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Prevalence and Molecular Detection of Quinolone-Resistant *E. coli* in Rectal Swab of Apparently Healthy Cattle in Bangladesh

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Authors' contributions

This work was carried out in collaboration between all authors. Authors KHMNHN, MAI and MTR designed the study. Author MMM carried out the experiment. Authors MBR and JH performed the statistical analysis. Authors MMM, KZ and MTR wrote the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Emergence of antibiotic resistance is a serious health problem both in human and animal all over the world. In this study, we investigated the prevalence of quinolone-resistant *E. coli* isolated from apparently healthy cattle in Mymensingh district, Bangladesh. A total of 137 rectal swabs was screened among which 95 was found positive for *E. coli.* Confirmation of isolation of *E. coli* was done by PCR targeting 16S rRNA gene of *E. coli* (prevalence 69.3%). Resistance against quinolone is primarily due to activities of *qnrS* and *qnrA* gene products. Among these *E. coli* quinolone-resistant gene *qnrS* was detected in 11 isolates. None of the isolates were found positive for *qnr A*gene. The overall prevalence of *qnrS* positive *E. coli* was 8.0%. Many of these quinolone-resistant

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E. coli was multidrug-resistant. Nucleotide sequence analysis of *qnrS* gene showed homology with the *qnrS* gene detected in China, Nigeria, Taiwan, Russia, Turkey and USA. All isolates that were resistant to multiple quinolones were found highly sensitive to imipenem, ertapenem and meropenem. The results of this study indicated that apparently healthy cattle harbor quinolone-resistant *E. coli* which have both clinical and public health significance. If strict regulation on the use of quinolones in food animals is not maintained, these quinolone-resistant *E. coli* may be transmitted to humans and other animals and may cause serious health problems in future. To the best of our knowledge, this is the first report on the molecular detection of quinolone-resistant *E. coli* in cattle in Bangladesh.

Keywords: Escherichia coli; healthy cattle; rectal swab; quinolone resistance; qnrS gene.

1. INTRODUCTION

Escherichia coli are intestinal commensal bacteria. Although most of the strains of *E. coli* are nonpathogenic, some strains can cause a variety of intestinal and extra-intestinal infections in humans and animals. Virulent strains of *E. coli* can cause gastroenteritis, urinary tract infections, and neonatal meningitis. In rare cases, virulent strains are also responsible for hemolytic-uremic syndrome, anemia, kidney failure, peritonitis, mastitis, septicemia and Gram-negative pneumonia [1]

Diseases caused by *E. coli* can be successfully treated with antibiotics such as fluoroquinolones and quinolones. Quinolones are broad-spectrum antimicrobial agents with excellent activity against *E. coli.* However, frequent uses of antimicrobial agents for control of bacterial infections are believed to be linked with the development of resistance of bacteria to antimicrobial agents. Quinolone-resistant *E. coli* isolates have become a major problem in infection control and treatment worldwide. Before the early 1990's quinolone resistance was rarely found in *E. coli* isolated from humans as well as animal. However, the frequency of resistance has significantly increased worldwide lately [2].

Several investigations on quinolone-resistant *E. coli* have been done in humans in Bangladesh [3,4]. Quinolone-resistant *E. coli* has been detected in many countries from healthy cattle and other animals [5,6]. Resistance against quinolone is primarily due to activities of *qnr* genes such as *qnrS* and *qnrA* genes. Antibiotic resistance genes can be transferred to quinolone sensitive bacteria by horizontal gene transfer. Cattle is one of the dominant components of livestock population of Bangladesh. Quinolone-resistant *E. coli* from cattle can be easily transmitted to other species of animal and into the environment. As per review search, no work has been performed for the investigation of quinolone-resistant *E. coli* isolated from apparently healthy cattle in Bangladesh. The present study was therefore carried out to investigate the prevalence and molecular detection of quinolone-resistant *E. coli* in rectal swab of apparently healthy cattle in Mymensingh.

2. MATERIALS AND METHODS

2.1 Sample Collection

A total 137 rectal swab samples were collected randomly from apparently healthy cattle for isolation of quinolone-resistant *E. coli*. Among these 137 samples, 55 were collected from Veterinary Teaching Hospital, Bangladesh Agricultural University, Mymensingh, 40 from Bangladesh Agricultural University Dairy Farm, Mymensingh, 18 from Upazila Veterinary Hospital, Muktagacha, Mymensingh and 24 from Zila Veterinary Hospital, Mymensingh.

These swab samples were collected under sterile conditions from four age groups of cattle of 0-1 year, 1-3 years, 3-5 years and above 5 years old. All samples originated from apparently healthy cattle. Areas selected for the experimental study were confined within Mymensingh districts of Bangladesh. Mymensingh is one of the largest district of Bangladesh, located about 120 km north of Dhaka, the capital of Bangladesh.

2.2 Isolation and Identification of *E. coli* and Detection of *qnrA* and *qnrS*

Isolation and identification of *E. coli* from the rectal swab were done based on morphology, staining, cultural and biochemical characteristics as described earlier by other [7].

Suspected *E. coli* isolates were confirmed as *E. coli* by PCR with primers specific for *E. coli* 16S rRNA gene. For PCR *E. coli* DNA was extracted

from pure culture by boiling method. In brief, 100 µl of deionized water was taken into an eppendorf tube. A pure bacterial colony from overnight culture on 37°C of EMB (Eosin Methylene Blue) agar was gently mixed with de-ionized water. The tube was transferred into boiling water and boiled for 10 minutes then immediately to ice for cold shock about 10 minutes and centrifuged at 10000 rpm for 10 minutes. Supernatant from the tube was collected and used as DNA template for PCR. The DNA sample was stored at -20°C until use.PCR for *E. coli* 16S rRNA gene was done using the primers ECO-1 and ECO-2 as described earlier by other [8]. PCR for amplification of quinolone resistant genes (*qnrA* and *qnrS*) was performed using the primers and procedure described previously [9].

2.3 Antibiogram

E. coli isolates were subjected to antibiotic sensitivity test by disc diffusion method against commonly used quinolones and three carbapenem antibiotics. Disk diffusion test was performed as described previously [10]. List of antibiotics used in the antibiogram and their concentration is presented in Table 1. Antimicrobial testing results were recorded as sensitive, intermediately sensitive or resistant and the zone of growth inhibition was compared with the zone size interpretative tables provided by Clinical and laboratory Standards Institute [11].

Table 1. Name of antibiotics with their disc concentration

Name of antibiotic	Disc concentration (µg)
Quinolone group	
Nalidixic Acid	30 µg
Gatifloxacin	5 µg
Levofloxacin	5 µg
Moxifloxacin	5 µg
Pefloxacin	5 µg
Oflofloxacin	5 µg
Ciprofloxacin	5 µg
Carbapenem group	
Ertapenem	10 µg
Imipenem	10 µg
Meropenem	10 µg

2.4 Sequencing and Sequence Analysis

The *qnrS* PCR products were purified by Wizard® SV Gel and PCR Clean-Up System (Promega, USA) and sent for sequencing commercially through the First BASE Laboratories SdnBhd, Malaysia. Sequence was analyzed (accession number KX752572) using BLASTx program available in PubMed.

2.5 Statistical Analysis

Data were analyzed where applicable using SPSS version 17 (Chicago: SPSS Inc.). We applied *Chi*-square ($\chi 2$) test to find out the significant relationship. The significance level was fixed at $P<0.05$.

3. RESULTS

3.1 Prevalence of *E. coli*

Prevalence of *E. coli* in rectal swab of apparently healthy cattle is presented in Table 2. The presence of *E. coli* in the rectal swab was confirmed by *E. coli* 16S rRNA gene specific PCR. The overall prevalence of *E. coli* was 69.3%.Thehighest prevalence was found in above 5 years of age group cattle (95.1%) whereas 0-1 year age group had the lowest prevalence (55.1%). The χ^2 test revealed that there was a significant relationship of prevalence of *E. coli* with the age of cattle.

Table 2. Prevalence of *E. coli* in rectal swab of apparently healthy cattle

Age group	No. of collected sample	Positive samples for *E. coli*	
		No.	%
0-1 year	69	38	55.1
1-3 years	19	13	68.4
3-5 years	8	5	62.5
Above 5 years	41	39	95.1
Total	137	95	69.3

3.2 Antibiogram

Ninety-five *E. coli* isolates was subjected to antibiotic sensitivity test against commonly used quinolone antibiotics and carbapenems. The results of antibiotic profiles are presented in Tables 3,4 and 5. Among the isolated *E. coli,* antibiotic resistance pattern was found higher in above 5 years old adult cattle compared to other age groups.

Table 3. Antibiogram profile of *E. coli* isolates according to age variation

Age (year)	*E. coli* (+)	Pattern	Number of *E. coli* isolates						
			Nali	Gati	Levo	Moxi	Peflo	Oflo	Cipro
0-1	38	Sensitive	27	29	33	30	34	35	34
		Intermediate	2	3	5	0	0	0	2
		Resistant	9	6	0	8	4	3	2
1-3	13	Sensitive	4	5	8	11	7	7	7
		Intermediate	0	0	1	0	1	0	3
		Resistant	9	8	4	2	5	6	3
3-5	5	Sensitive	1	2	3	1	3	4	1
		Intermediate	0	1	0	0	0	0	1
		Resistant	4	2	2	4	2	1	3
>5	39	Sensitive	12	18	24	17	10	14	19
		Intermediate	0	4	7	1	4	0	7
		Resistant	27	17	8	21	25	25	13

Table 4. The overall antibiogram profile of isolated *E. coli* according to different antibiotics

Antibiotic Name	Sensitive	Intermediate	Resistant
Nalidixic Acid	43 (45.3%)	3 (3.2%)	49 (51.6%)
Gatifloxacin	54 (56.8%)	8 (8.4%)	33 (34.7%)
Levofloxacin	68 (71.6%)	13 (13.7%)	14 (14.8%)
Moxifloxacin	59 (62.1%)	1 (1.0%)	35 (36.8%)
Pefloxacin	54(56.8%)	5 (5.3%)	36 (37.9%)
Ofloxacin	60 (63.2%)	0	35(36.8%)
Ciprofloxacin	61 (64.2)	13 (13.7%)	21 (22.1%)

Among 95 *E. coli* isolates, 17 isolates were resistant to all 7 types of quinolone antibiotics *i.e.*, they were multidrug-resistant. The prevalence of all resistant *E. coli* was 17.9%. It was also seen that all resistant *E. coli* isolates were highly sensitive to imipenem, ertapenem and meropenem (data not shown). The results also revealed that more than 50% of isolates were resistant to nalidixic acid, whereas the rates of resistance to ciprofloxacin and levofloxacin were lower, 22.1% and 14.8%, respectively. Antibiogram profile also revealed that all *E. coli* isolates that were resistant to multiple quinolones were highly sensitive to carbapenem group of antibiotics *e.g.,* imipenem, ertapenem and meropenem.

3.3 Molecular Detection of *qnrS gene*

All 95 *E. coli* isolates were subjected to PCR for *qnrA* and *qnrS* genes. Among these 95 isolates, 11 showed the presence of *qnrS* gene (prevalence 11.6%). None of the isolates was found positive for *qnrA.* The relation between antibiogram profile and the presence of gene responsible for antibiotic resistance is given in Table 5.

Table 5. Antibiotic sensitivity pattern of *E. coli* isolates with *qnrS* gene

Age group	Isolate no.	Resistance to antibiotic
0-1 year	**10**	NA, GAT, LE, MO, PF, OF, CIP
	32	NA, PF
	123	NA, MO, PF
1-3 years	11	NA, GAT
	115	NA
3-5 years	**97**	NA, GAT, LE, MO, PF, OF, CIP
	106	NA, GAT
>5 years	**21**	NA, GAT, LE, MO, PF, OF, CIP
	43	NA, GAT, LE, MO, PF, OF, CIP
	83	NA, GAT
	84	NA, GAT, LE, MO, PF, OF, CIP

NA-Nalidixic Acid, GAT-Gatifloxacin, LE-Levofloxacin, MO-Moxifloxacin, PF-Pefloxacin, OF-Ofloxacin, CIP-Ciprofloxacin.Isolates that are resistant to three or more antibiotics were considered as multi-drug resistant.

3.4 *qnrS* Sequencing

Sequencing of the *qnrS* gene of isolates 21, 83 and 123 was done commercially. These three sequences were identical. The sequence of isolate 21 was submitted to GenBank (accession number KX752572). BLASTx analysis of the sequences in NCBI database showed 100% identity of our *qnrS* gene with those isolated from China, Nigeria, Taiwan, Russia, Turkey and USA.

4. DISCUSSION

In the present study, we determined the prevalence and distribution of quinolone-resistant *E. coli* in apparently healthy cattle in Mymensingh area. In addition, the molecular basis of quinolone resistance was also revealed by PCR detection of quinolone resistant genes and their sequencing. To the best of our knowledge, this is the first record on molecular detection of quinolone resistant *E. coli* in cattle in Bangladesh.

A total of 95 out of 137 rectal swab samples were found positive for *E. coli.* The overall prevalence was 69.3%. Previously, Hassan et al. [8] detected 75% prevalence of *E. coli* in the rectal swab of healthy cattle, of which 43.33% were shiga-toxin producers [12] in Bangladesh and about 80% in the apparently healthy cattle of Nigeria [13].

On age basis, this study showed the highest prevalence of *E. coli* in cattle above 5 years of age. This may be due to more exposure of older animal to contaminated environment. Higher prevalence of *E. coli* in elder age group of animal has also been described in different studies [8].

Result of antibiotic sensitivity test according to age shows that most of the *E. coli* isolates from 0-1 age group of cattle were sensitive to all 7 antibiotics and few isolates were resistant to nalidixic acid and moxifloxacin, but none of them was resistant to levofloxacin. The almost contrasting situation was seen in above 5 years of age group where most of the *E. coli* isolates were resistant to nalidixic acid, moxifloxacin, pefloxacin and ofloxacin but sensitive to levofloxacin and ciprofloxacin. The observed difference in drug sensitivity of isolated *E. coli* against quinolones might be related with variation in the frequency of use of different quinolones.

The overall antibiotic sensitivity results of this study reveal that more than fifty percent (51.6%) of *E. coli* isolates were resistant to nalidixic acid. As nalidixic acid was the first of the synthetic quinolone antibiotics, it has been used indiscriminately and this may have contributed tothe development of resistance against it.

Bacteria show resistance against quinolones due to the presence of quinolone-resistance protein encoded by genes (*qnr* gene) such as *qnrS* and *qnrA* gene. The *qnr* genes have been identified worldwide with a high prevalence among enteric human bacteria, poultry and other food animals [14,15,16]. Based on PCR-based screening among the 95 *E. coli* isolates, 11 (11.6%) isolates were found positive for the *qnrS* gene and none for *qnrA.* Our findings are similar to those of others [17,18]. Detection of *qnrS* gene from the isolated *E. coli* confirmed that presence of quinolone-resistance protein in these *E. coli* isolates is the molecular basis for quinolone resistance.

Carbapenem is a member of beta lactum group of antibiotics. It is commonly used in human to treat multidrug-resistant bacteria. Imipenem, ertapenem and meropenem are members of the carbapenem group of antibiotics. An interesting finding of the present study is that all *E. coli* isolates that were found resistant to multiple antibiotics of quinolone group were highly sensitive to imipenem, ertapenem and meropenem. As far as we know, these antibiotics are not common in Bangladesh and not used in animals and hence were found highly sensitive against the isolated *E. coli.*

In Bangladesh, antibiotics are used indiscriminately in veterinary practice, resulting in the occurrence of antibiotic resistant *E. coli* [19]. Several multi-drug resistant *E. coli* were isolated from rectal swab of healthy cattle in this study. Five *E. coli* isolates among the 11 *qnrS*-positive *E. coli* were found resistant to seven types of quinolone antibiotic (multidrug-resistant) and other 6 *E. coli* isolates were resistant to one or two types of quinolone antibiotics. *E. coli* isolates resistant to 7 quinolones were most frequent in above 5 years of age where 3 of 4 isolates were multidrug-resistant. This may be the cause of more frequent exposure to quinolone antibiotics in their entire life. The prevalence of multidrug-resistant *E. coli* detected in this study area appears to be similar to earlier observations made by other authors [20,21].

Blast search (BLASTx) analysis of the *qnrS* gene detected in this study showed 100% identity to the *qnrS* containing region in quinolone-resistant protein gene (qnrS1) of China (KP773319.1, KP400525.1) [18,22], Nigeria (KM023153.1) [23], and Turkey (KP114562.1). These findings suggest that same type of *qnrS* gene is prevalent across the globe that may have a common ancestral origin.

The presence of quinolone-resistant *E. coli* in the healthy cattle has serious consequence on human health, particularly due to their multidrug resistant characteristics. Since humans remain in close contact with cattle in Bangladesh, particularly in rural areas, there is a great chance for the transmission of these antibiotic-resistant *E. coli* to humans and other animals. Measures therefore have to be adopted to limit the indiscriminate use of antibiotics in animal.

5. CONCLUSIONS

In this study *E. coli* was isolated from rectal swabs of healthy cattle and confirmed by PCR targeting *E. coli* 16S rRNA gene. Many of these isolated *E. coli* were found resistant to quinolone. *qnrS* gene responsible for quinolone resistance was also detected in the quinolone-resistant *E. coli* isolates and sequenced. Many of these *E. coli* isolates were also multidrug-resistant. The occurrence of quinolone-resistant *E. coli* in healthy cattle has great public health significance. Since in many cases people are in close contact with their cattle, especially in rural areas, these antibiotic resistant bacteria could transmit to them from healthy cattle. If strict regulation on the use of quinolone in food animals is not maintained, these quinolone-resistant *E. coli* may be transmitted to humans and other animals and may cause serious health problems in future.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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