**Original Research Article**

# Prevalence and molecular detection of quinolone resistant *E. coli* in rectal swab of apparently healthy cattle in Bangladesh


## 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.* Conformation of isolation of *E. coli* was done by PCR targeting 16S rRNA gene of *E. coli*(prevalence 69.34%). 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 *qnrA*gene. The overall prevalence of *qnrS* positive *E. coli* was 8.03%. Many of these quinolone resistant *E. coli* was multi-drug resistant. Nucleotide sequence analysis of *qnrS* gene showed homology with the *qnrS* gene detected in China, Nigeria, Taiwan, Russia, Turkey and USA. All these having resistance to multiple quinolone group of antibiotic were found highly sensitive to Imipenem, Ertapenem and Meropenem. The results of this study indicate that apparently healthy cattle harbor quinolone resistant *E. coli* which have both clinical and public health significance. If strict regulation on the use of quinolone in food animals is not maintained, these quinolone resistant *E. coli* may transmitted to human and other animal will 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 (Schmidt et al., 1996)

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. But the frequency of resistance has significantly increased worldwide lately (Jurado *et al.* 2008).

Several investigations on quinolone resistant *E. coli* have been done in humans in Bangladesh (Akter *et al.* 2011, Talukdar *et al.* 2013). Quinolone resistant *Escherichia coli* has been detected in many countries from healthy cattle and other animal (Todorović *et al*, 2017; Duse *et al.* 2016). 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, 35 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 by Begum *et al*, (2016).

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 de-ionized 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 by Hassan *et al.* (2014). PCR for amplification of quinolone resistant genes (*qnrA* and *qnrS*) were performed using the primers and procedure as described by Robicsek *et al.* (2006).

**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 by Bauer *et al.* (1966). 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 (CLSI, 2012),

**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.

2.6 STATISTICAL ANALYSIS

Data were statistically analyzed where applicable using SPSS version 17 (Chicago: SPSS Inc.). We applied *Chi*-square ($\chi_2$) test to find out the significant relationship

## 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%. The highest 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 $\chi^2$ test revealed that there was a significant relationship of prevalence of *E. coli* with the age of cattle.

### 3.2 ANTIBIOGRAM

Ninety-five *E. coli* isolates was subjected to antibiotic sensitivity test against commonly used quinolones 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.

Among 95 *E. coli* isolates, 17 isolates were resistant to all 7 types of quinolone antibiotics i.e., they were multi drugr esistant. 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.

### 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 into 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.* This overall prevalence was 69.3%. Previously, Hassan *et al.* (2014) detected 75% prevalence of *E. coli* in the rectal swab of healthy cattle in Bangladesh. Ogunleye *et al*. (2013) reported prevalence of *E. coli* about 80% in the apparently healthy cattle of Nigeria.

On age basis, this study showed the highest prevalence of *E. coli* was observed 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 (Anwarullah *et al.,* 2014, Hassan *et al.,* 2014).

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 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 in the 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 has been identified worldwide with a high prevalence among enteric human bacteria, poultry and other food animals (White *et al*,. 2000 and Fortini*et al*,. 2011; Nordmann and Poirel, 2005). 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 Yue *et al*. (2008) and Chen *et al.* (2012). 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* (Hussain *et al,*. 1982). 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 (Nazir *et al.*, 2005, Hossain *et al.*,2008).

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) (Chen *et al.* 2012; Liu *et al.*, 2015), Nigeria (KM023153.1) (Fortini *et al.* 2015), 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.

## 4. 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.

**ACKNOWLEDGEMENT**

The authors are very much thankful to Prof. Vincent Perreten, Institute of Veterinary Bacteriology, University of Berne, Switzerland for providing the carbapenem antibiotics disc.

CONFLICT OF INTEREST

The authors declared that they had no conflict of interests.

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**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 |

**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 |

290 **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 |

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293 **Table 4. The overall antibiogram profile of isolated *E. coli* according to different**
294 **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%) |

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297 **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 |

298 NA-Nalidixic Acid, GAT-Gatifloxacin, LE-Levofloxacin, MO-Moxifloxacin, PF-Pefloxacin,
299 OF-Ofloxacin, CIP-Ciprofloxacin. Isolates that are resistant to three or more antibiotics were
300 considered as multi-drug resistant.
301