chloramphenicol; *S*, streptomycin.

Phenotypic MDR Nature of ESBL-Producing E. coli

All the ESBL-producing *E. coli* isolates displayed a MDR phenotype. In total, seven resistance patterns were identified among the ESBL-producing *E. coli* isolates. The most common MDR phenotype was ciprofloxacin, erythromycin, tetracycline, ampicillin, chloramphenicol, and streptomycin (CIP-E-TE-AMP-C-S) which was found in 11 (52.38%) of the ESBL-producing *E. coli* isolates. Furthermore, one isolate (BB-30) was resistant against 7 antibiotics (6 classes) (Table 2). By bivariate analysis, a highly significant correlation was observed between resistance patterns of ciprofloxacin and tetracycline (Pearson correlation coefficients, $\rho = 0.868$, $p = < 0.001$), ciprofloxacin and chloramphenicol ($\rho = 0.791$, $p = < 0.001$), and tetracycline and chloramphenicol ($\rho = 0.686$, $p = 0.001$). A moderately significant correlation was identified between tetracycline and meropenem ($\rho = -0.461$, $p = < 0.035$) and streptomycin and meropenem ($\rho = -0.548$, $p = < 0.01$) (Table 3).

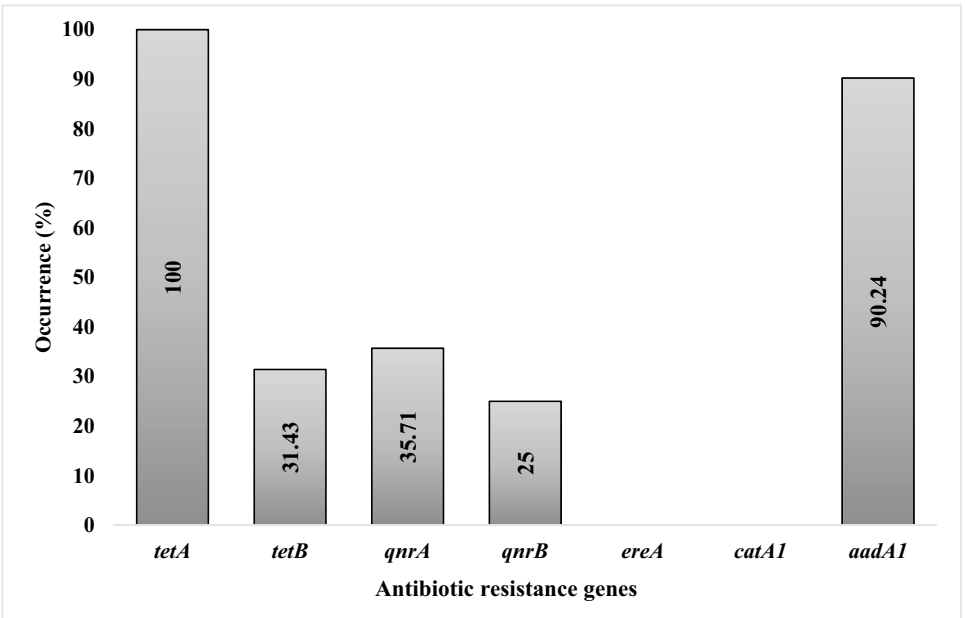

Fig. 1 Occurrence of various antibiotic resistance genes in the isolates *Escherichia coli* from fecal materials of migratory birds

Table 2 Phenotypic and genotypic resistance profiles of ESBL-producing *E. coli* isolated from migratory birds

Isolate name	Resistance phenotypes	Resistance genotype					
		Beta-lactamase genes	TE	CIP	S	E	C
BB-2	CIP, E, TE, AMP, C	bla_{TEM}, bla_{CMY}, bla_{CTX-M}, bla_{SHV}	*tetA*, *tetB*	*qnrA*, *qnrB*	-	-	-
BB-4	CIP, E, TE, AMP, C, S	bla_{TEM}, bla_{CMY}, bla_{CTX-M}, bla_{SHV}	*tetA*, *tetB*	-	*aadA1*	-	-
BB-6	CIP, E, TE, AMP, S	bla_{CTX-M}, bla_{SHV}	*tetA*, *tetB*	*qnrB*	*aadA1*	-	-
BB-9	CIP, E, TE, AMP, C, S	bla_{TEM}, bla_{CMY}, bla_{CTX-M}, bla_{SHV}	*tetA*, *tetB*	-	*aadA1*	-	-
BB-11	CIP, E, TE, AMP, C, S	bla_{TEM}, bla_{CMY}, bla_{CTX-M}	*tetA*	*qnrA*, *qnrB*	-	-	-
BB-14	CIP, E, TE, AMP, C, S	bla_{TEM}, bla_{CMY}, bla_{CTX-M}	*tetA*	-	*aadA1*	-	-
BB-17	CIP, E, TE, AMP, C	bla_{TEM}, bla_{CMY}, bla_{CTX-M}	*tetA*, *tetB*	*qnrA*	-	-	-
BB-19	E, AMP, S	bla_{TEM}, bla_{CMY}, bla_{CTX-M}	-	-	*aadA1*	-	-
BB-26	CIP, E, TE, AMP, C, S	bla_{TEM}, bla_{CMY}, bla_{CTX-M}, bla_{SHV}	*tetA*	*qnrB*	*aadA1*	-	-
BB-27	CIP, E, TE, AMP, C, S	bla_{TEM}, bla_{CMY}	*tetA*	*qnrA*	*aadA1*	-	-
BB-29	E, TE, AMP, S	bla_{TEM}, bla_{CMY}, bla_{CTX-M}	*tetA*	-	*aadA1*	-	-
BB-30	CIP, GEN, E, TE, AMP, C, S	bla_{TEM}, bla_{CTX-M}, bla_{SHV}	*tetA*, *tetB*	-	*aadA1*	-	-
BB-36	E, AMP, S	bla_{TEM}, bla_{CMY}, bla_{CTX-M}, bla_{SHV}	-	-	*aadA1*	-	-
BB-37	CIP, E, TE, AMP, C, S	bla_{TEM}, bla_{CMY}, bla_{CTX-M}	*tetA*, *tetB*	-	*aadA1*	-	-
BB-40	CIP, E, TE, AMP, S	bla_{TEM}, bla_{CMY}	*tetA*	*qnrA*	*aadA1*	-	-
BB-41	CIP, E, TE, AMP, C, S	bla_{TEM}, bla_{CMY}, bla_{CTX-M}, bla_{SHV}	*tetA*, *tetB*	-	*aadA1*	-	-
BB-51	CIP, E, TE, AMP, C, S	bla_{TEM}, bla_{CMY}, bla_{CTX-M}	*tetA*	*qnrA*, *qnrB*	*aadA1*	-	-
BB-55	CIP, E, TE, AMP, C, S	bla_{TEM}, bla_{CMY}	*tetA*	*qnrA*	*aadA1*	-	-
BB-59	CIP, E, TE, AMP, C, S	bla_{TEM}, bla_{CMY}, bla_{CTX-M}	*tetA*	-	*aadA1*	-	-
BB-60	E, AMP, S	bla_{TEM}, bla_{CMY}, bla_{CTX-M}	-	-	*aadA1*	-	-
BB-63	E, MEM, AMP	bla_{TEM}, bla_{CMY}, bla_{CTX-M}, bla_{SHV}	-	-	-	-	-

A p-value less than 0.05 ($p < 0.05$) was deemed as significant. **Correlation is significant at the 0.01 level (2-tailed); *Correlation is significant at the 0.05 level (2-tailed); [a]Cannot be computed because at least one of the variables is constant. *CIP*, ciprofloxacin; *GEN*, gentamicin; *E*, erythromycin; *TE*, tetracycline; *MEM*, meropenem; *AMP*, ampicillin; *C*, chloramphenicol; *S*, streptomycin.

Table 3 Pearson correlation coefficients showing the correlation between resistances to antibiotics in ESBL-producing *Escherichia coli* isolates

		CIP	GEN	E	TE	CL	MEM	AMP	C	S
CIP	Pearson correlation	1								
	Sig. (2-tailed)	-								
GEN	Pearson correlation	0.125	1							
	Sig. (2-tailed)	0.589								
E	Pearson correlation	[a]	[a]	[a]						
	Sig. (2-tailed)	-	-	-						
TE	Pearson correlation	0.868**	0.108	[a]	1					
	Sig. (2-tailed)	0.000	0.640	-	-					
CL	Pearson correlation	[a]	[a]	[a]	[a]	[a]				
	Sig. (2-tailed)	-	-	-	-	-				
MEM	Pearson correlation	−0.400	−0.050	[a]	−0.461*	[a]	1			
	Sig. (2-tailed)	0.072	0.830	-	0.035	-	-			
AMP	Pearson correlation	[a]	[a]	[a]	[a]	[a]	[a]	[a]		
	Sig. (2-tailed)	-	-	-	-	-	-	-		
C	Pearson correlation	0.791**	0.158	[a]	0.686**	[a]	−0.316	[a]	1	
	Sig. (2-tailed)	0.000	0.494	-	0.001	-	0.163	-	-	
S	Pearson correlation	0.091	0.091	[a]	0.149	[a]	−0.548*	[a]	0.000	1
	Sig. (2-tailed)	0.694	0.694	-	0.521	-	0.010	-	1.000	-

Prevalence of Beta-Lactamase Genes in E. coli

Among the 21 ESBL-producing *E. coli* isolates, all harbored two or more beta-lactamase genes with bla_{TEM} as the significantly dominant (chi-square test, $p < 0.001$) (20/21; 95.24%; 95% CI, 77.33–99.76%), followed by bla_{CMY} (19/21; 90.48%; 95% CI, 71.09–98.31%), bla_{CTX-M} (18/21; 85.71%; 95% CI, 65.36–95.02%), and bla_{SHV} (9/21; 42.86%; 95% CI, 24.47–63.45%). The most common pattern was bla_{TEM}–bla_{CMY}–bla_{CTX-M} presenting in nine ESBL-producing *E. coli* isolates. In addition, seven isolates were found positive for all four beta-lactamase genes (bla_{TEM}–bla_{CMY}–bla_{CTX-M}–bla_{SHV}) and four for any of two genes (Table 2).

Comparison of Pearson Correlation Coefficients Among ESBL-Producing E. coli

The Pearson correlation coefficients (ρ) derived from bivariate statistical analysis revealed a highly significant correlation between the bla_{TEM} and bla_{CMY} beta-lactamase genes of *E. coli* ($\rho = 0.689$; $p = 0.001$). However, the other genes did not show any significant correlation ($p > 0.05$) (Table 4).

Here, a *p*-value less than 0.05 was deemed statistically significant. **Correlation is significant at the 0.01 level (two-tailed).

Discussion

There is an acknowledged failure to detect antibiotic resistance and the associated genes from migratory birds in Bangladesh. Although several studies were conducted previously in Bangladesh to detect ESBL-producing *E. coli* [30–32], until now their association with migratory birds has not been routinely reported. In this study, we therefore reported antibiotic resistance and beta-lactamase genes in *E. coli* isolated from migratory birds travelling to Bangladesh.

Table 4 Comparison of Pearson correlation coefficients between beta-lactamase genes of *Escherichia coli* isolated from fecal materials of migratory birds

		bla_{TEM}	bla_{CMY}	bla_{SHV}	bla_{CTX-M}
bla_{TEM}	Pearson correlation	1	-	-	-
	Sig. (2-tailed)	-	-	-	-
bla_{CMY}	Pearson correlation	0.689**	1	-	-
	Sig. (2-tailed)	0.001	-	-	-
bla_{SHV}	Pearson correlation	−0.258	−0.375	1	-
	Sig. (2-tailed)	0.258	0.094	-	-
bla_{CTX-M}	Pearson correlation	−0.091	−0.132	0.079	1
	Sig. (2-tailed)	0.694	0.567	0.735	-

The *E. coli* strains isolated from migratory birds harbored several resistance genes including *tetA*, *tetB*, *qnrA*, *qnrB*, and *aadA1* genes; of these, *tetA* was found in all tetracycline-resistant phenotypes. However, there was an absence of *ereA* and *catA1* genes in the *E. coli* isolates. Previously, Dolejska et al. [33] detected the high occurrence of different resistance genes from black-headed gulls in the Czech Republic. In addition, Radhouani et al. [34] recorded several antibiotic resistance genes from migratory birds, which is consistent with the present study. The occurrence of resistant *E. coli* in migratory birds may be associated with the transmission of antibiotic resistance genes to other environments. Water contaminated with feces of migratory birds could be a significant risk factor for the dissemination of resistant *E. coli* pathogens and their resistance genes in the environments. In addition, the spread of *E. coli*-carrying antibiotic resistance genes from migratory birds to humans and animals can occur via contaminated fresh and seawater systems [35].

The near pandemic spreading of ESBL-producing bacteria is of great public health concern across the globe. The occurrence of ESBL-producing *E. coli* has been increasing in many countries, not only restricted to humans but also reported in environmental niches like migratory birds, livestock, water, and in soils [32]. The present study indicates that many migratory birds (38.18%) are carriers of ESBL-producing *E. coli* in Bangladesh. This high prevalence is comparable to the previous studies detecting ESBL-producing *E. coli* in 30% of wild ducks [30] and in 17.3% of migratory gulls [31] in Bangladesh and in 17% of wild migratory birds in Pakistan [18]. These results and the data presented here indicate that migratory birds play a significant role in transmitting and spreading ESBL-producing *E. coli* in Asia via the Indus migration route. Globally, multiple studies detected ESBL-producing *E. coli* [4, 8, 36–38], illustrating the global importance of migratory birds as potential carriers and reservoirs. The results found in the present study suggest that migratory birds may contribute to the dissemination of ESBL-producing *E. coli* in Bangladesh.

In the current study, all the ESBL-producing *E. coli* isolates were phenotypically MDR in nature. Previously, Mohsin et al. [18] detected MDR ESBL-producing *E. coli* from 88.84% of wild migratory birds travelling to Pakistan. Here, highly significant positive correlations between resistance patterns of tetracycline and ciprofloxacin, chloramphenicol and ciprofloxacin, and chloramphenicol and streptomycin were shown by bivariate analysis. These significant associations might be due to the hazardous use of antimicrobial agents in areas usually inhabited by migratory birds. Environmental contamination might also play a pivotal role in such cases. The high occurrence of MDR ESBL-producing

E. coli from our study is of great concern and may be due to the deterioration of the surrounding environments.

In the present study, bla_{TEM} was detected at a predominant rate compared to other ESBL genotypes, as previously reported [37]. In addition, ESBL genotypes bla_{CMY} and $bla_{CTX\text{-}M}$ were also frequently detected in our study. The beta-lactamase gene bla_{SHV} was detected in more than 40% of ESBL-producing *E. coli* isolates. These results are in agreement with previous studies [8, 18, 30–32, 38, 39]. ESBL genotypes $bla_{CTX\text{-}M}$ are increasingly distributed in humans, animals, and environmental sources which is revealing an urgent problem in infectious disease treatment [40]. The genes $bla_{CTX\text{-}M}$ are very common in wild birds and are generally found in human- and veterinary-origin ESBL isolates [41]. Though the AmpC-type beta-lactamase gene bla_{CMY}-producing *E. coli* is commonly associated with humans, companion, and food-producing animals, it is also commonly detected in avian wildlife [40]. In addition to bla_{CMY} and $bla_{CTX\text{-}M}$, the beta-lactamase genes bla_{TEM} and bla_{SHV} also confer resistance to beta-lactam classes of antibiotics in humans, livestock, and other animals [37]. The high prevalence of beta-lactamase genes in *E. coli* isolated from migratory birds poses a serious threat to human health. This is because migratory birds are directly connected to environmental features, especially water, and contaminated water plays a significant role in the dissemination of beta-lactamase-producing *E. coli* in the human community.

In this study, we found that 95.24% ESBL-producing *E. coli* harbored one or more antibiotic resistance genes along with beta-lactamase genes. Previously, Mohsin et al. [18] detected similar resistance genes in ESBL-producing *E. coli* isolates from migratory birds in Pakistan. The occurrence of MDR ESBL-producing *E. coli* isolates in the migratory birds travelling to Bangladesh in winter is alarming, as possible consequences would be severe clinical outcomes concomitant with serious limitations in antimicrobial treatment.

To the best of our knowledge, this is the first study in Bangladesh to detect *E. coli*-carrying beta-lactamase *blaCMY* and *blaSHV* genes from migratory birds; however, the study has several limitations. Here, we did not focus on environmental samples contaminated with the migratory birds' fecal materials to confirm transmission of AMR from these birds to the environment. In addition, a limited number of samples were analyzed in this study. A further detailed study comprising more samples and in-depth genetic analysis of the resistant isolates could have been more informative.

Conclusions

The present study confirms the detection of ESBL-producing *E.* coli harboring clinically important bla_{CMY} and bla_{SHV} and other resistance genes from migratory birds for the first time in Bangladesh. The findings indicate that migratory birds are potential carriers and spreaders of AMR- and ESBL-producing organisms in humans, animals, and vulnerable environments in Bangladesh and pose a serious threat to one-health components. Therefore, these birds need to be kept under antibiotic resistance surveillance for better management of AMR-related hazards in Bangladesh.

Acknowledgements We would like to thank Dr. Jayedul Hassan, Department of Microbiology and Hygiene, Bangladesh Agricultural University, for providing the PCR-positive controls. We also thank to Dr. AMM Taufiquer Rahman, Zilla Hospital, Naogaon, Bangladesh, for comments and suggestions on this this study.

Author Contribution Conceptualization, MSI, and MTR; methodology, MSI; software, MSI; validation, MTR; formal analysis, MSI, MAS, SI, and MTR; investigation, MSI, MPS and SI; data curation, MSI; writing—original draft preparation, MSI, MAS, and MTR; writing—review and editing, MSI, MAS, MR, FMB, MPS, and MTR; supervision, MAK and MTR; project administration, SR and MTR; critical revisions and writing, MTR.

Funding We would like to thank the Bangladesh Agricultural University Research System (BAURES; 2019/8/BAU) and University Grants Commission of Bangladesh (2020/28/UGC) for facilitating parts of the study.

Declarations

Ethics Approval The methodologies and related protocols used in this study were approved by the institutional ethical committee (AWEEC/BAU/2019(14)).

Conflict of Interest The authors declare no competing interests.

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