

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*. 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 *qnrA* gene. The overall prevalence of *qnrS* positive *E. coli* was 8.0%. Many of these quinolone-resistant *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.

26 1. INTRODUCTION

27 *Escherichia coli* are intestinal commensal bacteria. Although most of the strains of *E. coli* are
28 nonpathogenic, some strains can cause a variety of intestinal and extra-intestinal infections in
29 humans and animals. Virulent strains of *E. coli* can cause gastroenteritis, urinary tract
30 infections, and neonatal meningitis. In rare cases, virulent strains are also responsible for
31 hemolytic-uremic syndrome, anemia, kidney failure, peritonitis, mastitis, septicemia and
32 Gram-negative pneumonia (Schmidt et al., 1996)

33 Diseases caused by *E. coli* can be successfully treated with antibiotics such as
34 fluoroquinolones and quinolones. Quinolones are broad-spectrum antimicrobial agents with
35 excellent activity against *E. coli*. However, frequent uses of antimicrobial agents for control of
36 bacterial infections are believed to be linked with the development of resistance of bacteria to
37 antimicrobial agents. Quinolone-resistant *E. coli* isolates have become a major problem in
38 infection control and treatment worldwide. Before the early 1990's quinolone resistance was
39 rarely found in *E. coli* isolated from humans as well as animal. However, the frequency of
40 resistance has significantly increased worldwide lately (Jurado et al. 2008).

41 Several investigations on quinolone-resistant *E. coli* have been done in humans in
42 Bangladesh (Akter et al. 2011, Talukdar et al. 2013). Quinolone-resistant *E. coli* has been
43 detected in many countries from healthy cattle and other animals (Todorović et al,
44 2017; Duse et al. 2016). Resistance against quinolone is primarily due to activities of *qnr* genes
45 such as *qnrS* and *qnrA* genes. Antibiotic resistance genes can be transferred to quinolone
46 sensitive bacteria by horizontal gene transfer. Cattle is one of the dominant components of
47 livestock population of Bangladesh. Quinolone-resistant *E. coli* from cattle can be easily
48 transmitted to other species of animal and into the environment. As per review search, no
49 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.

53

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 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*) was performed using the primers and procedure 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 Sdn Bhd, Malaysia. Sequence was analyzed (accession number KX752572) using BLASTx program available in PubMed.

2.6 STATISTICAL ANALYSIS

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

98 3. Results

99 3.1 PREVALENCE OF *E. coli*

100 Prevalence of *E. coli* in rectal swab of apparently healthy cattle is presented in Table 2. The
101 presence of *E. coli* in the rectal swab was confirmed by *E. coli* 16S rRNA gene specific
102 PCR. The overall prevalence of *E. coli* was 69.3%. The highest prevalence was found in above
103 5 years of age group cattle (95.1%) whereas 0-1 year age group had the lowest prevalence
104 (55.1%). The χ^2 test revealed that there was a significant relationship of prevalence of *E. coli*
105 with the age of cattle.

106 3.2 ANTIBIOGRAM

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

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

119 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 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*. 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* 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 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 to 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 have 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.*

168 *coli* isolates that were found resistant to multiple antibiotics of quinolone group were highly
169 sensitive to imipenem, ertapenem and meropenem. As far as we know, these antibiotics are
170 not common in Bangladesh and not used in animals and hence were found highly sensitive
171 against the isolated *E. coli*.

172 In Bangladesh, antibiotics are used indiscriminately in veterinary practice, resulting in the
173 occurrence of antibiotic resistant *E. coli* (Hussain *et al.*, 1982). Several multi-drug resistant *E.*
174 *coli* were isolated from rectal swab of healthy cattle in this study. Five *E. coli* isolates among
175 the 11 *qnrS*-positive *E. coli* were found resistant to seven types of quinolone antibiotic
176 (multidrug-resistant) and other 6 *E. coli* isolates were resistant to one or two types of
177 quinolone antibiotics. *E. coli* isolates resistant to 7 quinolones were most frequent in above 5
178 years of age where 3 of 4 isolates were multidrug-resistant. This may be the cause of more
179 frequent exposure to quinolone antibiotics in their entire life. The prevalence of multidrug-
180 resistant *E. coli* detected in this study area appears to be similar to earlier observations made
181 by other authors (Nazir *et al.*, 2005, Hossain *et al.*, 2008).

182 Blast search (BLASTx) analysis of the *qnrS* gene detected in this study showed 100% identity
183 to the *qnrS* containing region in quinolone-resistant protein gene (*qnrS1*) of China
184 (KP773319.1, KP400525.1) (Chen *et al.* 2012; Liu *et al.*, 2015), Nigeria (KM023153.1)
185 (Fortini *et al.* 2015), and Turkey (KP114562.1). These findings suggest that same type of *qnrS*
186 gene is prevalent across the globe that may have a common ancestral origin.

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

192

193 **4. CONCLUSIONS**

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

204

205 **ACKNOWLEDGEMENT**

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

208 **CONFLICT OF INTEREST**

209 The authors declare that they have no conflict of interests.

210

211

212 **REFERENCES**

213 Akter F, Amin MR, Osman KT, Anwar MN, Karim MM and MA Hossain, 2011.
 214 Ciprofloxacin-resistant *Escherichia coli* in hospital wastewater of Bangladesh and
 215 prediction of its mechanism of resistance. World J. Microbiol Biotech. 28; 827-834.

216 Anwarullah M, Khan JA, Khan MS, Ashraf K and M Avais, 2014. Prevalence of *Salmonella*
 217 and *Escherichia coli* associated with diarrhea in buffalo and cow calves. Buffalo
 218 Bullet. 33: 332-336.

219 Bauer AW, Kirby WM, Sherris JC and Turck M, 1966: Antibiotic susceptibility testing by a
 220 standardized single disk method. Am J Clin Pathol. 45: 493-496.

221 Begum, F, Islam, MM, Sohidullah, M, Kabir, SML, Islam M, and Rahman MT,
 222 2016. Molecular identification and antibiogram profiles of *Escherichia coli* isolated
 223 from apparently healthy and diarrheic goats. Bangladesh J. Vet. Med. 14: 203-208.

224 Chen X, Zhang W, Pan W, Yin J, Pan Z, Gao S, and Jiao X, 2012. Prevalence of *qnr*,
 225 *aac(6')-Ib-cr*, *qepA*, and *oqxAB* in *Escherichia coli* isolates from humans, animals,
 226 and the environment. Antimicrob. Agents Chemother. 56: 3423-3427.

227 CLSI 2012. Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved
 228 Standard—Eleventh Edition. CLSI document M02-A11. Wayne, PA: Clinical and
 229 Laboratory Standards Institute.

230 Duse A, Wallera, KP, Emanuelson U, Unnerstada, HE, Persson Y and Bengtsson B,
 231 2016. Occurrence and spread of quinolone-resistant *Escherichia coli* on dairy
 232 farms. Appl. Environ. Microbiol. 82: 3765-3773.

233 Fortini D, Fashae K, Garcia-Fernandez A, Villa L, and A Caratolli, 2011. Plasmid mediated
 234 quinolone resistance and beta lactamases in *E coli* from healthy animals from Nigeria.
 235 J. Antimicrob. Chem. 6: 1269-1272.

236 Fortini, D., Fashae, K., Villa, L., Feudi, C., García-Fernández, A. and A Carattoli, 2015. A
 237 novel plasmid carrying blaCTX-M-15 identified in commensal *Escherichia coli* from
 238 healthy pregnant women in Ibadan. Nigeria. J. Glob. Antimicrob. Resist. 3:9-12.

239 Hassan J, Parvej MS, Rahman MB, Khan MSR, Rahman MT, Kamal T and KHMNH Nazir,
 240 2014. Prevalence and characterization of *Escherichia coli* from rectal swab of
 241 apparently healthy cattle in Mymensingh, Bangladesh. Microb.Health, 3: 12-14.

242 Hossain MT, Siddique MP, Hossain FMA, Zinnah MA, Hossain MM, Alam MK, Rahman
 243 MT and Choudhury KA, 2008. Isolation, identification, toxin profile and antibiogram
 244 of *Escherichia coli* isolated from broilers and layers in Mymensingh district of
 245 Bangladesh. Bangladesh Vet. Med. 6:01-05.

246 Hussain MM, Glass RJ and Khan MR, 1982: Antibiotic used in a rural community in
 247 Bangladesh. Int. J. Epidemiol. 11: 402-405.

248 Jurado S, Orden JA, Horcajo P, Fuente RDL, Ruiz-Sanat-Quiteria JA, Martínez-Pulgarin S
 249 and Domínguez-Bernal G, 2008. Characterization of fluoroquinolone resistance in
 250 *Escherichia coli* strains from ruminants. J. Vet. Diagnost.Invest. 20: 342-345.

251 Liu Y, Feng Y, Wu W, Xie Y, Wang X, Zhang X, Chen X and Z Zong, 2015. First report of
 252 OXA-181-producing *Escherichia coli* in China and characterization of the isolate
 253 using whole-genome sequencing. Antimicrob. Agents Chemother. 59; 5022-5025.

254 Nazir KHMNH, Rahman MB, Nasiruddin KM, Akhtar F, Khan MFR and Islam MS, 2005:
 255 Antibiotic sensitivity of *Escherichia coli* isolated from water and its relation with
 256 plasmid profile analysis. Pakistan J. Biolog.Sci. 8: 1610-1613.

257 Nordmann P, Poirel L 2005: Emergence of plasmid-mediated resistance to quinolones in
 258 *Enterobacteriaceae*. J. Antimicrobial Chem. 56: 463-469.

259 Ogunleye AO, Okunlade AO, Jeminlehin FO, Ajuwape ATP, 2013: Antibiotic resistance in
 260 *Escherichia coli* isolated from healthy cattle at a major cattle market in Ibadan, Oyo
 261 State, South Western, Nigeria. African J. Microbiol. Res. 7:4572-4575.

262 Robicsek A, Strahilevitz J, Sahm DF, Jacoby GA, Hooper DC, 2006: *qnr* Prevalence in
 263 ceftazidime-resistant *Enterobacteriaceae* isolates from the United States. Antimicrob.
 264 Agents Chemother.. 50: 2872-2874.

265

266 Schmidt H, Maier E, Karch H, Benz R, 1996: Pore-forming properties of the plasmid-
 267 encoded hemolysin of enterohemorrhagic *Escherichia coli* O157:H7. European Journal of
 268 Biochemistry, 241: 594-601.

269

270 Talukdar PK, Rahman M, Rahman M, Nabi A, Islam Z, Hoque MM, Endtz HP, Islam MA,
 271 2013: Antimicrobial resistance, virulence factors and genetic diversity of *Escherichia*
 272 *coli* isolates from household water supply in Dhaka, Bangladesh. PLoS ONE, 8:
 273 e61090.

274 Todorović D, Velhner M, Grego E, Vidanović D, Milanov D, Krnjaić D, Kehrenberg C.
 275 2017.Molecular characterization of multidrug-resistant *Escherichia coli* isolates from
 276 bovine clinical mastitis and pigs in the Vojvodina Province, Serbia.
 277 Microb Drug Resist. 2017. doi: 10.1089/mdr.2017.0016.

278 White DG, Piddock LJV, Maurer JJ 2000: Characterization of fluoroquinolone-resistance
 279 among veterinary isolates of avian *Escherichia coli*. Antimicrob.Agents
 280 Chemother.44:2897-2899.

281 Yue L, Jiang HX, Liao XP, Liu JH, Li SJ, Chen XY, Chen CX, Lü DH, Liu YH 2008:
 282 Prevalence of plasmid-mediated quinolone resistance *qnr* genes in poultry and swine
 283 clinical isolates of *Escherichia coli*. Vet. Microbiol.132; 414-420.

284

285

286 **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
Ofloxacin	5 µg
Ciprofloxacin	5 µg
Carbapenem Group	
Ertapenem	10 µg
Imipenem	10 µg
Meropenem	10 µg

287

288

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

Age group	No. of collected sample	Positive samples for <i>E. coli</i>	
		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

291

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

Age (year)	<i>E. coli</i> (+)	Pattern	Number of <i>E. coli</i> 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

293

294

295 **Table 4. The overall antibiogram profile of isolated *E. coli* according to different**
 296 **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%)

297

298

299 **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

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

303