

Original Research Article

Prevalence and molecular detection of quinolone resistant *E. coli* in rectal swab of apparently healthy cattle in ~~Mymensingh-Bangladesh and their public health~~ significance

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 69.34%). 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* were 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 be transmitted to human and other animals and 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.

26 Keywords: *Escherichia coli*, healthy cattle, rectal swab, quinolone resistance, *qnrS* gene.

27 1. INTRODUCTION

28 *Escherichia coli* are intestinal commensal bacteria. Although most of the strains of *E. coli* are
29 nonpathogenic, some strains can cause a variety of intestinal and extra-intestinal infections in
30 men and animals. Virulent strains of *E. coli* can cause gastroenteritis, urinary tract infections,
31 and neonatal meningitis. In rare cases, virulent strains are also responsible for hemolytic-
32 uremic syndrome, anemia, ~~or~~ kidney failure, peritonitis, mastitis, septicemia and ~~gram~~ Gram-
33 negative pneumonia (Todar, 2007).

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

43 Several investigations on quinolone resistant *E. coli* have been done in humans in Bangladesh
44 (Akter *et al.* 2011, Talukdar *et al.* 2013). Quinolone ~~resistance-resistant~~ *Escherichia coli* has
45 been detected in many countries from healthy cattle and other animal (Todorović *et al.* 2017;
46 Dusea *et al.* 2016). Resistance against quinolone is primarily due to activities of *qnr* genes
47 such as *qnrS* and *qnrA* genes. Antibiotic resistance genes can be transferred to quinolone
48 sensitive bacteria by horizontal gene transfer. Cattle is one of the dominant components of
49 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 ~~was~~has been yet performed for the investigation of quinolone resistant ~~in~~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

A total 137 rectal swab samples were collected randomly from apparently healthy cattle for isolation of quinolone ~~resistance~~resistant *E. coli*. These swab samples were collected ~~maintaining under~~ sterile conditions from ~~on~~ four age groups of cattle of 0-1 year, 1-3 years, 3-5 years and above 5 years old. All ~~the~~ samples ~~were~~ originated from apparently healthy cattle. Areas selected for the experimental study were confined within Mymensingh districts of Bangladesh. The source of sample and location is presented in Table 1.

2.1 ISOLATION AND IDENTIFICATION OF *E. coli*

Isolation and identification of *E. coli* from the rectal swab ~~was~~were done based on morphology, staining, cultural and biochemical characteristics as described by Begum *et al*, (2016).

2.2 DNA EXTRACTION AND PCR FOR *E. coli* 16S rRNA GENE

Suspected ~~*E. coli* isolated~~isolates *E. coli* were ~~conformed~~confirmed as *E. coli* by PCR with primers specific for *E. coli* 16S rRNA gene. For ~~the~~ 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 agar was gently mixed with ~~the~~ de-ionized water. The tube was ~~then~~ 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 ~~kept in~~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). List of primers used for the detection of *E. coli* 16S rRNA gene is presented in Table 2.

2.3 PCR ~~FOR~~ for *qnrA* ~~AND~~ and *qnrS*

PCR for amplification of quinolone resistant genes (*qnrA* and *qnrS*) were performed following the procedure ~~mentioned~~described by Robicsek *et al.* (2006).

2.4 ANTIBIOGRAM

~~Isolated~~ *E. coli* isolates were subjected to antibiotic sensitivity test by disc diffusion method against commonly used ~~seven~~quinolones ~~antibiotics~~ and three carbapenem antibiotics. Disk diffusion test was ~~done~~performed as described by Bauer *et al.* (1966). List ~~of~~if antibiotics used in the antibiogram and their concentration is presented ~~in~~ Table 3. 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), ~~Comite~~Comité De de L'antibiogramme l'antibiogramme De de La la Societesociété Francaise française De de Microbiologie microbiologie (report 2000-2001) and Product Catalog.

2.5 SEQUENCING AND SEQUENCE ANALYSIS

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

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 4. The
101 pPresence of *E. coli* in the rectal swab was confirmed by *E. coli* -16S rRNA gene specific
102 PCR (Figure 1). The overall prevalence of *E. coli* was 69.34% (Table 4), among themThe
103 highest prevalence was found in above 5 years of age group cattle (95.12%) whereas 0-1 year
104 age group belongs had the lowest prevalence (55.107%). χ^2 test revealed that, there was a
105 significant relationship of prevalence of *E. coli* with the age of cattle.

106 3.2 ANTIBIOGRAM

107 Ninety-Ninety-five *E. coli* isolates was subjected to antibiotic sensitivity test against
108 commonly used seven-quinolones antibiotics and three-carbapenems antibiotics. The results
109 of antibiotic profiles are presented in Tables 5 and 6 and Figures 2 and Figure 4. Among the
110 isolated *E. coli*, antibiotic resistance pattern was found higher in above 5 years old adult
111 cattle compared to other age groups.

112 Among 95 *E. coli* isolates, 17 isolates were resistant to all (7 types of quinolone antibiotics)
113 antibiotic g. i.e., they were multi drug-resistant. The prevalence of all antibiotic-resistant *E.*
114 *coli* was (17.89%). It was also seen that, all antibiotic-resistant *E. coli* isolates were highly
115 sensitive to Imipenem-imipenem, Ertapenem-ertapenem and Meropenem-meropenem (data not
116 shown). The results of antibiotic sensitivity of *E. coli* also revealed that more than 50% of
117 isolates are-were resistant to Nalidixic-nalidixic acid, where-as the rates of resistance to
118 cCiprofloxacin and Levofloxacin were showing less percentage of resistance; lower, 22.11%
119 and 14.876%, respectively. Antibioqram profile also revealed that all the *E. coli* isolates that
120 were resistant to multiple quinolones group of antibiotic were highly sensitive to carbapenem

group of antibiotics e.g., ~~Imipenem~~imipenem, ~~Ertapenem~~ertapenem and ~~Meropenem~~meropenem.

3.3 MOLECULAR DETECTION OF *qnrS* GENE

All ~~the~~ 95 *E. coli* isolates ~~was~~were subjected to PCR for *qnrA* and *qnrS* genes. Among these 95 isolates, 11 ~~*E. coli*~~ showed (Figure 3) the presence of *qnrS* gene (prevalence 11.~~657~~%). 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 ~~below~~ in Table 7.

3.4 *qnrS* SEQUENCING

Sequencing of the *qnrS* gene of isolates 21, 83 and 123 was done commercially. ~~All~~These three sequences ~~showed 100% similarities among themselves~~were identical. The ~~s~~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~~quinolone-resistant *E. coli* in apparently healthy cattle in Mymensingh area. In addition, the molecular basis of quinolone ~~resistant~~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 69.34%. 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 ~~that the~~ highest prevalence of *E. coli* ~~was observed in~~ cattle above 5 years of age. This may be due to more exposure of ~~these~~ older animal to contaminated environment. Higher prevalence of *E. coli* in elder age group of animal has also been described ~~by in~~ different studies (Anwarullah *et al.*, 2014, Hassan *et al.*, 2014).

Result of antibiotic sensitivity test according to age ~~variation~~ shows that most of the *E. coli* isolates from 0-1 age group of cattle ~~was were~~ sensitive to all 7 antibiotics and ~~only fewer~~ ~~isolates was were~~ resistant to ~~Nalidixic~~ nalidixic acid and ~~Moxifloxacin~~ moxifloxacin, but none of them ~~are was~~ resistant to ~~Levofloxacin~~ levofloxacin. The almost ~~opposite figure~~ contrasting situation was seen in above 5 years of age group where most of the *E. coli* isolates were resistant to ~~Nalidixic~~ nalidixic acid, ~~Moxifloxacin~~ moxifloxacin, ~~Pefloxacin~~ pefloxacin and ~~Ofloxacin~~ ofloxacin but sensitive to ~~Levofloxacin~~ levofloxacin and slightly to ~~Ciprofloxacin~~ ciprofloxacin.

The overall antibiotic sensitivity results of this study reveals that more than fifty percent (51.586%) of *E. coli* isolates were resistant to ~~Nalidixic~~ nalidixic acid. As ~~Nalidixic~~ 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 world-wide with a high prevalence among enteric human bacteria, poultry and other food animals (White *et al.*, 2000 and Fortiniet *al.*, 2011; Nordmann and Poirel, 2005).

Based on ~~PCR-PCR~~ based screening among the 95 *E. coli* isolates, 11 (11.657%) isolates were found positive for the *qnrS* gene and none for *qnrA*. Our findings ~~is are almost~~ similar to ~~those~~ of Yue *et al.* (2008) and Chen *et al.* (2012). Detection of *qnrS* gene from the isolated *E.*

167 *coli* ~~conformed~~confirmed that presence of quinolone-resistance protein in these ~~isolated~~E.
168 *coli* ~~isolates are~~is the molecular basis for quinolone resistance.

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

177 In Bangladesh, antibiotics are used indiscriminately in veterinary practice, resulting in the
178 occurrence of antibiotic resistant *E. coli* (Hussain *et al.*, 1982). Several multi-drug resistant *E.*
179 *coli* were isolated from rectal swab of healthy cattle in this study (~~Table 7, Figure 2~~). Five *E.*
180 *coli* isolates among the 11 ~~qnrS-PCR~~-positive *E. coli* were found resistant to seven types of
181 quinolone antibiotic (multi-~~drug drug~~-resistant) and other 6 *E. coli* isolates were resistant to
182 one or two types of quinolone antibiotics. ~~Seven types of quinolone antibiotic resistant~~ *E. coli*
183 isolates resistant to 7 quinolones were most frequent in above 5 years of age where 3 of 4
184 isolates were ~~multidrug multidrug~~-resistant ~~out of 4 isolates~~. This may be the cause of more
185 frequent exposure to quinolone antibiotics ~~more and more~~ in their entire life. The prevalence
186 of multi-~~drug drug~~-resistant *E. coli* detected in this study ~~region area~~ appears to be ~~analogous~~
187 similar to earlier observations made by ~~many previous studies~~other authors (Nazir *et al.*,
188 2005, Hossain *et al.*,2008).

189 Blast search (BLASTx) analysis of the *qnrS*_gene detected in this study showed 100%
190 identity to the *qnrS*_containing region in ~~quinolone~~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), ~~China (KP400525.1) (Liu et al., 2015)~~ and Turkey (KP114562.1). These findings suggesting that same type of qnrS gene is prevalent across the globe that may have a common ancestral origin.

~~The p~~ Presence of ~~quinolone-quinolone~~-resistant *E. coli* in the healthy cattle has serious consequence on human health, particularly due to their multi-drug resistant characteristics. Since humans remain in close contact with cattle in Bangladesh, particularly in ~~the~~-rural areas, there is a great chance for the transmission of these ~~antibiotic-antibiotic~~-resistant *E. coli* to humans and other animals. Measures therefore ~~has-have~~ to be adopted to ~~stop-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* ~~was-were~~ found resistant- to quinolone. *qnrS* gene responsible for quinolone [resistance](#) was also detected in the ~~quinolone~~ [quinolone](#)-resistant *E. coli* [isolates](#) and sequenced. Many of these *E. coli* [isolates](#) were also multi-~~drug-drug~~-resistant. ~~The o~~ Occurrence of ~~quinolone-quinolone~~-resistant *E. coli* in healthy cattle ~~E. coli~~ 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-quinolone~~-resistant *E. coli* may [be](#) transmitted to humans and other animals [s and may will](#) cause serious health problems in future. ~~This is the fast report on the detection and sequencing of qnrS gene responsible for quinolone resistant in E. coli isolated from healthy cattle in Bangladesh.~~

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Table 1. Source and location of samples used in this study.

SL. No.	Source and Location	Total No. of Samples collected
1.	Veterinary Teaching Hospital, Bangladesh Agricultural University, Mymensingh	55
2.	Bangladesh Agricultural University Dairy Farm, Mymensingh	40
3.	Upazila Veterinary Hospital, Muktagacha, Mymensingh	18
4.	Zila Veterinary Hospital, Mymensingh	24
	Total	137

Table 2. List of primers used to amplify *E. coli* 16S rRNA gene, *qnrA* and*qnrS*gene.

Name of the primer	Target gene	Primer Sequence (5'--3')	Amplicon size (bp)	Reference
ECO-1	16S rRNA gene	GACCTCGGTTTAGTTCACAGA	585	Hassan <i>et al.</i> 2014
ECO-2		CACACGCTGACGCTGACCA		
<i>qnrS</i> _F	<i>qnrS</i>	ATTTCTCACGCCAGGATTG	516	Robicseket <i>al.</i> 2006
<i>qnrS</i> _R		GATCGGCAAAGGTTAGGTCA		
<i>qnrA</i> _F	<i>qnrA</i>	ACGACATTCGTCAACTGCAA	417	Robicseket <i>al.</i>

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<i>qnrA_R</i>	TAAATTGGCACCTGTAGGC	2006
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Table 3. 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

Table 4. Prevalence of *E. coli* in rectal swab of apparently healthy cattle.

Age group	No. of collected sample	Positive samples for <i>E. coli</i>	
		Sample Number	%
0-1 year	69	38	55.07

1-3 years	19	13	68.42
3-5 years	08	05	62.5
Above 5 years	41	39	95.12
Total	137	95	69.34

Table 5. Antibigram profile of isolated *E. coli* isolates according to age variation.

Age (year)	<i>E. coli</i> (+)	Pattern	Number of <i>E. coli</i> isolates against different antibiotics						
			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	05	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 6. The overall antibiogram profile of isolated *E. coli* according to different antibiotics.

Antibiotic Name	Sensitive	Intermediate	Resistant
Nalidixic Acid	43 (45.326%)	3 (3.162%)	49 (51.586%)
Gatifloxacin	54 (56.84%)	8 (8.42%)	33 (34.74%)
Levofloxacin	68 (71.658%)	13 (13.768%)	14 (14.876%)
Moxifloxacin	59 (62.11%)	1 (1.05%)	35 (36.84%)
Pefloxacin	54(56.84%)	5 (5.326%)	36 (37.989%)
Ofloxacin	60 (63.216%)	0 (0.00%)	35(36.84%)
Ciprofloxacin	61 (64.21)	13 (13.768%)	21 (22.11%)

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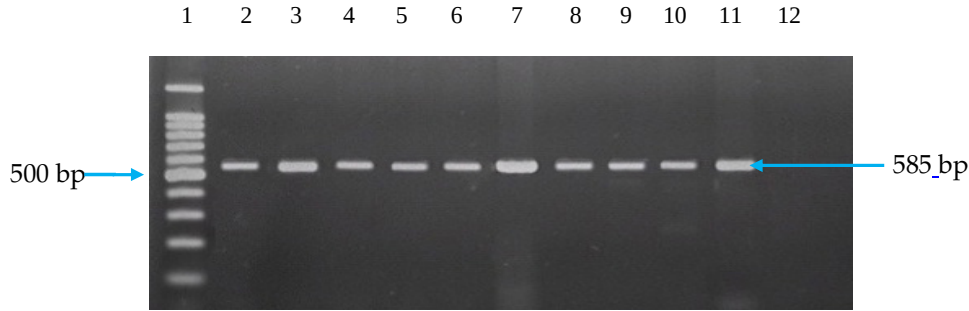
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Table 7. Antibiotic sensitivity pattern of *E. coli* isolates having with *qnrS* gene. Isolate having resistance against three or more antibiotics are considered as multi drug resistant.

SL No.	Age group	Isolate No.	Resistance to antibiotic	Presence of gene
1	0-1 year	10	NA, GAT, LE, MO, PF, OF, CIP	<i>qnrS</i>
2		32	NA, PF	<i>qnrS</i>
3		123	NA, MO, PF	<i>qnrS</i>
4	1-3 years	11	NA, GAT	<i>qnrS</i>
5		115	NA	<i>qnrS</i>
6	3-5 years	97	NA, GAT, LE, MO, PF, OF, CIP	<i>qnrS</i>
7		106	NA, GAT	<i>qnrS</i>
8	>5 years	21	NA, GAT, LE, MO, PF, OF, CIP	<i>qnrS</i>
9		43	NA, GAT, LE, MO, PF, OF, CIP	<i>qnrS</i>

10	83	NA, GAT	<i>qnrS</i>
11	84	NA, GAT, LE, MO, PF, OF, CIP	<i>qnrS</i>

304 NA-Nalidixic Acid, GAT-Gatifloxacin, LE-Levofloxacin, MO-Moxifloxacin, PF-Pefloxacin,
 305 OF-Ofloxacin, CIP-Ciprofloxacin. Isolates that are resistant to three or more antibiotics
 306 were considered as multi-drug resistant.
 307



308 Figure 1. PCR amplification of 16S rRNA of *E. coli*. Lane 1: 100bp size DNA marker;
 309 lane 2-10: representative *E. coli* isolates from rectal swab; lane 11: Positive control and
 310 lane 12: Negative control without DNA.
 311

312

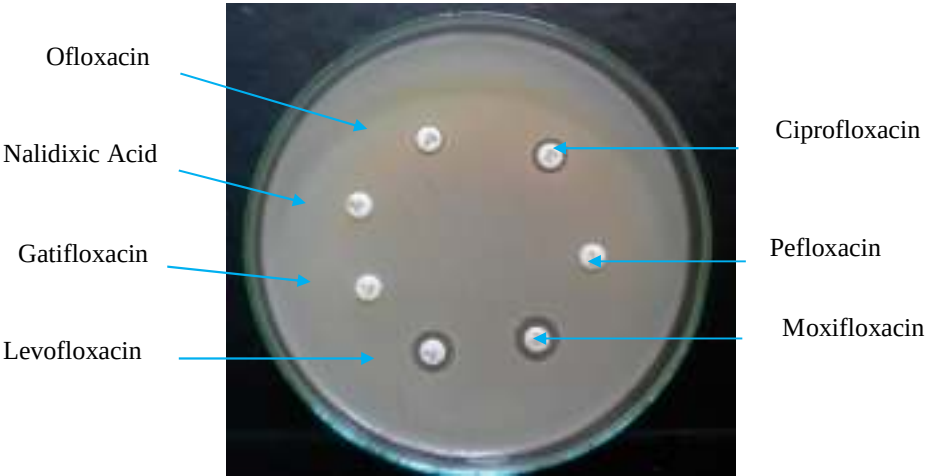


Figure 2. Antibiotic sensitivity test showing a multidrug resistant *E. coli*

313

314

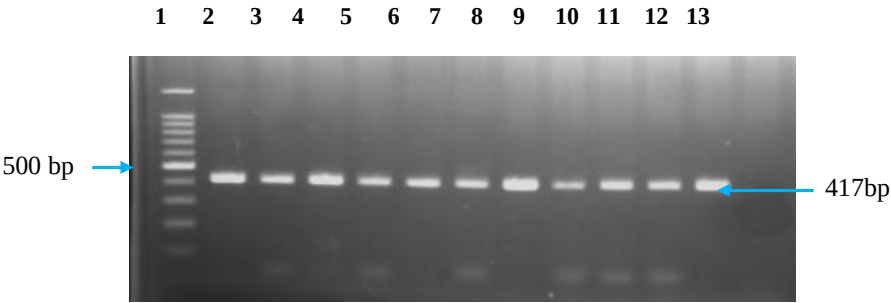
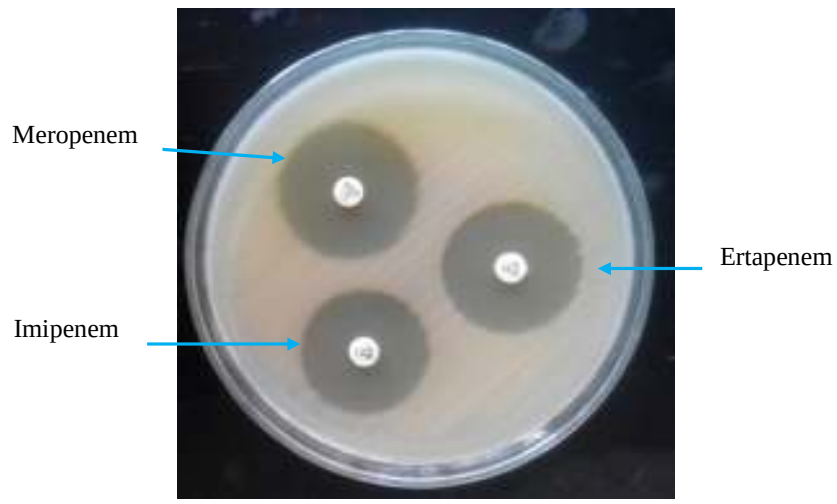


Figure 3. PCR assay to amplify *qnrS* gene of *E. coli* isolates recovered from apparently healthy cattle. Lane 1: 100bp size DNA marker; lane 2- 12: representative *E. coli*, and lane 13: Negative control without DNA.

318



319 **Figure 4. Antibiogram of multiple quinolone group of antibiotic resistant *E. coli***
320 **showing high sensitive to carbapenem group of antibiotic (Imipenem, Ertapenem and**
321 **Meropenem)**