Results

The present study was conducted in the Department of Microbiology, Assam University, Silchar, Assam, India. The duration of study was May 2013 to April 2015. The bacterial strains were collected from Silchar Medical College and Hospital, Silchar, Assam and different community health centres on Silchar town. This tertiary referral hospital serves around 38,26,110 numbers of populations of Assam and neighbouring states like North Tripura, Mizoram, Meghalaya and Manipur. Environmental samples were collected from different sites of five different rivers of southern Assam, water bodies near waste disposal site and food samples were collected from food vendor shops of Silchar town.

A total of 967 consecutive non-duplicate different clinical specimens have been studied during the period of May 2013 to April 2015 and a total of 130 consecutive non-duplicate different environmental samples were studied for the period of September 2014 to August 2015.

4.1 Isolation and identification of isolates from clinical specimens and environmental samples:

Clinical specimens were streaked on Mac-conkey agar, Blood agar and CLED agar (for urine specimen), whereas the environmental samples were streaked on Mac-conkey agar and their microscopical observation by Gram staining, motility and cultural characteristics were observed as mentioned (Table 16). All the enterobacterial isolates were rod shaped and and stained pink in colour. They were further investigated with different biochemical reactions and interpreted accordingly. Lactose fermenting pink colonies were observed for *Escherichia coli* and *Klebsiella* spp.(Figure 9a,9b,9c).

Table 16: Cultural characteristics:

Organisms	**Colony morphology on MacConkey agar, Blood agar and CLED agar**	**Motility**
Escherichia coli	Shiny, pink coloured lactose fermenting colonies on Macconkey agar and beta haemolytic colonies on blood agar.	Motile
Klebsiella spp.	Mucoid, pink coloured lactose fermenting colony and non haemolytic mucoid colony on blood agar.	Non-Motile
Proteus spp.	Colourless, non-lactose fermenting on CLED agar.	Motile

4.1.1 Identification of isolates by biochemical test:

As per interpretation of the results of biochemical reaction, a total number of 212 isolates were found belonging to the member of Enterobacteriaceae family. Among them, predominant type was *Escherichia coli* (n=139), followed by *Klebsiella pneumoniae* (n=53), *Klebsiella oxytoca* (n=7) and *Proteus* spp. (n=12) (Table 17).

Table 17: List of biochemical test:

Organisms	***Escherichia coli***	***Klebsiella pneumoniae***	***Klebsiella oxytoca***	***Proteus vulgaris***	***Proteus mirabilis***
Catalase	+	+	+	+	+
Oxidase	-	-	-	-	-
Indole	+	-	+	+	-
Methyl red	+	-	-	+	-
Voges proskauer	-	+	+	-	+
Simmons citrate	-	+	+	-	+
Urease	-	+	+	+	+
Triple sugar iron	A/A Gas+ H_2S^-	A/A Gas+ H_2S^-	A/A Gas^+ H_2S^-	K/A Gas^+ H_2S^+	K/A Gas^+ H_2S^+
Phenyl alanine Diaminase	-	-	-	+	+
Lysine decarboxylase	+	+	+	–	–
Orinthine decarboxylase	-	-	-	-	+
Arginine Dehydrolase	+	-	-	-	-
Glucose	Acid+ Gas+	Acid+ Gas+	Acid+ Gas+	Acid+ Gas+	Acid+ Gas+
Lactose	+	+	+	-	-
Sucrose	+	+	+	+	-
Mannitol	+	+	+	-	-
Oxidative Fermentative	F	F	F	F	F
Nitrate reduction	+	+	+	+	+

+positive, -negative, A acid, K alkaline, F- fermentative

4.2 Samples:

4.2.1 Clinical isolates:

A total of 864 bacterial isolates were obtained of which 212 were identified as members of Enterobacteriaceae comprising 80 isolates from Silchar Medical College and Hospital and 132 from different community health centres (Table 18).

Table 18: Different isolates obtained from clinical sample in the study**.**

Name of Organisms	**Hospital**	**Community**	**Total**
Escherichia coli	42/80 (Urine=38, stool=3, Pus=1)	97/132 (Urine=94, stool=3)	139
Klebsiella pnuemoniae	30/80 (Blood=6, Throat swab=1, sputum=7, Tracheal aspirate=1, Pus=9, urine=6)	23/132 (sputum=10, pus=5, throat swab=8)	53
Klebsiella oxytoca	2/80 (Ear swab=2)	5/132 (Blood=3, pus=2)	7
Proteus vulgaris	4/80 (pus=2, stool=2)	4/132 (pus=3, stool=1)	8
Proteus mirabilis	1/80 (urine=1)	3/132 (urine=3)	4
Citrobacter Spp.	1/80 (urine=1)	_	1
Total	80 (37.74%)	132 (62.26%)	212

Fig.9a Colony morphology of *E. coli* on Macconkey agar

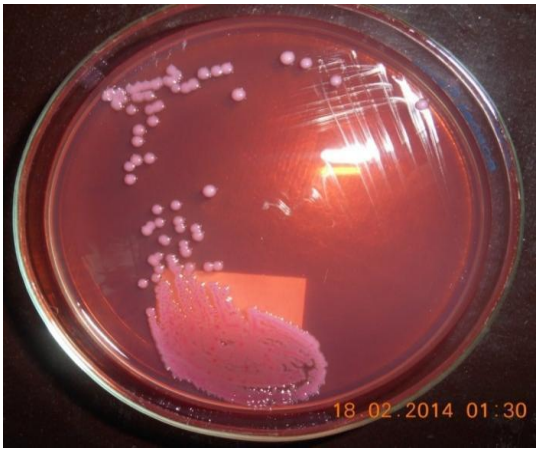


Fig.9b Colony morphology of *Klebsiella* spp.on Macconkey agar

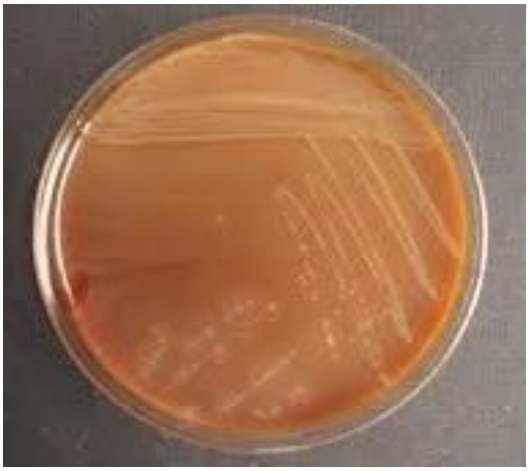


Fig.9c Colony morphology of *Proteus* spp.on Macconkey agar

4.2.2 Environmental isolates:

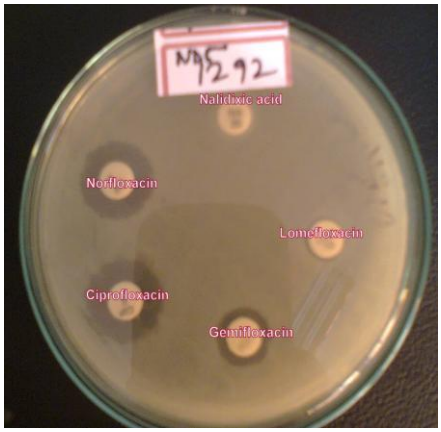
A total of 153 bacterial isolates were isolated of which 68 (44.44%) were identified as Enterobacteriaceae (Table19).

Table 19: Different isolates obtained from environmental samples in the study

Name of organisms	**Water samples from river**	**Water samples near waste disposal sites**	**Food samples from vendor shops**	**Total**
Escherichia coli	10	10	3	23
Klebsiella pnuemoniae	1	3	-	4
Klebsiella oxytoca	5	16	1	22
Proteus mirabilis	10	6	3	19
Total	26	35	7	68

4.3 Phenotypic screening of quinolone resistance:

When screened for quinolone resistance nalidixic acid showed highest resistance (88.68%) followed by lomefloxacin (87.74%), ciprofloxacin(76.42%) and sparfloxacin (74.06 %) (Table 18; Figure 9 and 10).



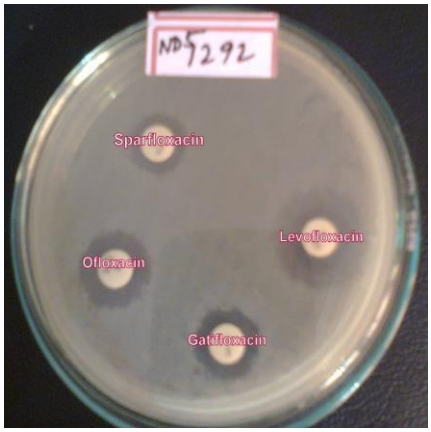


Fig 10: Antibiotic susceptibility plates against quinolone antibiotics

Table no 20: Phenotypic screening results of quinolone resistance among clinical isolates

Organism / Antibiotics	*E.coli* (n=139)		*Klebsiella oxytoca*(n=7)		*Klebsiella pneumoniae* (n=53)		*Proteus mirabilis* (n=4)		*Proteus vulgaris* (n=8)		*Citrobacter* spp.(n=1)	
	N	%	N	%	N	%	N	%	N	%	N	%
Nalidixic acid	129	92.81	2	_	49	92.45	1	_	7	_	_	_
Norfloxacin	102	73.38	3	_	32	60.38	1	_	4	_	_	_
Ofloxacin	101	72.66	4	___–___	26	49.06	-	_–	3	_–	_—	_–
Ciprofloxacin	114	82.01	4	_–	37	69.81	_—	_–	7	_–	_–	_–
Lomefloxacin	126	90.65	3	_–	48	90.57	2	_–	7	_–	_–	_–
Levofloxacin	82	58.99	1	_–	23	43.40	_—	_–	2	_–	_–	_–
Sparfloxacin	110	79.14	4	-	38	71.70	_	_	5	_	_	_
Gatifloxacin	94	67.63	3	_	33	62.26	1	_	4	_	_	_
Gemifloxacin	122	87.77	4	_	43	81.13	1	_	5	_	_	_

n=Total no.of isolate, N=No. of resistant isolate, %=Percentage

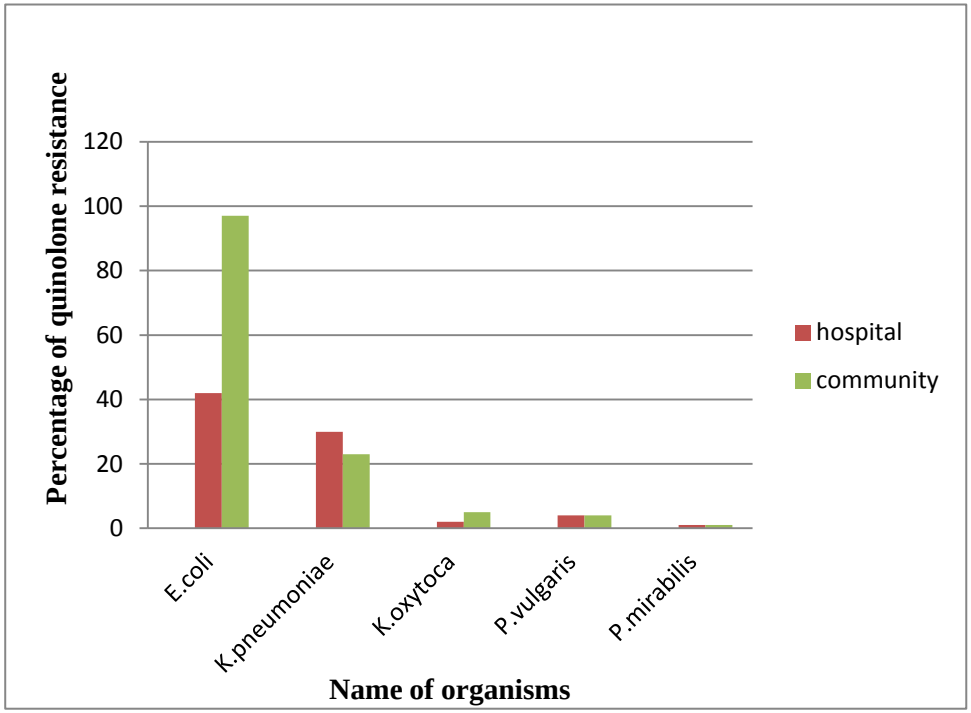


Figure 11: Proportion of quinolone resistant enterobacterial hospital (n=79) and community (n=130) isolates at species level

4.3.1: Resistance tree based on quinolone antibiotic resistance pattern

Based on the quinolone resistance profile of the clinical isolates a total of 17 different resistance pattern were observed; among which 12 patterns were obtained for *E. coli*, 7 for *Klebsiella pneumoniae*, 4 for *Klebsiella oxytoca*, 3 for *Proteus vulgaris* and 2 for *Proteus mirabilis.* Pattern1 wsa observed to the most prevalent in all organisms except *Proteus mirabilis.* In *Proteus mirabilis* pattern 3 and pattern 6 were observed (Table 21).

Table 21: Antibiogram pattern (tree) of quinolone resistant clinical isolates

E. coli	*Klebsiella pneumoniae*	*Klebsiella oxytoca*	*Proteus vulgaris*	*Proteus mirabilis*
NAL,CIP,NOR,OFX,SPX, LOM,GEM (n=71)				
NAL,CIP,SPX,LOM,GEM (n=31)	NAL,CIP,NOR,OFX,SPX ,LOM,	NAL,CIP,NOR,OFX,SPX,	NAL,CIP,NOR,,OFX,SPX,LOM,	NAL,OFX,SPX,LOM ,GEM (n=1)
NAL,CIP,SPX,LVX,GEM (n=11)	GEM (n=11)	LOM,GEM (n=4)	GEM (n=4)	NAL, OFX, LOM, GEM (n=1)
NAL,CIP,LOM,GEM (n=7)	NAL,CIP,OXF,SPX,LOM, GEM		NAL,OFX,SPX,LOM,GEM	
NAL,LOM,GEM (n=3)	(n=10)	NAL,CIP,OFX,SPX,LOM,GE	(n=3)	
NAL,CIP,LOM (n=3)	NAL,CIP,SPX,LOM,GEM	M (n=1)	NAL, OFX, LOM, GEM (n=1)	
NAL,NOR,LOM (n=3)	(n=10)	NAL,CIP,SPX,LOM,GEM		
SPX,LOM (n=2)	NAL,CIP,NOR,GEM (n=9)	(n=1)		
CIP,LOM (n=2)	NAL, CIP,LOM (n=7)	NAL,CIP,NOR,GEM (n=1)		
NAL,LOM (n=2)	NAL, LOM (n=4)			
LOM (n=2)	NAL (n=2)			
NOR (n=2)				

CIP-ciprofloxacin, GAT- gatifloxacin, LVX- levofloxacin, LOM- lomefloxacin, NAL- nalidixic acid, NOR- norfloxacin, OFX- ofloxacin, SPX- sparfloxacin, GEM- gemifloxacin, n= no. of isolates.

Pattern 1: NAL,CIP,NOR,OFX,SPX, LOM,GEM
Pattern 2: NAL,CIP,OXF,SPX,LOM, GEM
Pattern 3: NAL,OFX,SPX,LOM ,GEM
Pattern 4: NAL,CIP,SPX,LOM,GEM
Pattern 5: NAL,CIP,SPX,LVX,GEM
Pattern 6: NAL, OFX, LOM, GEM
Pattern 7: NAL,CIP,NOR,GEM
Pattern 8: NAL,CIP,LOM,GEM
Pattern 9: NAL,LOM,GEM
Pattern 10: NAL,NOR,LOM
Pattern 11: NAL,CIP,LOM
Pattern 12:SPX,LOM
Pattern 13: NAL,LOM
Pattern 14: CIP,LOM
Pattern 15: LOM
Pattern 16: NOR
Pattern 17: NAL

Table 22: Phenotypic screening results of quinolone resistance among environmental isolates

Organism / **Antibiotics**	***Escherichia coli*** **(N=23)**		***Klebsiella pneumoniae*** **(N=4)**		***Klebsiella oxytoca*****(N=22)**		***Proteus mirabilis*** **(N=19)**	
	n	**%**	n	%	n	%	n	%
Nalidixic acid	19	82.61	3	_	21	95.45	17	89.47
Norfloxacin	17	73.91	3	_	19	86.36	16	84.21
Ofloxacin	14	60.87	2	_	17	77.27	13	68.42
Ciprofloxacin	15	65.22	2	_	18	81.81	14	73.68
Lomefloxacin	14	60.87	3	_	17	77.27	14	73.68
Levofloxacin	13	56.52	1		14	63.63	10	52.63

4.4 Minimum inhibitory concentration

4.4.1 Minimum inhibitory concentration against quinolones in clinical isolates.

Among all the fluoroquinolone resistant clinical isolates, highest susceptibility was observed against levofloxacin 36.36% (n=76) and gatifloxacin 35.89% (n=75) followed by ofloxacin 33.49% (n=70); norfloxacin 32.54% (n=68); ciprofloxacin 22.01% (n=46) and rest of the isolates were above the MIC break point (Table 23-27 and Figure 12).

Table23: MIC of the screened clinical isolates against norfloxacin

Organisms	**Antibiotic concentration (µg/ml)**													
	≤0.5	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**256**	**≥256**	**MIC_{50}**	**MIC_{90}**
E.coli **(n=139)**	8	3	3	7	14	3	2	3	6	7	13	70	256	**≥256**
Klebsiella pneumoniae **(n=53)**	9	_	5	4	8	_	2	1	1	2	1	20	16	**≥256**
Klebsiella oxytoca **(n=7)**	_	_	1	_	2	_	_	_	1	_	_	3	64	**≥256**
Proteus vulgaris **(n=8)**	_	1	1	1	_	_	_	_	1	_	1	3	64	**≥256**
Proteus mirabilis **(n=2)**	_	_	_	1	_	_	_	_	_	_	_	1	2	**256**

Sensitive, Intermidiate resistance, resistant.

Table 24: MIC of the screened clinical isolates against ciprofloxacin

Organisms	Antibiotic concentration (µg/ml)													
	≤0.5	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**256**	**≥256**	**MIC_{50}**	**MIC_{90}**
E.coli **(n=139)**	16	1	7	5	10	5	8	14	16	20	19	18	64	**≥256**
Klebsiella pneumoniae **(n=53)**	10	6	1	10	3	_	2	1	2	4	5	9	8	**≥256**
Klebsiella oxytoca **(n=7)**	1	_	2	_	_	_	_	_	_	1	_	3	128	**≥256**
Proteus vulgaris **(n=8)**	_	1	_	_	_	_	_	2	_	1	_	4	128	**≥256**
Proteus mirabilis **(n=2)**	_	_	1	1	_	_	_	_	_	_	_	_	1	2

Sensitive, Intermidiate resistance, resistant.

Table 25: MIC of the screened clinical isolates against ofloxacin

Organism	Antibiotic concentration (µg/ml)													
	≤0.5	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**256**	**≥256**	**MIC_{50}**	**MIC_{90}**
E.coli **(n=139)**	7	10	7	13	_	6	14	16	25	27	11	3	32	256
Klebsiella pneumoniae **(n=53)**	14	4	4	5	6	1	2	_	4	6	3	4	4	256
Klebsiella oxytoca **(n=7)**	2	_	_	1	_	_	_	_	_	2	1	1	128	≥256
Proteus vulgaris **(n=8)**	1	_	_	_	_	1	1	2	_	2	1	_	32	256
Proteus mirabilis **(n=2)**	1	_	_	1	_	_	_	_	_	_	_	_	≤0.5	2

Sensitive, Intermidiate resistance, resistant.

Table 26: MIC of the screened clinical isolates against gatifloxacin

Organism	**Antibiotic concentration (µg/ml)**													
	≤0.5	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**256**	**≥256**	**MIC_{50}**	**MIC_{90}**
E.coli **(n=139)**	10	4	13	13	19	19	19	18	9	7	5	3	8	128
Klebsiella pneumoniae **(n=53)**	2	9	9	7	5	2	2	4	4	3	3	3	2	256
Klebsiella oxytoca **(n=7)**	5	_	_	_	_	2	_	_	_	_	_	_	≤0.5	8
Proteus vulgaris **(n=8)**	1	_	1	_	_	1	1	1	2	_	_	1	16	≥256
Proteus mirabilis **(n=2)**	1	_	_	_	_	_	_	1	_	_	_	_	≤0.5	32

Sensitive, **Intermidiate resistance**, **resistant.**

Table 27: MIC of the screened clinical isolates against levofloxacin

Organism	**Antibiotic concentration (µg/ml)**													
	≤0.5	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**256**	**≥256**	**MIC_{50}**	**MIC_{90}**
E.coli **(n=139)**	8	10	16	14	10	9	5	13	18	14	16	6	16	256
Klebsiella pneumoniae **(n=53)**	18	4	_	_	6	3	3	3	7	9	_	_	4	128
Klebsiella oxytoca **(n=7)**	3	_	1	_	1	2	_	_	_	_	_	_	1	8
Proteus vulgaris **(n=8)**	_	_	1	_	_	_	_	3	1	1	1	1	32	≥256
Proteus mirabilis **(n=2)**	_	_	1	_	1	_	_	_	_	_	_	_	1	4

Sensitive, **Intermidiate resistance**, **resistant.**

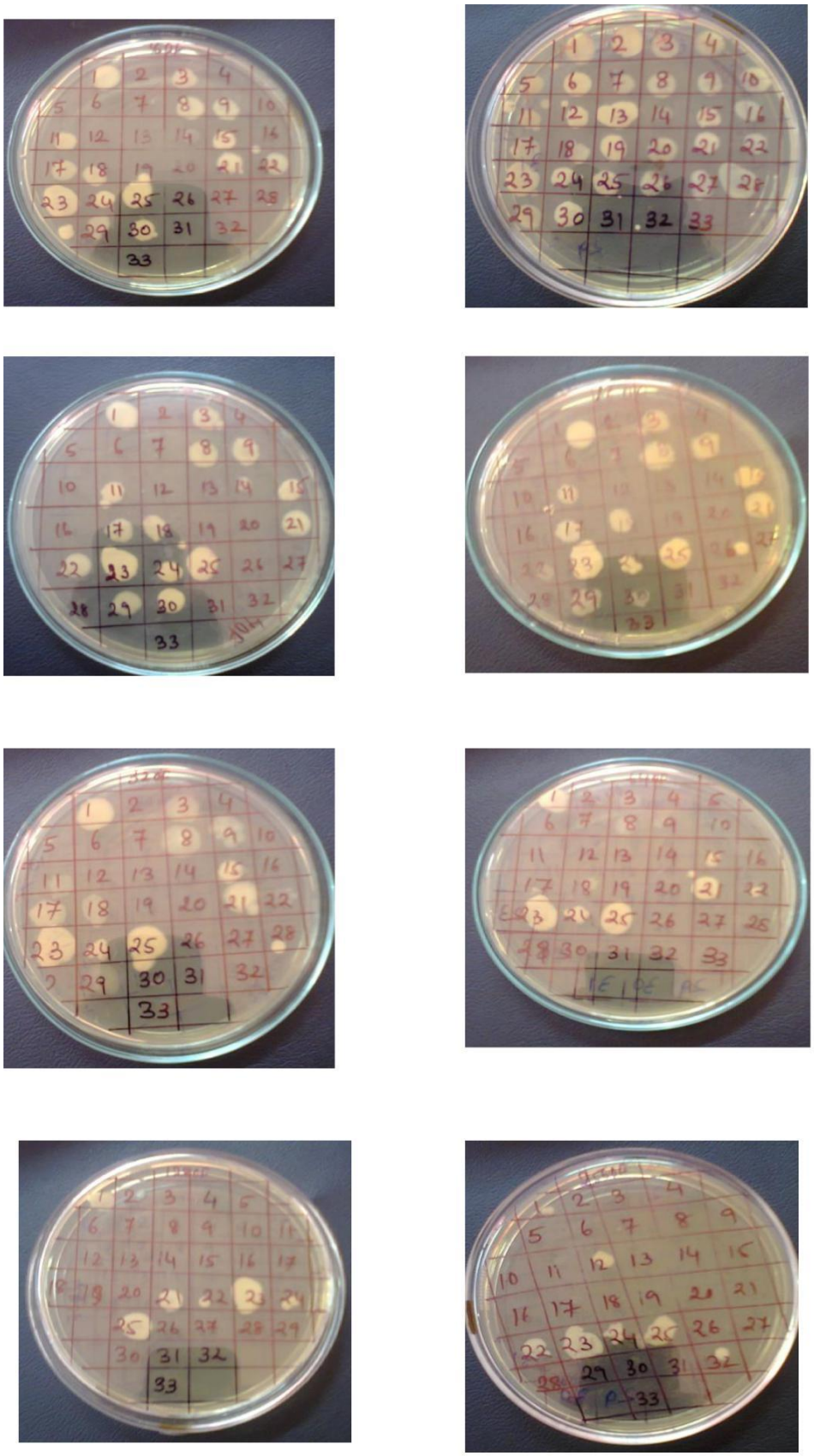


Fig 12: Minimum inhibitory concentration of quinolone resistant isolates against ofloxacin at 2-256µg/ml.

4.4.2 Minimum inhibitory concentration against quinolones in environmental isolates.

Among all the fluoroquinolone resistant environmental isolates, highest susceptibility was observed against levofloxacin 27.94% (n=19) and ofloxacin 22.06% (n=15) followed by norfloxacin 20.59% (n=14); ciprofloxacin 11.76% (n=8) and rest of the isolates were above the MIC break point (Table 28-31).

Table 28: MIC of screened environmental isolates against norfloxacin.

Isolates	MIC range of quinolone resistant isolate(µg/ml)									
	2	**4**	**8**	**16**	**32**	**64**	**128**	**256**	**MIC_{50}**	**MIC_{90}**
E.coli **(n=23)**	2	4	1	_	2	3	10	1	64	128
Klebsiella pneumoniae **(n=4)**	_	1	_	_	1	2	_	_	32	64
Klebsiella oxytoca **(n=22)**	_	3	1	6	2	9	1	_	32	64
Proteus mirabilis **(n=19)**	1	3	_	1	3	2	8	1	128	128

Sensitive, Intermidiate resistance, resistant.

Table 29: MIC of screened environmental isolates against ciprofloxacin

Isolates	MIC range of quinolone resistant isolate(µg/ml)										
	1	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**256**	**MIC_{50}**	**MIC_{90}**
E.coli **(n=23)**	6	2	_	2	1	6	4	2	_	32	64
Klebsiella pneumoniae **(n=4)**	1	_	_	_	_	_	2	1	_	64	128
Klebsiella oxytoca **(n=22)**	1	5	_	_	4	_	11	1	_	64	64
Proteus mirabilis **(n=19)**	_	4	_	4	_	_	2	8	1	64	128

Table 30: MIC of screened environmental isolates against ofloxacin

Isolates	MIC range of quinolone resistant isolate(µg/ml)									
	2	**4**	**8**	**16**	**32**	**64**	**128**	**256**	**MIC_{50}**	**MIC_{90}**
E.coli **(n=23)**	7	2	1	6	5	2	_	_	16	32
Klebsiella pneumoniae **(n=4)**	1	_	_	1	1	1	_	_	32	64
Klebsiella oxytoca **(n=22)**	4	_	1	6	9	2	_	_	16	32
Proteus mirabilis **(n=19)**	3	3	_	3	10	_	_	_	32	32

Sensitive, Intermidiate resistance, resistant.

Table 31: MIC of screened environmental isolates against levofloxacin

Isolates	MIC range of quinolone resistant isolate(µg/ml)									
	2	**4**	**8**	**16**	**32**	**64**	**128**	**256**	**MIC_{50}**	**MIC_{90}**
E.coli **(n=23)**	6	2	2	6	5	2	_	_	16	32
Klebsiella pneumoniae **(n=4)**	1	_	_	3	1	_	_	_	16	32
Klebsiella oxytoca **(n=22)**	6	2	4	_	9	1	_	_	8	32
Proteus mirabilis **(n=19)**	6	2	2	_	9	_	_	_	8	32

Sensitive, **Intermidiate resistance**, **resistant**

4.5 Genotypic characterization of quinolone resistant isolates:

4.5.1 Genotypic characterization of quinolone resistant clinical isolates:

Multiplex PCR results showed presence of different quinolone resistance determinants, among them *aac(6')-lb cr* (n= 23) was most common followed by *qnr*D (n=18), *qnr*A (n=7), *qnr*S (n= 4), *qnr*B (n= 2) (Figure 13). A total of 54 (25.83%) isolates were harbouring single *qnr* gene (Table 32), while in 18 (8.61%) isolates multiple *qnr* genes were found (Table 33). There were 137 isolates which did not show any amplification with target primers.

4.5.2 Genotypic characterization of quinolone resistant environmental isolates:

Multiplex PCR results showed presence of different quinolone resistance determinants, among them *aac(6')-lb cr* (n= 25) was most common, followed by *qnr*D (n=7), *qnr*A (n=5) and *qnr*S (n= 2) (Table 32; Figure 14).

Table 32: Distribution of *qnr* genes among tested clinical isolates (harbouring single *qnr* gene)

***qnr* gene type** / **organisms**	***qnr*A**	***qnr*A**	***qnr*A**	***qnr*A**	***aac(6')-Ib-cr***	**Total (%)**	**None**
***E. coli* (n=139)**	2	1	14	2	16	25.18%	96
***Klebsiella pneumoniae* (n=53)**	4	2	5	2	4	32.07%	25
***Klebsiella oxytoca* (n=7)**	1	--	1	--	--	28.57%	4
***Proteus vulgaris* (n=8)**	--	--	1	--	1	25%	6
***Proteus mirabilis* (n=2)**	--	--	--	--	1	50%	1
Total (n=209)	7	3	21	4	22	27.27%	132

Table 33: Distribution of *qnr* genes among test clinical isolates (harbouring multiple *qnr* gene)

***qnr* genes** / Organisms	***qnr*A+ *aac*(6')*Ib-cr***	***qnr*D+ *aac*(6') *Ib-cr***	***qnr*A+ *qnr*D**	***qnr*B+ aac(6')*Ib-cr***	***qnr*A+ *qnr*B**	***qnr*A + *qnr*B + *qnr*D**	***qnr*A+ *qnr*D+ *aac*(6') *Ib-cr***
***E.coli* (n=139)**	1	3	1	1	1	1	--
***Klebsiella pneumoniae* (n=53)**	--	8	1	1	--	--	1
***Klebsiella oxytoca* (n=7)**	--	--	1	--	--	--	--
***Proteus vulgaris* (n=8)**	--	--	--	--	--	--	--
***Proteus mirabilis* (n=2)**	--	--	--	--	--	--	--
Total (n=209)	1	11	3	2	1	1	1

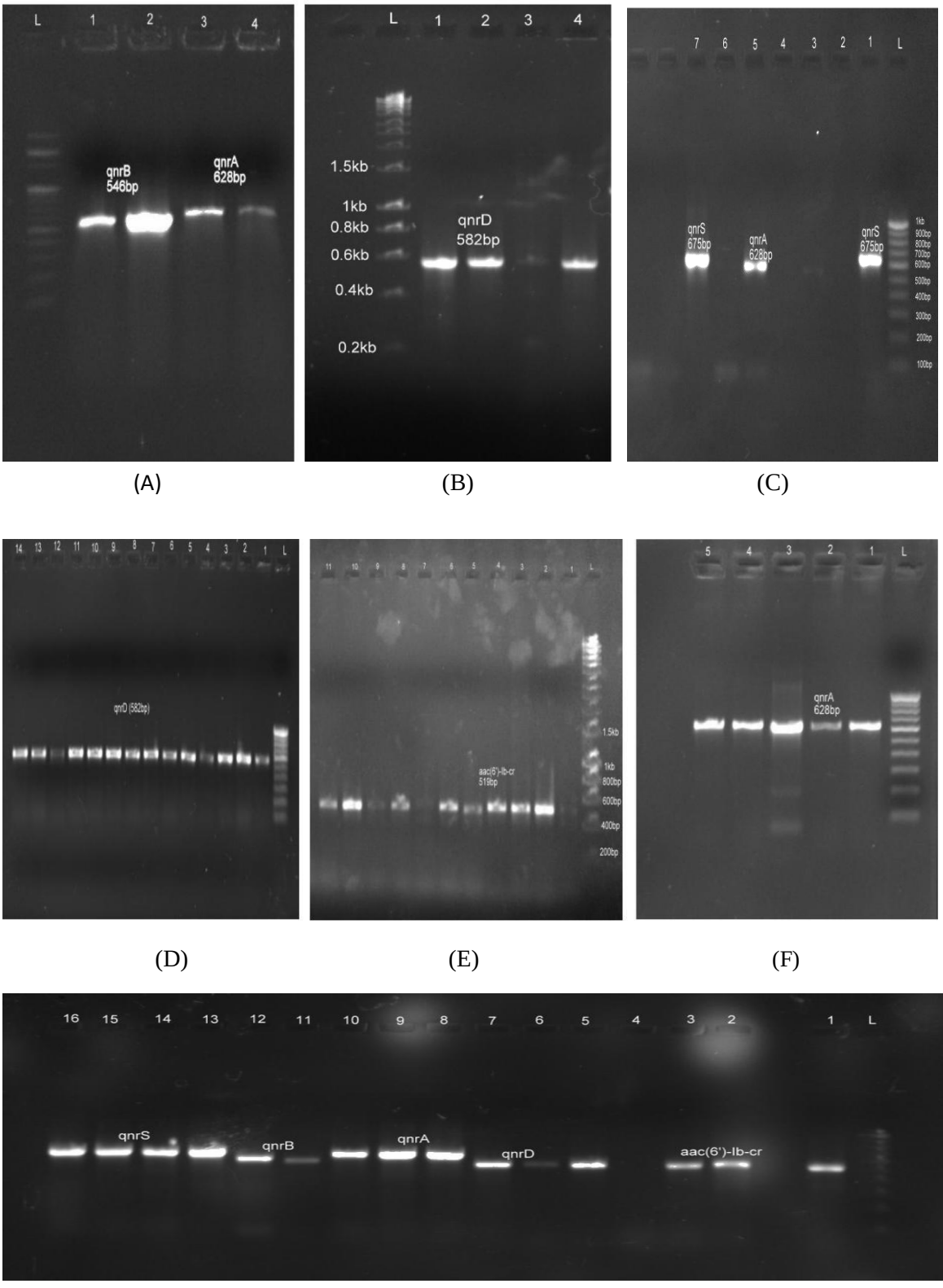
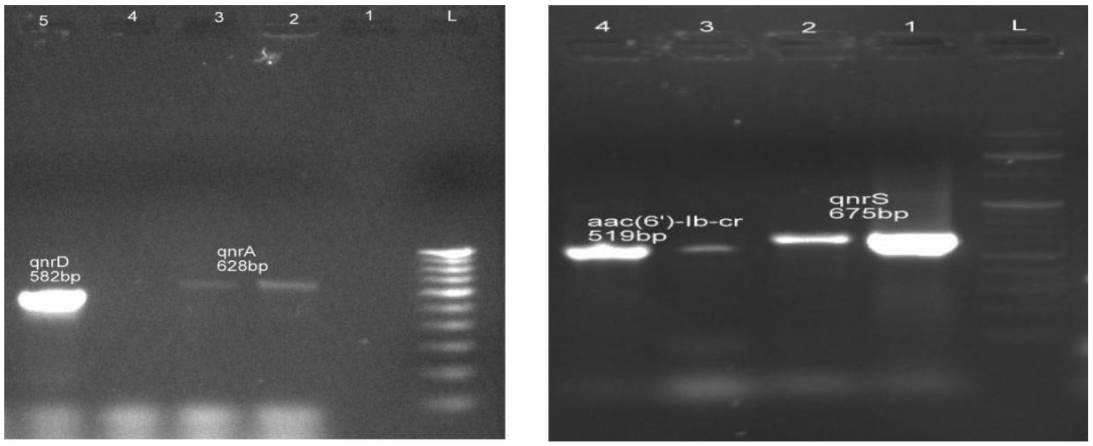


Figure 13:PCR amplification of *qnr* and *aac(6')-Ib-cr*. (A) *qnr*B (546bp) and *qnr*A (628bp); (B) *qnr*D (582bp); (C) *qnr*S (675bp) and *qnr*A(628bp); (D)*qnr*D (582bp); (E) *aac(6')-Ib-cr* (519bp); (F) *qnr*A and (G) L-ladder (100bp), lane 1, 2, 3, 5- *aac(6')-Ib-cr*, lane 6 and 7- *qnr*D, lane 8-10- *qnr*A, 11-12- *qnr*B, lane13-16- *qnr*S

Table 34: Distribution of *qnr* genes among test environmental isolates.

***qnr* gene type** / **Organisms**	***qnr*A**	***qnr*D**	***qnr*S**	***aac*(6')*Ib-cr***	**None**
E.coli **(n=23)**	2	4	1	16	--
Klebsiella pneumoniae **(n=4)**	2	1	1	--	--
Klebsiella oxytoca **(n=22)**	1	2	--	3	16
Proteus mirabilis **(n=19)**	--	--	--	6	13
Total (n=68)	5	7	2	25	29



(A) (B)

Figure 14: PCR amplification of *qnr* and *aac(6')-Ib-cr*.(A) *qnr*A (628bp), *qnr*D (582bp); (B) *qnr*S (675bp) and *aac(6')-Ib-cr* (519bp)

4.6 Sequencing of all *qnr* genes and *aac*(6')*Ib-cr*:

Sequencing of the PCR products of *qnr* and *aac(6')-Ib-cr* showed that isolates harboured *qnr*A1, *qnr*B7, *qnr*B8, qnrS1, qnrD1and *aac(6')-Ib-cr* variants in this study area (Figure15 to19).

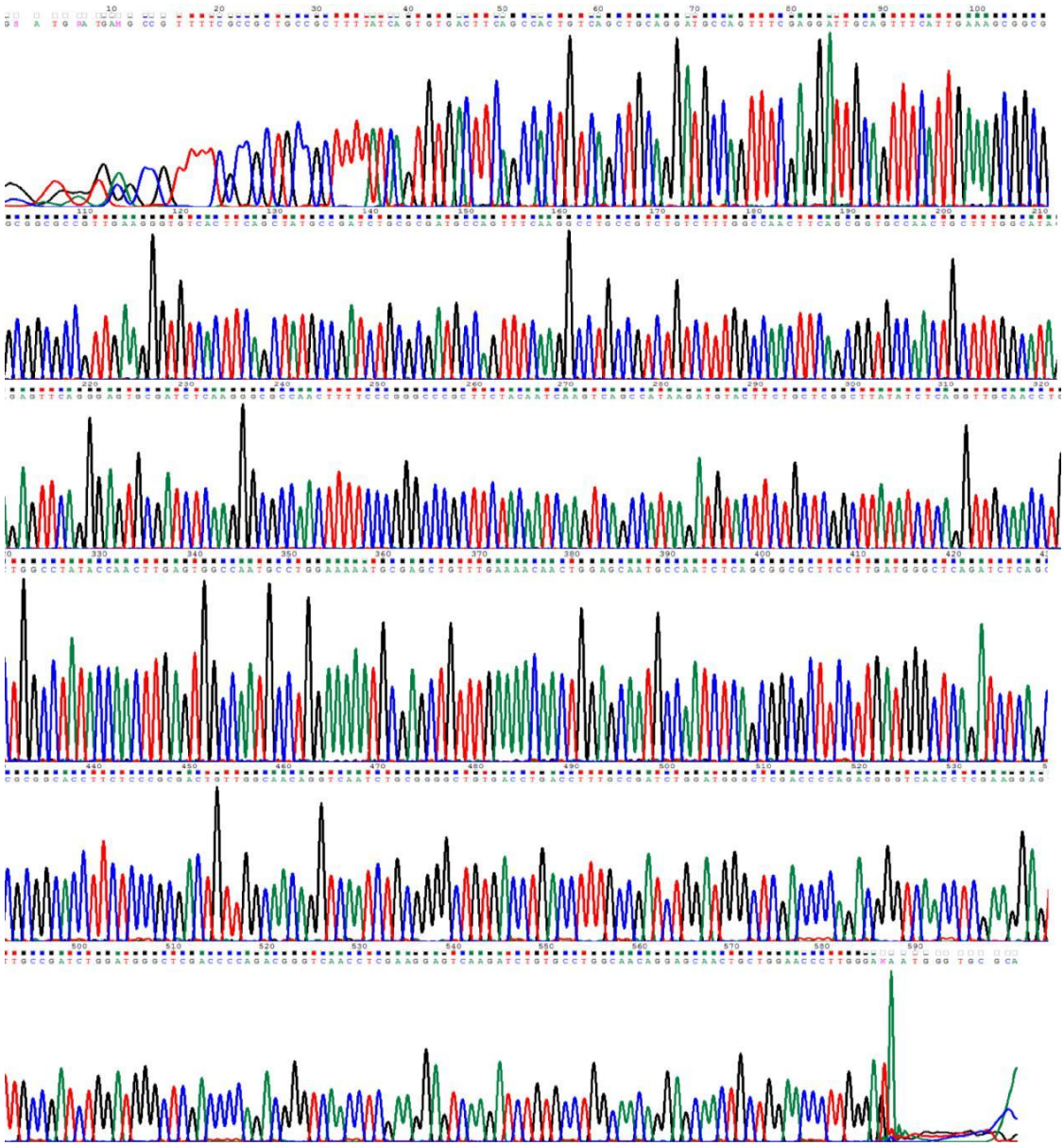


Figure 15: Electropherogram of *qnr*A1 PCR amplicon sequence

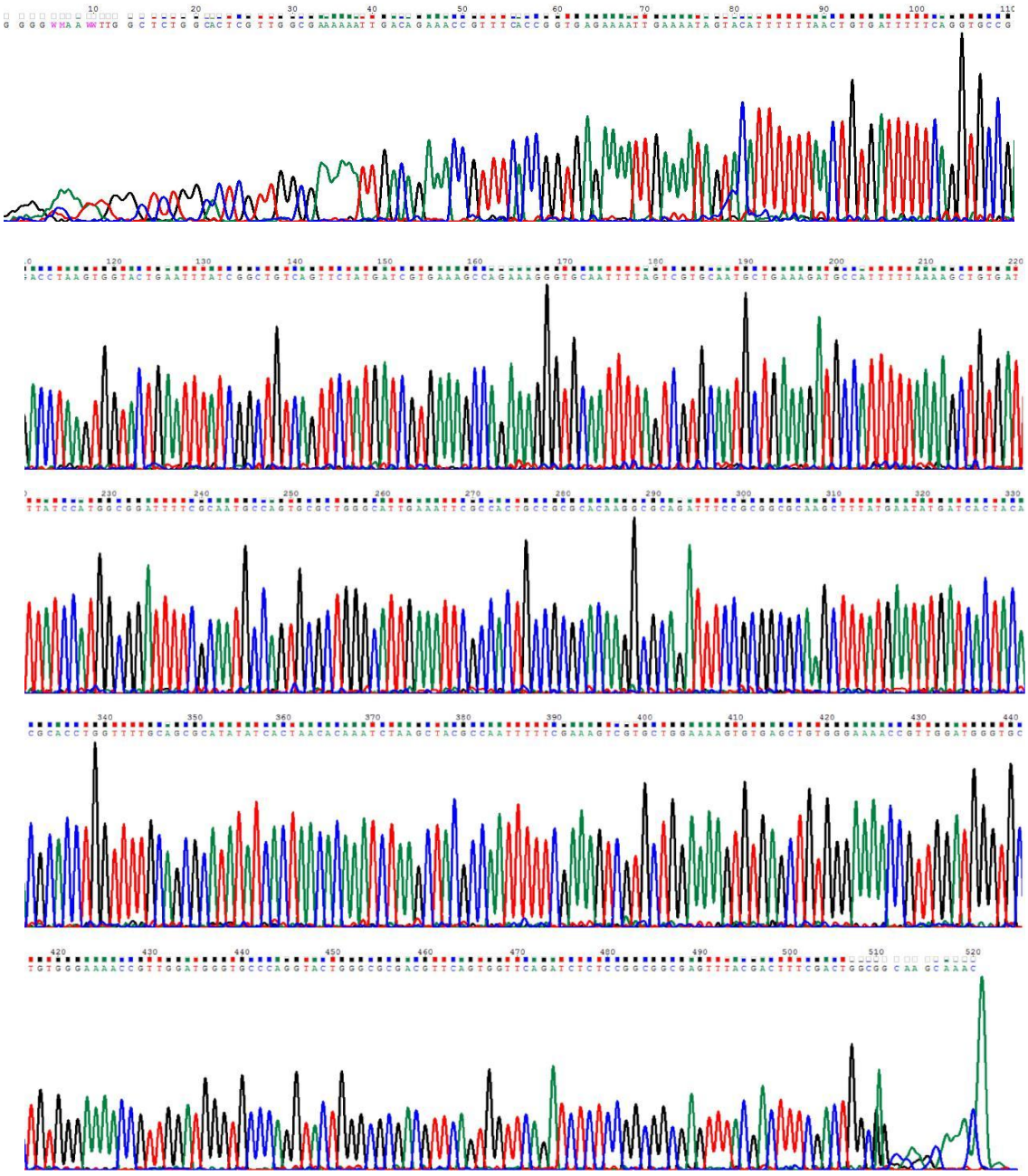


Figure 16 : Electropherogram of *qnr*B7 PCR amplicon sequence

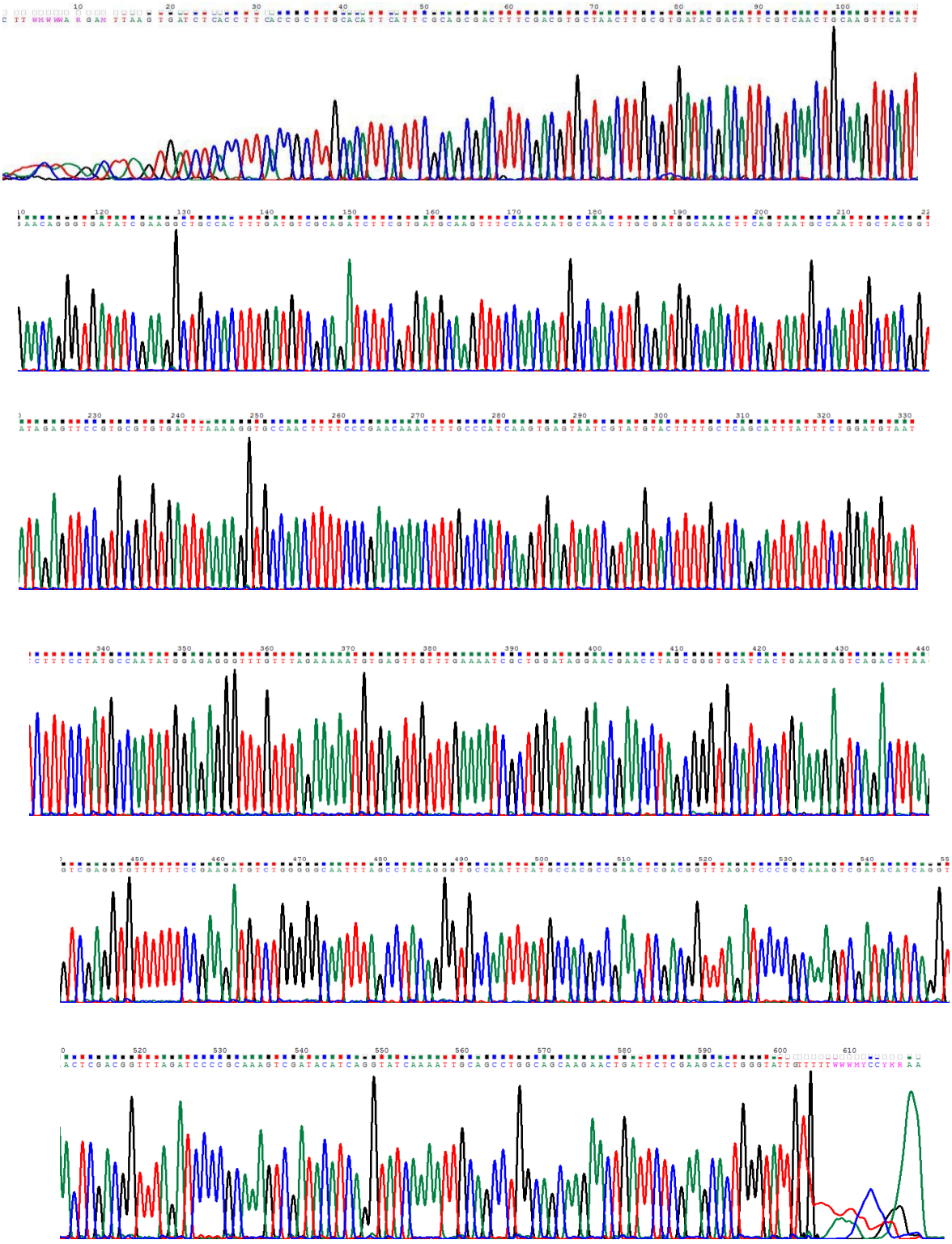


Figure 17 : Electropherogram of *qnr*S1 PCR amplicon sequence

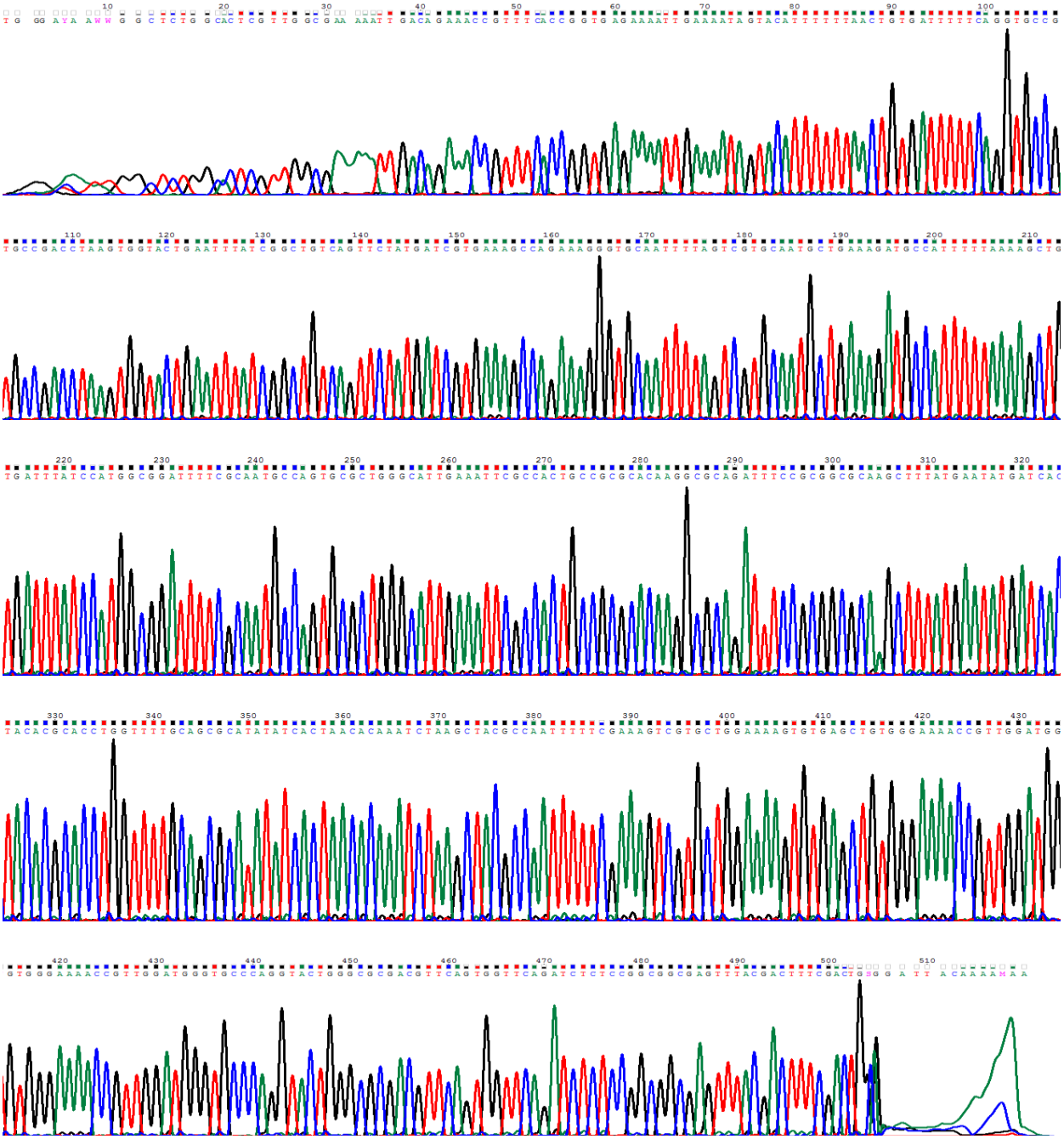


Figure 18: Electropherogram of *qnr*D1 PCR amplicon sequence

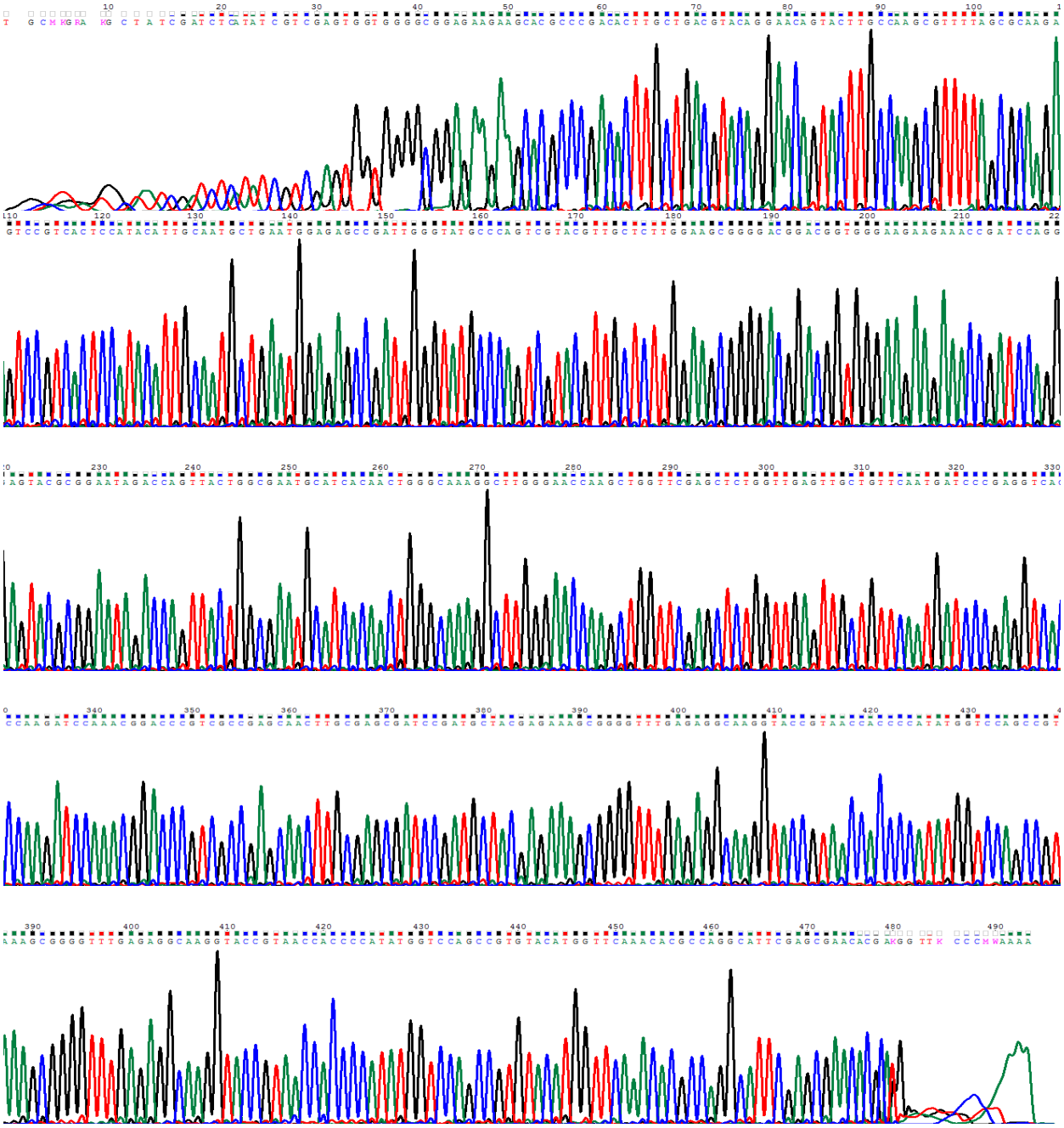


Figure 19: Electropherogram of *aac(6')-Ib-cr* variants PCR amplicon sequence

4.7 Transferability of *qnr* genes and *aac*(6')*Ib-cr* genes:

4.7.1 PCR detection of *qnr* genes and *aac*(6')*Ib-cr* genes in transformants:

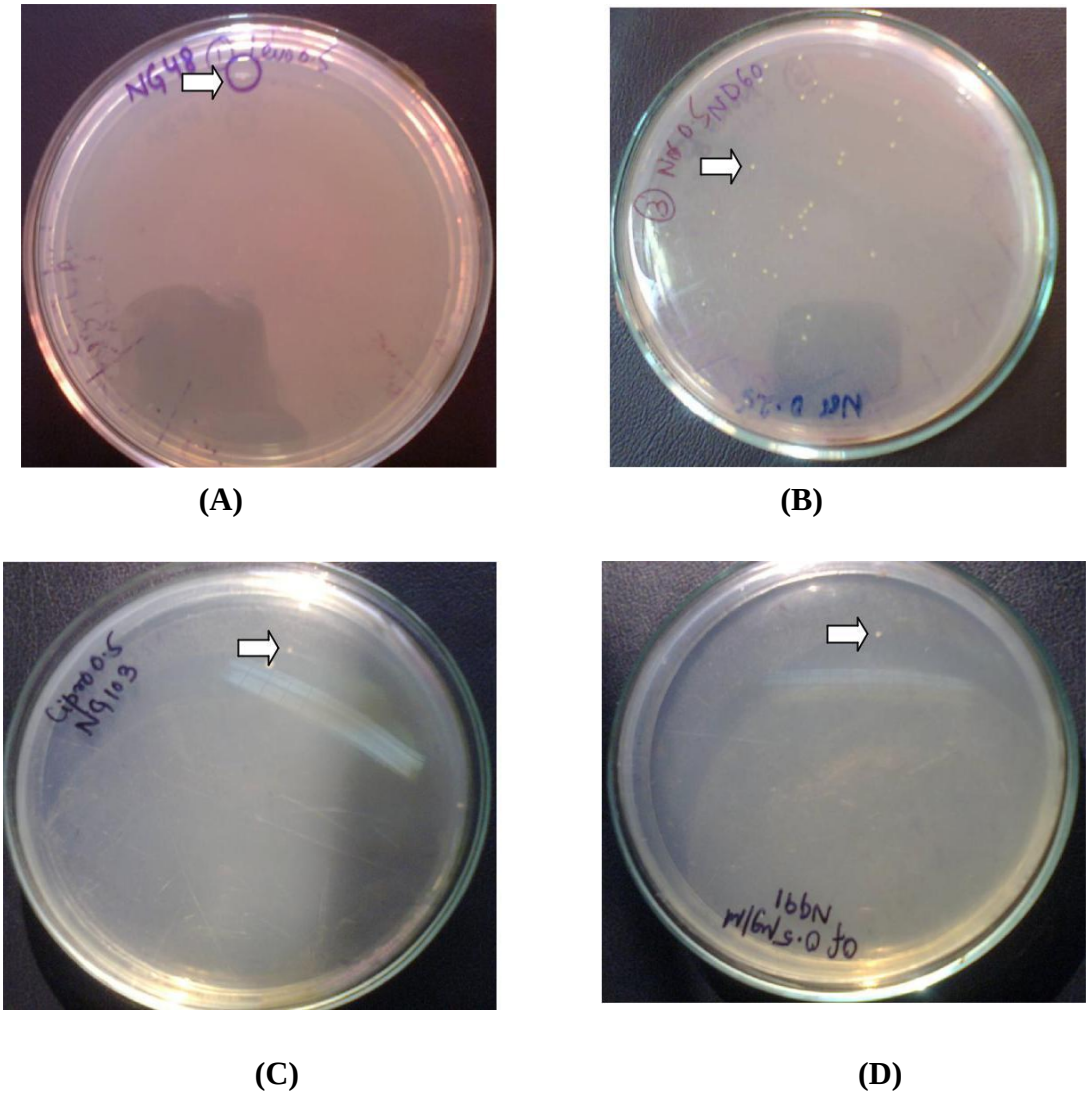
A total of 98 clinical isolates and 36 environmental isolates were subjected to transformation assay, of which transformation was successful with 82 (83.67%) clinical and 36 (100%) environmental isolates (Figure20). PCR was performed for all the transformants and results indicative that all the *qnr* and *aac*(6')*Ib-cr* genes could be transformed successfully to the recipient strain in case of clinical isolates while in case of environmental isolates only *qnr*S and *aac*(6') *–Ib-cr* could be successfully transformed.

4.7.2 Selection specificity of *qnr* genes:

The transformants carrying the PMQR determinants showed biasness during selection towards the quinolone antibiotics. Transformants which carried the *qnr*A determinants were selected from the media containing norfloxacin, ciprofloxacin and levofloxacin; *qnr*B positive transformants were selected from the media containing norfloxacin, ciprofloxacin; *qnr*D positive transformants were selected from the media containing norfloxacin,ofloxacin; *qnr*S positive transformants were selected from the media containing levofloxacin and the transformants which carried the *aac*(6')-*Ib-cr* genes were selected from the media containing norfloxacin and ciprofloxacin (Table 35).

4.7.3 MIC of transformants:

High MIC_{50} and MIC_{90} was observed against all tested fluoroquinolones in transformants of members of Enterobacterial isolates harbouring single and multiple *qnr* genes (Table 36-40). MIC_{50} and MIC_{90} of the transformants against norfloxacin and ciprofloxacin ranged between (2-4 µg/ml), against ofloxacin and gatifloxacin ranged between (1-4 µg/ml), whereas for levefloxacin the range is 0.5-2 µg/ml.



(A) **(B)**

(C) **(D)**

Figure 20 (A-D): Transformants in *E.coli* JM107 recepient strain selected against quinolone antibiotics.

Table 35: MIC_{50} and MIC_{90} of transformants harbouring *qnr*A

Antibiotics	**Isolates harbouring single *qnr* genes**		**Isolates harbouring multiple *qnr* genes**	
	MIC_{50} (µg/ml)	**MIC_{90} (µg/ml)**	**MIC_{50} (µg/ml)**	**MIC_{90} (µg/ml)**
Norfloxacin	2	4	4	4
Ciprofloxacin	2	4	4	4
Ofloxacin	1	2	1	2
Gatifloxacin	2	2	2	4
Levofloxacin	0.5	1	0.5	1

Table36: MIC_{50} and MIC_{90} of transformants harbouring *qnr*B

Antibiotics	**Isolates harbouring single *qnr* genes**		**Isolates harbouring multiple *qnr* genes**	
	MIC_{50} (µg/ml)	**MIC_{90} (µg/ml)**	**MIC_{50} (µg/ml)**	**MIC_{90} (µg/ml)**
Norfloxacin	2	4	4	4
Ciprofloxacin	2	2	2	4
Ofloxacin	2	2	1	2
Gatifloxacin	2	2	2	2
Levofloxacin	0.5	1	0.5	1

Table 37: MIC_{50} and MIC_{90} of transformants harbouring *qnr*D

Antibiotics	**Isolates harbouring single *qnr* genes**		**Isolates harbouring multiple *qnr* genes**	
	MIC_{50} (µg/ml)	**MIC_{90} (µg/ml)**	**MIC_{50} (µg/ml)**	**MIC_{90} (µg/ml)**
Norfloxacin	4	4	4	4
Ciprofloxacin	2	4	2	4
Ofloxacin	2	4	4	4
Gatifloxacin	1	2	2	2
Levofloxacin	1	1	1	1

Table 38: MIC_{50} and MIC_{90} of transformants harbouring *qnr*S

Antibiotics	**Isolates harbouring single *qnr* genes**		**Isolates harbouring multiple *qnr* genes**	
	MIC_{50}(µg/ml)	**MIC_{90}(µg/ml)**	**MIC_{50}(µg/ml)**	**MIC_{90}(µg/ml)**
Norfloxacin	2	2	2	4
Ciprofloxacin	2	4	2	4
Ofloxacin	1	2	1	2
Gatifloxacin	2	2	2	2
Levofloxacin	2	2	2	2

Table 39: MIC_{50} and MIC_{90} of transformants harbouring *aac*(6')*Ib-cr*

Antibiotics	**Isolates harbouring single *qnr* genes**		**Isolates harbouring multiple *qnr* genes**	
	MIC_{50}(µg/ml)	**MIC_{90}(µg/ml)**	**MIC_{50}(µg/ml)**	**MIC_{90}(µg/ml)**
Norfloxacin	4	4	4	4
Ciprofloxacin	4	4	4	4
Ofloxacin	1	2	2	2
Gatifloxacin	1	1	2	2
Levofloxacin	0.5	1	1	1

4.8 Analysis of plasmids:

Plasmid was analysed for the transformants harboring *qnr* gene of each type and the observation was as follows: A 25kb plasmid was found in the isolates carrying *qnr*A, 15kb and 20kb was found carrying *qnr*D, 20kb was found carrying *qnr*B, 25kb was found carrying *qnr*S and 18 kb and 20kb plasmid was found in the isolates carrying *aac*(6')*Ib-cr* genes (Figure 21).

4.9 Plasmid Incompatibility typing:

Plasmid incompatibility group typing in transformants suggested that *qnr*A was located within P, HI1, B/o, T, F_{rep}B, K/B and I1 Inc type; *qnr*B was located within F_{rep}B, K and I1 Inc type; *qnr*D was located within FrepB, FIB, K/B, P Inc type; *qnr*S was located within F_{rep}B and K/B Inc type; *aac(6')-Ib-cr* was located within HI2, FIIs, K/B, P, FrepB, FIB Inc type (Figure22;Table 41-45). Isolates harbouring multiple *qnr* genes were originated through diverse Inc group types *viz*: HI1, I1, W, Y, P, FrepB, K, B/o (Table 44).FIB, F_{rep}B, K/B and HI2 were the most predominant

Inc type present among the isolates. $F_{rep}B$ Inc type was found common among *K.pneumoniae.*

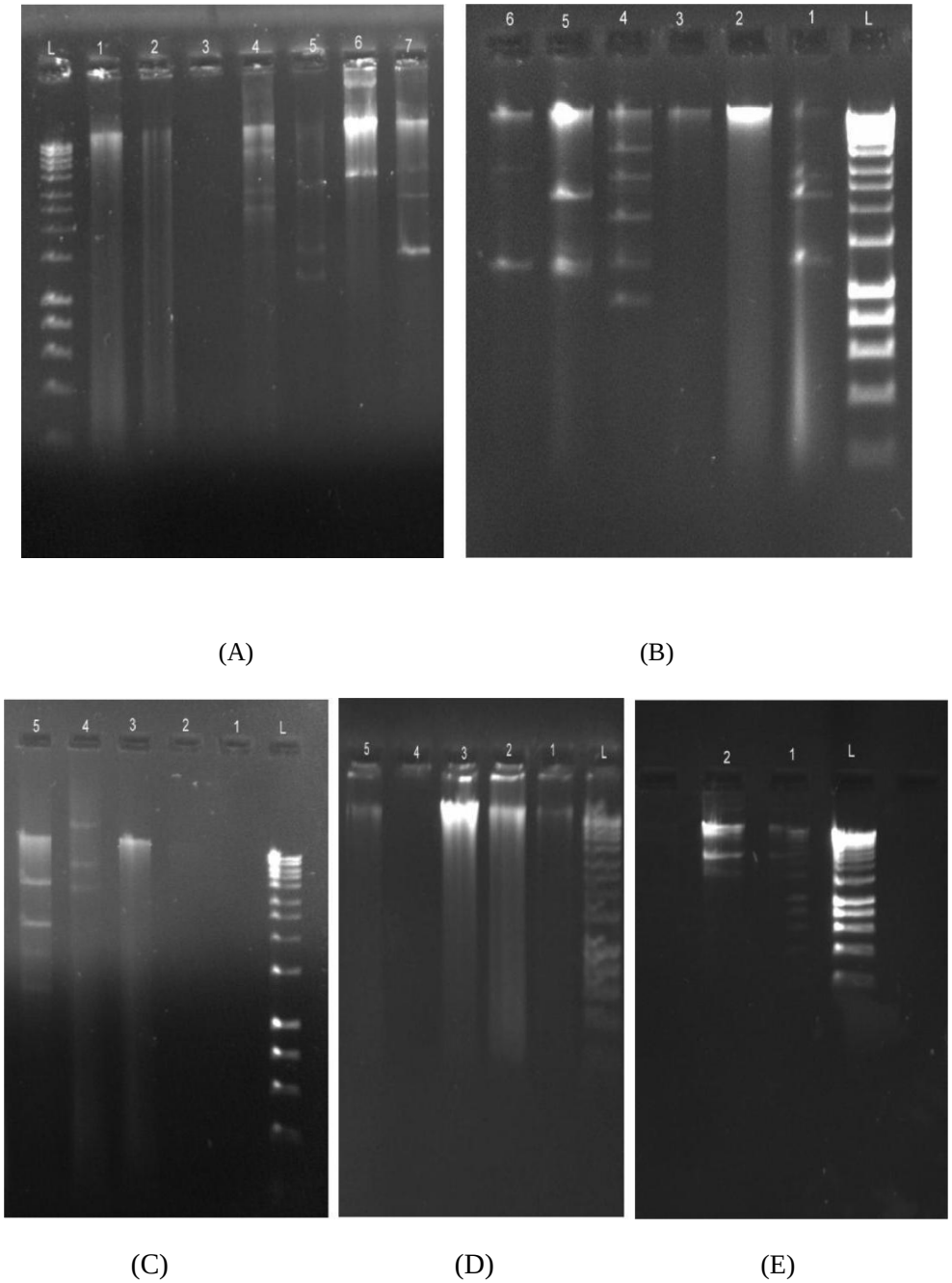


Figure 21: Analysis of plasmid of transformants harbouring *qnr* genes. (A) Plasmid in *qnr*A (B) Plasmid in *qnr*D (C) Plasmid in *qnr*B (D) Plasmid in *qnr*S (E) Plasmid in *aac*(6')*Ib-cr*

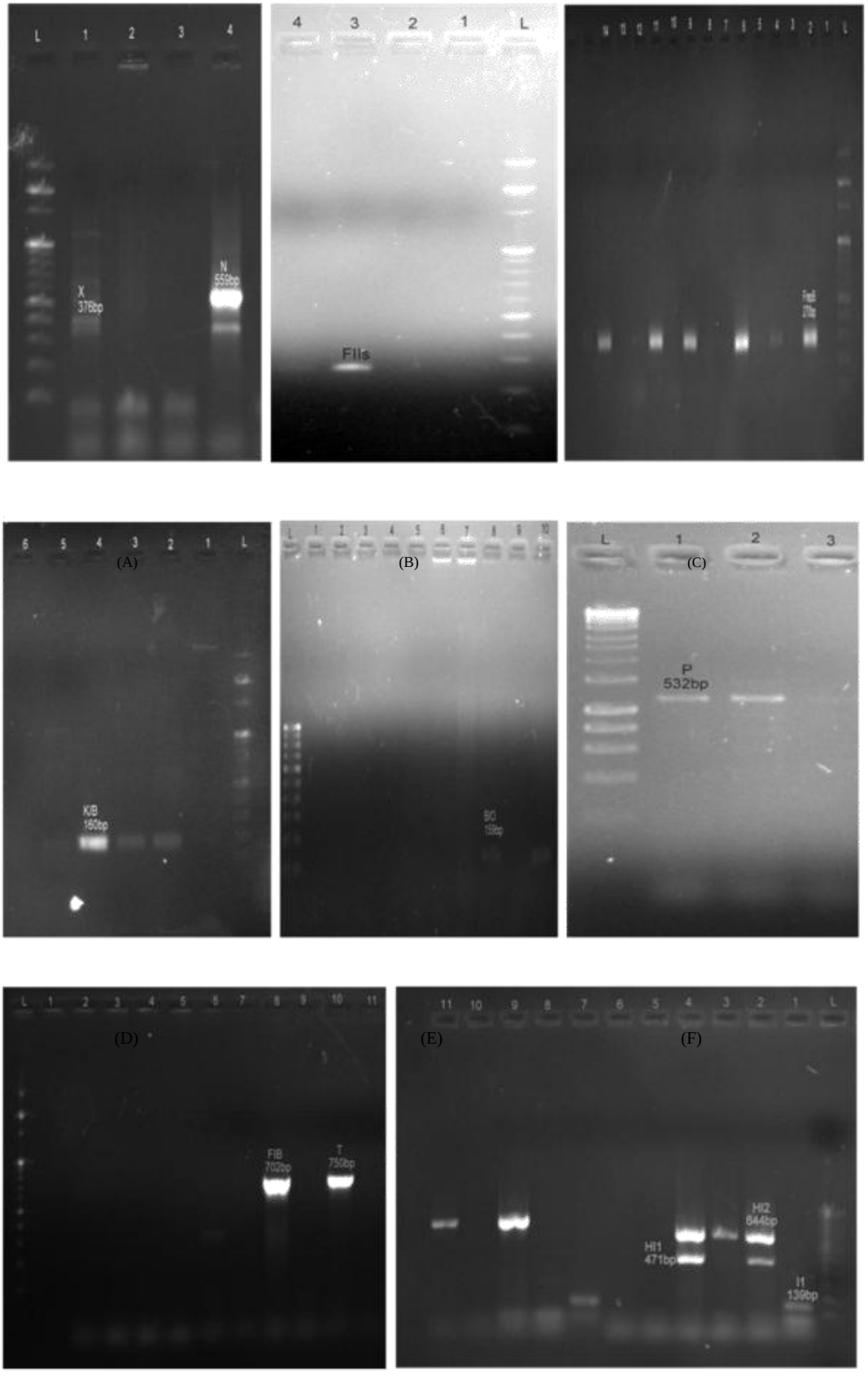


Figure 22 :PCR detection of Inc groups in transformants (A) 376bp Inc X, 559bp IncN; (B) 270bp Inc FIIs; (C) 270bp Inc FrepB; (D) 160bp Inc K/B; (E) 159bp Inc B/O; (F) 532bp Inc P; (G) 702 bp Inc FIB, 750bp Inc T; and (H) 471bp Inc HI1, 644bp Inc HI2, 139bp Inc I1

Table 40: Incompatibility typing of transformant harbouring *qnr*A

Replicon Types	**Organisms**				
	E. coli	***Klebsiella pneumoniae***	***Klebsiella oxytoca***	***Proteus vulgaris***	***Proteus mirabilis***
HI1	--	**1**	--	--	--
HI2	--	--	--	--	--
I1	--	--	1	--	--
X	--	--	--	--	--
L/M	--	--	--	--	--
N	--	--	--	--	--
FIA	--	--	--	--	--
FIB	--	--	--	--	--
W	--	--	--	--	--
Y	--	--	--	--	--
P	1	--	--	--	--
FIC	--	--	--	--	--
A/C	--	--	--	--	--
T	--	1	--	--	--
FIIS	--	--	--	--	--
FrepB	--	1	--	--	--
K/B	--	1	--	--	--
B/O	1	--	--	--	--

Table 41: Incompatibility typing of transformant harbouring *qnr*B

Replicon Types	**Organisms**				
	E. coli	***Klebsiella pneumoniae***	***Klebsiella oxytoca***	***Proteus vulgaris***	***Proteus mirabilis***
HI1	--	--	--	--	--
HI2	--	--	--	--	--
I1	--	1	--	--	--
X	--	--	--	--	--
L/M	--	--	--	--	--
N	--	--	--	--	--
FIA	--	--	--	--	--
FIB	--	--	--	--	--
W	--	--	--	--	--
Y	--	--	--	--	--
P	--	--	--	--	--
FIC	--	--	--	--	--
A/C	--	--	--	--	--
T	--	--	--	--	--
FIIS	--	--	--	--	--
FrepB	--	1	--	--	--
K/B	1	--	--	--	--
B/O	--	--	--	--	--

Table 42: Incompatibility typing of transformant harbouring *qnr*D

Replicon Types	**Organisms**				
	E. coli	***Klebsiella pneumoniae***	***Klebsiella oxytoca***	***Proteus vulgaris***	***Proteus mirabilis***
HI1	--	--	--	--	--
HI2	--	--	--	--	--
I1	--	--	--	--	--
X	1	--	--	--	--
L/M	--	--	--	--	--
N	1	--	--	--	--
FIA	--	--	--	--	--
FIB	8	--	1	1	--
W	--	--	--	--	--
Y	--	--	--	--	--
P	2	--	--	--	--
FIC	--	--	--	--	--
A/C	--	--	--	--	--
T	--	--	--	--	--
FIIS	--	--	--	--	--
FrepB	--	3	--	--	--
K/B	2	2	--	--	--
B/O	--	--	--	--	--

Table 43: Incompatibility typing of transformant harbouring *qnr*S

Replicon Types	**Organisms**				
	E. coli	***Klebsiella pneumoniae***	***Klebsiella oxytoca***	***Proteus vulgaris***	***Proteus mirabilis***
HI1	--	--	--	--	--
HI2	--	--	--	--	--
I1	--	--	--	--	--
X	--	--	--	--	--
L/M	--	--	--	--	--
N	--	--	--	--	--
FIA	--	--	--	--	--
FIB	--	--	--	--	--
W	--	--	--	--	--
Y	--	--	--	--	--
P	--	--	--	--	--
FIC	--	--	--	--	--
A/C	--	--	--	--	--
T	--	--	--	--	--
FIIS	--	--	--	--	--
FrepB	2	--	--	--	--
K/B	--	2	--	--	--
B/O	--	--	--	--	--

Table 44: Incompatibility typing of transformant harbouring *aac*(6')*Ib-cr*

Replicon Types	**Organisms**				
	E. coli	***Klebsiella pneumoniae***	***Klebsiella oxytoca***	***Proteus vulgaris***	***Proteus mirabilis***
HI1	--	--	--	--	--
HI2	9	--	--	--	--
I1	--	--	--	--	--
X	--	--	--	--	--
L/M	--	--	--	--	--
N	--	--	--	--	--
FIA	--	--	--	--	--
FIB	--	--	--	1	--
W	--	--	--	--	--
Y	--	--	--	--	--
P	--	2	--	--	1
FIC	--	--	--	--	--
A/C	--	--	--	--	--
T	--	--	--	--	--
FIIS	5	--	--	--	--
FrepB	--	2	--	--	--
K/B	2	--	--	--	--
B/O	--	--	--	--	--

Table 45: Incompatibility typing of transformant harbouring multiple *qnr* genes

Replicon Types	**Organisms**				
	E. coli	***Klebsiella pneumoniae***	***Klebsiella oxytoca***	***Proteus vulgaris***	***Proteus mirabilis***
HI1	2	1	--	--	--
HI2	--	--	--	--	--
I1	--	--	1	--	--
X	--	--	--	--	--
L/M	--	--	--	--	--
N	--	--	--	--	--
FIA	--	--	--	--	--
FIB	--	--	--	--	--
W	--	6	--	--	--
Y	5	--	--	--	--
P	1	--	--	--	--
FIC	--	--	--	--	--
A/C	--	--	--	--	--
T	--	--	--	--	--
FIIS	--	--	--	--	--
FrepB	--	1	--	--	--
K/B	--	1	--	--	--
B/O	--	2	--	--	--

4.10 Analysis of conjugative transferability of plasmid

Conjugation experiment was performed with 82 isolates and it was found that 76 (92.68%) isolates were conjugatively transferable (Figure23). Resistance pattern of these transconjugant against quinolone antibiotics were confirmed by disc diffusion test (Figure 24 A and B).

4.10.1 PCR detection of *qnr* among transconjugants:

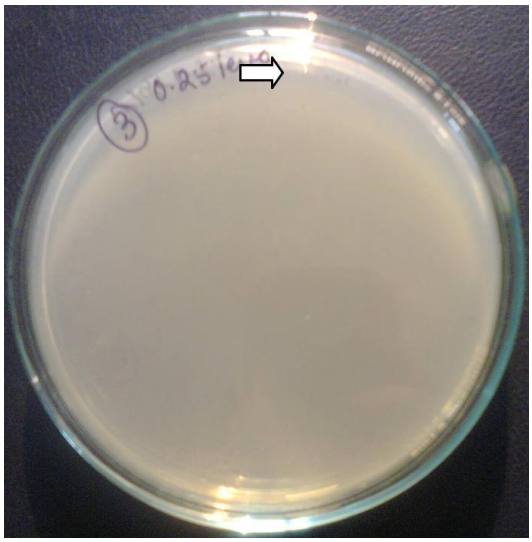
PCR results were indicative that *qnr* genes were present in all the transconjugant plasmids.

4.10.2 MIC of transconjugants:

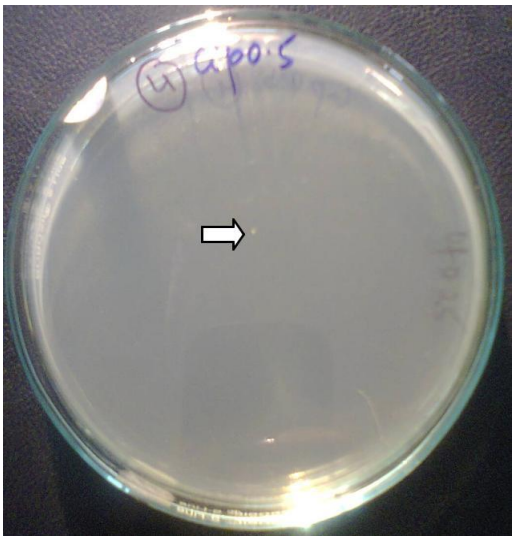
High MIC_{50} and MIC_{90} were observed in all transconjugants against all tested quinolones antibiotics (Table47-51). MIC_{50} and MIC_{90} for all the transconjugants against norfloxacin, ciprofloxacin and ofloxacin ranged between 1-4 µg/ml whereas against gatifloxacin and levofloxacin MIC_{50} and MIC_{90} ranged between 0.5-2 µg/ml (Figure 24C and D).

Table 46: MIC_{50} and MIC_{90} of transconjugants harbouring *qnr*A

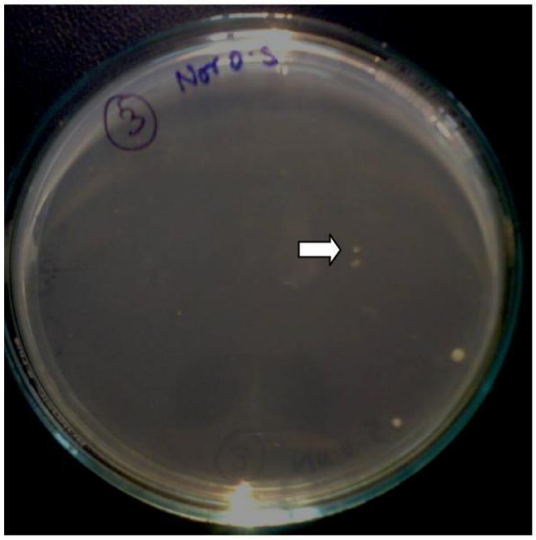
Antibiotics	**Isolates harbouring single *qnr* genes**		**Isolates harbouring multiple *qnr* genes**	
	MIC_{50}(µg/ml)	**MIC_{90}(µg/ml)**	**MIC_{50}(µg/ml)**	**MIC_{90}(µg/ml)**
Norfloxacin	2	2	2	4
Ciprofloxacin	2	2	2	4
Ofloxacin	1	2	1	2
Gatifloxacin	1	2	2	4
Levofloxacin	0.5	1	0.5	1



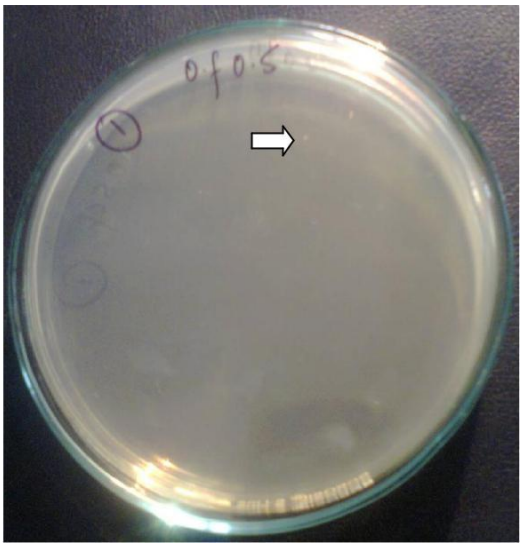
(A)



(B)



(C)



(D)

Figure 23 (A-D): Transconjugants in *E.coli* F^- strain (small white colonies).

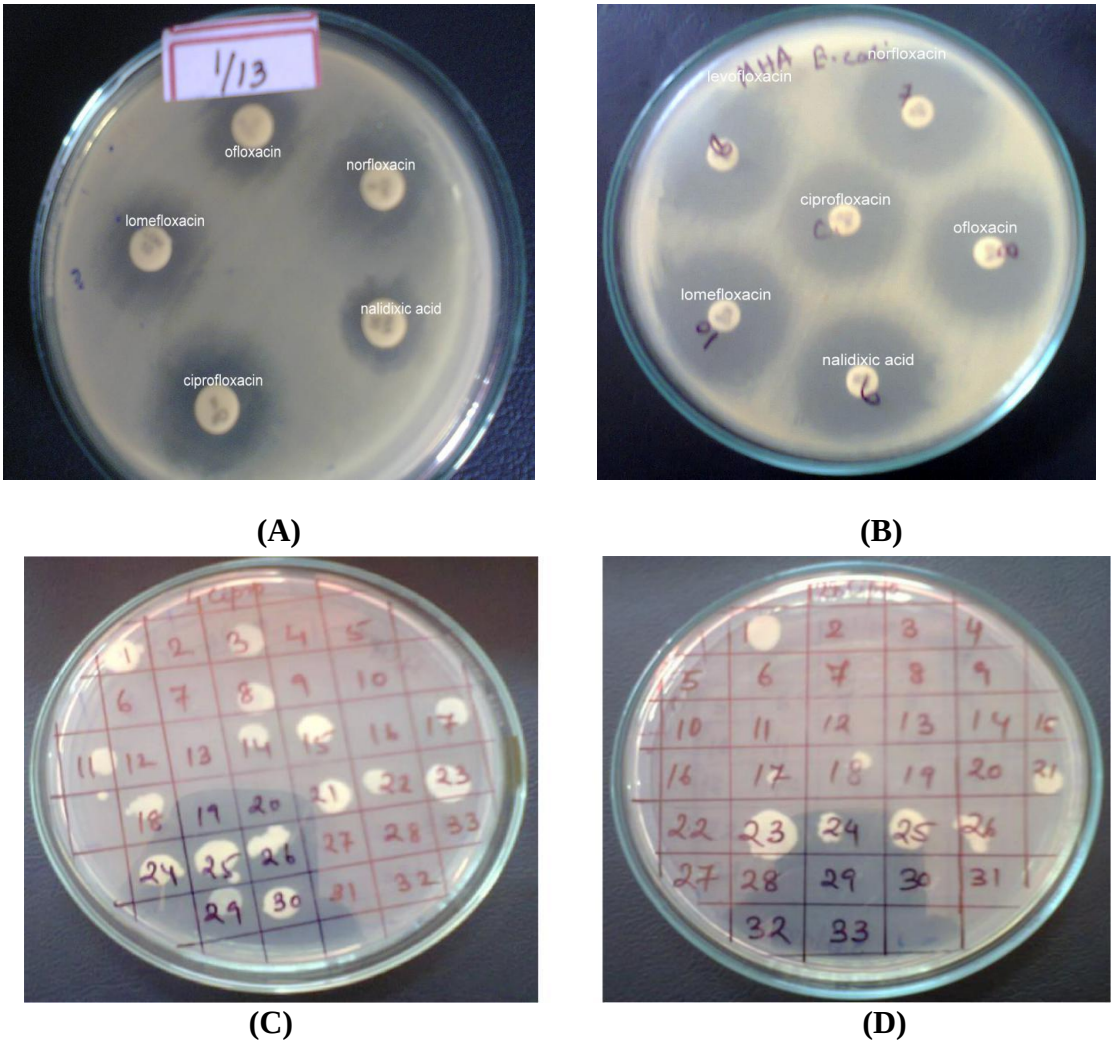


Figure 24(A): Antibiotic susceptibility of transformant against quinolone antibiotics. (B) Antibiotic susceptibility of *E. coli* JM 107 without plasmid against quinolone antibiotics. (C) MIC of transformants against quinolone antibiotic. (D) MIC of transconjugants against quinolone antibiotic**.**

Table 47: MIC_{50} and MIC_{90} of transconjugants harbouring *qnr*B

Antibiotics	**Isolates harbouring single *qnr* genes**		**Isolates harbouring multiple *qnr* genes**	
	MIC_{50}(µg/ml)	**MIC_{90}(µg/ml)**	**MIC_{50}(µg/ml)**	**MIC_{90}(µg/ml)**
Norfloxacin	2	2	4	4
Ciprofloxacin	2	2	2	4
Ofloxacin	1	2	1	2
Gatifloxacin	1	2	2	2
Levofloxacin	0.5	1	0.5	1

Table 48: MIC_{50} and MIC_{90} of transconjugants harbouring *qnr*D

Antibiotics	**Isolates harbouring single *qnr* genes**		**Isolates harbouring multiple *qnr* genes**	
	MIC_{50}(µg/ml)	**MIC_{90}(µg/ml)**	**MIC_{50}(µg/ml)**	**MIC_{90}(µg/ml)**
Norfloxacin	2	2	2	4
Ciprofloxacin	2	2	2	4
Ofloxacin	2	4	2	4
Gatifloxacin	1	1	2	2
Levofloxacin	1	1	1	1

Table 49: MIC_{50} and MIC_{90} of transconjugants harbouring *qnr*S

Antibiotics	**Isolates harbouring single *qnr* genes**		**Isolates harbouring multiple *qnr* genes**	
	MIC_{50}(µg/ml)	**MIC_{90}(µg/ml)**	**MIC_{50}(µg/ml)**	**MIC_{90}(µg/ml)**
Norfloxacin	1	2	2	4
Ciprofloxacin	1	2	2	2
Ofloxacin	1	2	1	2
Gatifloxacin	0.5	2	1	2
Levofloxacin	0.5	2	1	2

Table 50: MIC_{50} and MIC_{90} of transconjugants harbouring *aac*(6')*Ib-cr*

Antibiotics	**Isolates harbouring single *qnr* genes**		**Isolates harbouring multiple *qnr* genes**	
	MIC_{50}(µg/ml)	**MIC_{90}(µg/ml)**	**MIC_{50}(µg/ml)**	**MIC_{90}(µg/ml)**
Norfloxacin	2	4	2	4
Ciprofloxacin	2	4	2	4
Ofloxacin	1	1	2	2
Gatifloxacin	1	1	2	2
Levofloxacin	0.5	1	1	1

4.11 DNA fingerprinting of quinolone resistant isolates by ERIC PCR

After performing ERIC PCR, 74 types of *Escherichia coli*, 21 types *Klebsiella pnemoniae*, 3 types of *Klebsiella oxytoca*,3 types *Proteus vulgaris* and 2 types *Proteus mirabilis* were found in the study (Figure 25A,B,C).

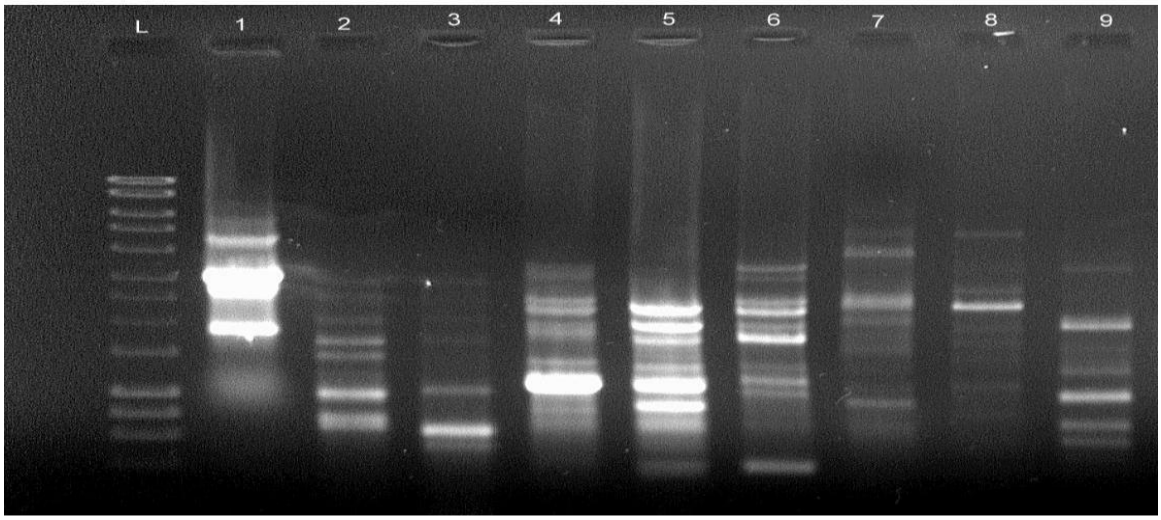
4.12 Determination of genetic environment:

4.12.1 Detection of the location of *qnr* gene within integron region:

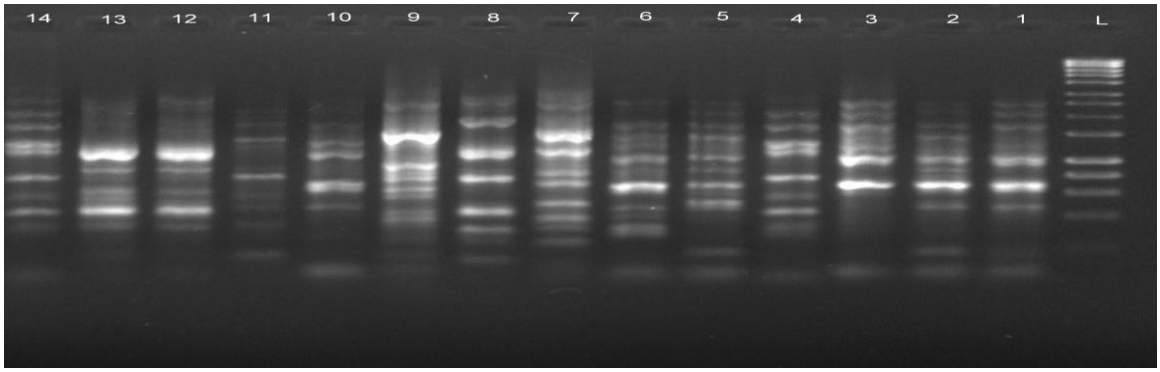
Sequencing of the amplified products revealed that *qnr*D and *aac*(6')*Ib-cr* were located within the variable region of class 1 integron whereas other quinolone determinants showed no associoation with the gene capture mechanism.

4.12.2 Association of *qnr* and *aac*(6')*Ib-cr* genes with mobile element:

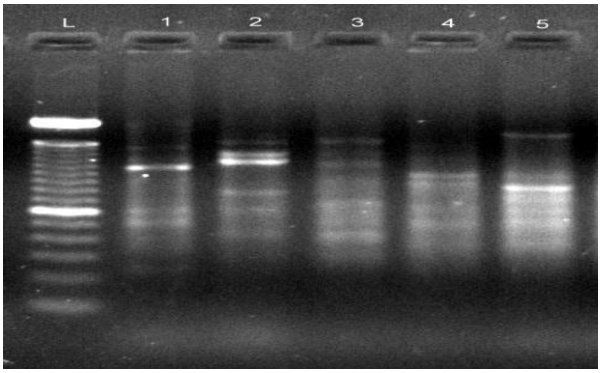
Sequencing results showed that *qnr*A genes were linked with *tnISEcp1*; *tnIS26;* and *IS26* in the upstream region while in case of *aac*(6')*Ib-cr* the upstream region showed the presence of *tnIS26*. No association was observed in other quinolone resistance determinants associated with mobile genetic elements.



(A)



(B)



(C)

Figure 25 : (A) DNA finger printing of *E. coli* by ERIC PCR. Lane L: 10Kb DNA hyper ladder; Lane 1-9: ERIC pattern of *E. coli* Type 1-9 **(B)** DNA finger printing of *Klebsiella pneumonia* and *Klebsiella oxytoca* by ERIC PCR.Lane L: 10Kb DNA hyper ladder; Lane 1-11: ERIC pattern of *Klebsiella pneumonia* Type 1-11; Lane 12-14: ERIC pattern of *Klebsiella oxytoca* Type 1-3. **(C)** DNA finger printing of *Proteus vulgaris* and *Proteus mirabilis* by ERIC PCR.Lane L: 10Kb DNA hyper

ladder; Lane 1-3: ERIC pattern of *Proteus vulgaris* Type 1-3; Lane 4-5: ERIC pattern of *Proteus mirabilis* Type 1-2.

4.13 Study of mutation in the quinolone resistance determining region by denaturing gradient gel electrophoresis:

All the quinolone resistant isolates that were devoid of quinolone resistance determinants were subjected to PCR amplification using primers listed in table 17. All the isolates showed positive results for PCR amplification with the primers used (Figure 26). Altogether 96 isolates were studied for mutation in the quinolone resistance determining region (QRDR) by DGGE. Four band patterns were obtained for each gene when compared with the positive control (Figure 27).

4.13.1 Sequencing of *gyr*A and *par*C genes:

The amplified products were subjected to DGGE. Analysis of the gel revealed four types (A-D) of band patterns of each of *gyr*A and *par*C genes. In order to determine the contribution of mutation in QRDR region which attributes fluoroquinolone resistance, sequencing of *gyr*A and *par*C patterns were done. When the DNA sequence of the *gyr*A was compared with *gyr*A subunit of EC493/89, it revealed nucleotide differences at many positions (Figure 28a and 28b). Pattern A were found to have 9 point mutations (EGYMU1), Pattern B with 13 point mutations (EGYMU2), Pattern C with11 point mutations (EGYMU3) and Pattern D was found to have 10 point mutations (EGYMU4). Two transition mutations were common in all the isolates. Three insertion mutations were found between 166^{th} and 167^{th} base by T (starting with position 1 at the A of the start codon of *gyr*A) of *gyr*A pattern EGYMU1. At 181^{th} and 190^{th} base, deletion of single nucleotide A was observed in all *gyr*A pattern (Figure28a). Mutation in codons 83 and 87 in *gyr*A displayed the most common alteration in clinical isolates. Transition mutation at codon 83 was a C-T that resulted in the substitution of leucine for serine in pattern A, B and D but in pattern C (EGYMU3) transition mutation at codon 83 resulted in the substitution of glycine for serine. Another transition mutation was C-A at position 87, which resulted in an Asp87Leu and Asp87 Asn. Twenty one different types of mutation in *gyr*A were found amongst the isolates analysed. They were Trp56Met, Asn57Thr,

Asn57His, Trp59Met, Asn60Thr, Asn60Asp, Lys61Gly, Lys61Ser, Ala62Thr, Ala62Pro, Tyr63Ser, Tyr63Ile, Lys64Leu, Lys65Ile, Ser83Leu, Ser83Gly, Val85Ala, Asp87Asn, Asp87Leu, Arg91Tyr and Ser111Asn.

The isolates showing the four mutation pattern of *par*C were named as EPRMU1, EPRMU2, EPRMU3, EPRMU4.Pattern EPRMU1 were found to have 10 point mutations, of which 5 insertion mutation with T. Transition mutation of A-G at base 251st and transversion mutation of G-C at base 325th were found in EPRMU2. Two transversion mutations C-A and two transition mutation C-T were observed in EPRMU3 (Figure 28a and 28b). Two transversion mutation G-T and C-A were detected in EPRMU4. Codons 74,76, 77, 80, 82, 84, 89, 91, 105, 107 showed alterations in the QRDR of the *par*C gene. The replacements were Tyr74Leu, Pro76Phe, His77Arg, Ser80Ile, Ser80Arg, Cys82Leu, Glu84Gly, Glu84Lys, Met89Leu, Gln91His, Asn105Thr and Gly107Ala. Mutations outside the QRDR were also observed during the study. A deletion of G at 390th base and a transversion of G-T at 393rd base have resulted in the substitution of Glu130Asp and Leu131Phe respectively (Figure 29a and 29b)

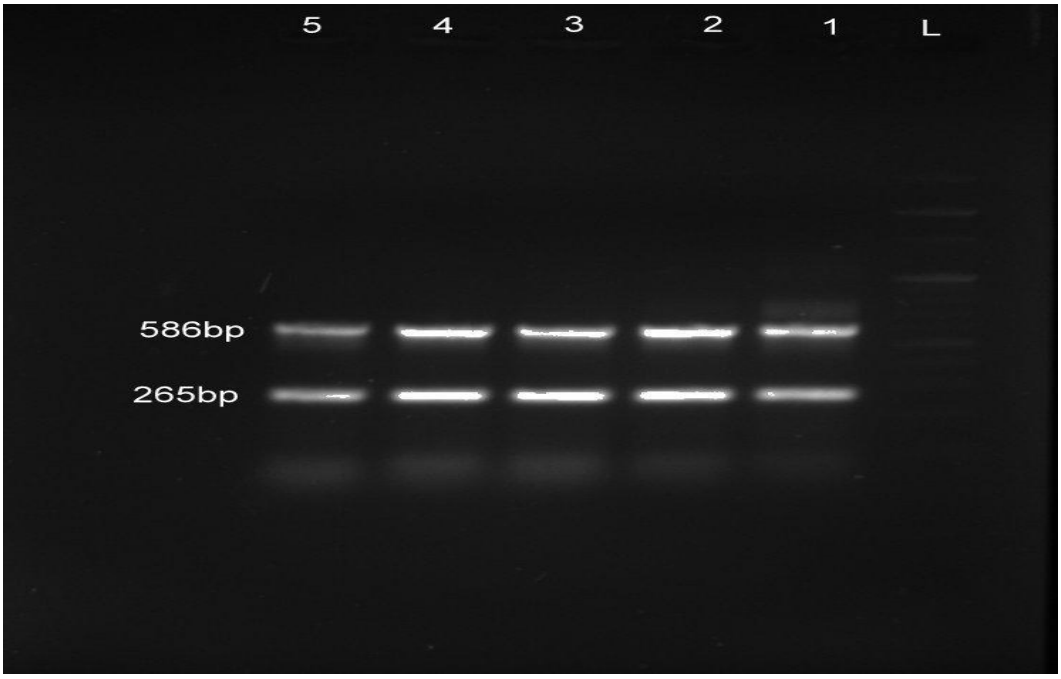
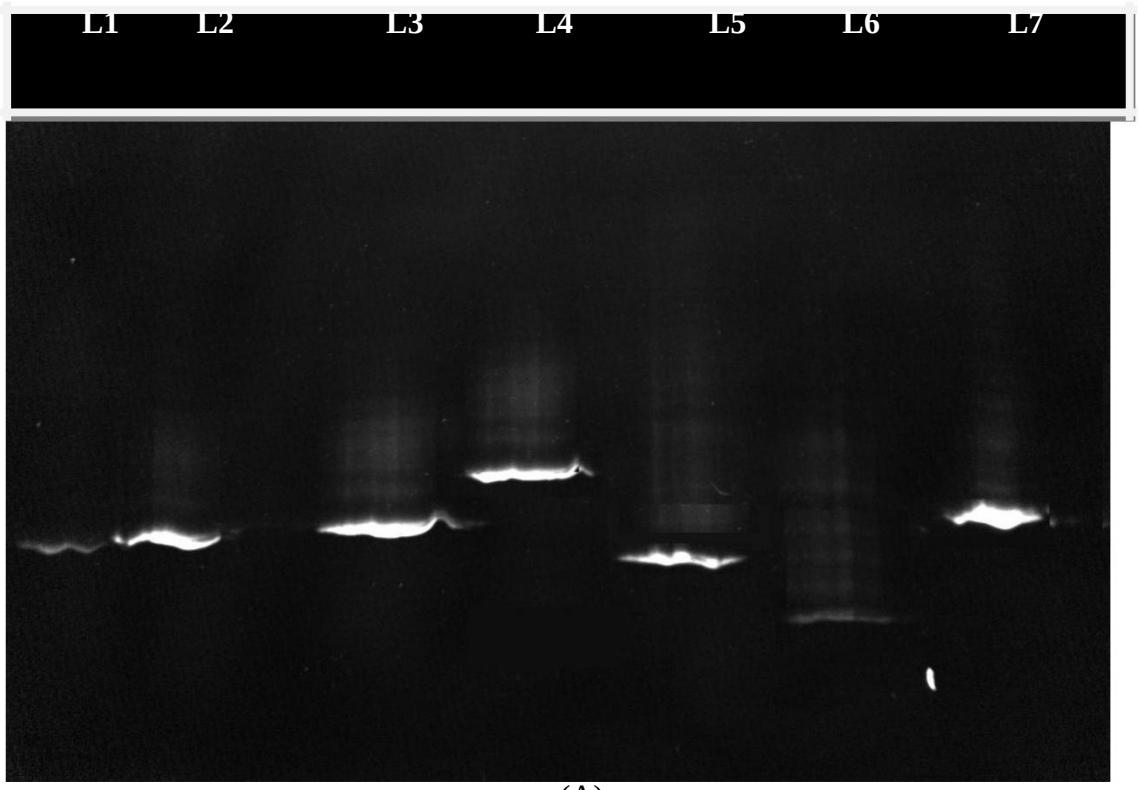
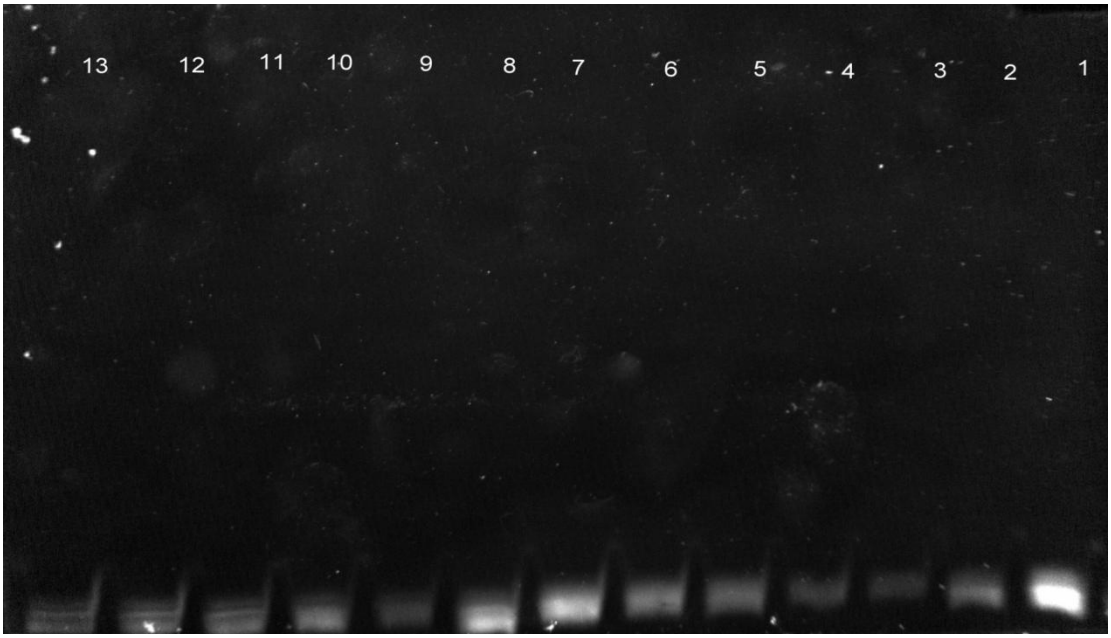


Figure 26: PCR amplification of *gyr*A (586bp) and *par*C (265bp)



(A)



(B)

Figure 27: (A) Denaturing gradient gel electrophoresis pattern of *gyr*A gene

(B) Denaturing gradient gel electrophoresis pattern of *par*Cgene

Figure 28a: Sequence alignment of the four type of mutational pattern *gyr*A sequences with the sequence of *Escherichia coli* strain 493/89(EC493/89)

```
EPRMU1181  AAAAAATCGGCCCGTACCGTCGGTGACGTACTGGGTAAATTACCATTCCGCA-GGCGATC  239
EPRMU2181  AAAAAATCGGCCCGTACCGTCGGTGACGTACTGGGTAAAT-ACCAT-CCGCACGGCGATA  238
EC493/89181AAAAAATCGGCCCGTACCGTCGGTGACGTACTGGGTAAAT-ACCAT-CCGCACGGCGATA  238
EPRMU3181  AAAAAATCGGCCCGTACCGTCGGTGACGTACTGGGTAAAT-ACCAT-CCGCACGGCGATA  238
EPRMU4181 AAAAAATCGGCCCGTACCGTCGGTGACGTACTGGGTAAAT-ACCAT-CCGCACGGCGATA 238

EPRMU1240  GCGCCTTGTTATGAAGCGATGGTCCTTGATTGGCGCAGCCGTTCTCTTACCGTTATCCGC  299
EPRMU2239  GCGCCT-GTTATGGAGCGATGGTCCT-GAT-GGCGCAGCCGTTCTCTTACCGTTATCCGC  295
EC493/89239GCGCCT-GTTATGAAGCGATGGTCCT-GAT-GGCGCAGCCGTTCTCTTACCGTTATCCGC  295
EPRMU3239  GAGCCTTGTTATAAAGCGATGGTCCT-GAT-GGCGCAGCCGTTCTCTTACCGTTATCCGC  296
EPRMU4239 GCGCCT-GTTATGAAGCGATGGTCCT-GAT-GGCGCATCCGTTCTCTTACCGTTATCCGC 295

EPRMU1300  TGGTTGATGGTCAGGGGAACTCGGGGCGCGCCGGACGATCCGAAATCGTTCGCGGCAATG  359
EPRMU2301  TGGTTGATGGTCAGGGGAACT-GGGCCGCGCCGGACGATCCGAAATCGTTCGCGGCAATG  360
EC493/89296TGGTTGATGGTCAGGGGAACT-GGGGCGCGCCGGACGATCCGAAATCGTTCGCGGCAATG  354
EPRMU3301  TGGTTGATGGTCAGGGGAACT-GGGGTGCGCCGGACGATCCGAAATCGTTCGCGGCAATG  360
EPRMU4301 TGGTTGATGGTCAGGG-AACT-GGGGCGCGCCGGACGATCCGAAATCGTTCGCGGCAATG 359

EPRMU1360  CGTTACACCGAATCCCGGTTGTCGAAATATTCCGA-CTT-CTATTGAGCGAGTTGGGGCAG  418
EPRMU2361  CGTTACACCGAATCCCGGTTGTCGAAATATTCCGAGCTG-CTATTGAGCGAGTTGGGGCAG  420
EC493/89355CGTTACACCGAATCCCGGTTGTCGAAATATTCCGAGCTG-CTATTGAGCGAGTTGGGGCAG  414
EPRMU3361  CGTTACACCGAATCCCGGTTGTCGAAATATTCCGAGTTGGCTATTGAGAGAGTTGGGGCAG  420
EPRMU4360 CGTTACACCGAATCCCGGTTGTCGAAATATTCCGAGCTG-CTATTGAGAGAGTTGGGGCAG 402
```

Figure 28b: Sequence alignment of the four type of mutational pattern *par*C sequences with the sequence of *Escherichia coli* strain 493/89(EC493/89)

Figure 29a: Protein sequence alignment of four mutational patterns of GyrA with the sequence of *Escherichia coli* strain 93/89 (EC493/89).

Figure 29b: Protein sequence alignment of four mutational patterns of ParC with the sequence of *Escherichia coli* strain 493/89(EC493/89).

4.14 Therapeutic option:

In case of antibiotics other than quinolone antibiotics for treatment options, susceptibility was high against polymixinB 83.25% (n=174) followed by imipenem 73.68% (n=154) and cefotaxime 66.99% (n=140) (Table 52; Figure30).

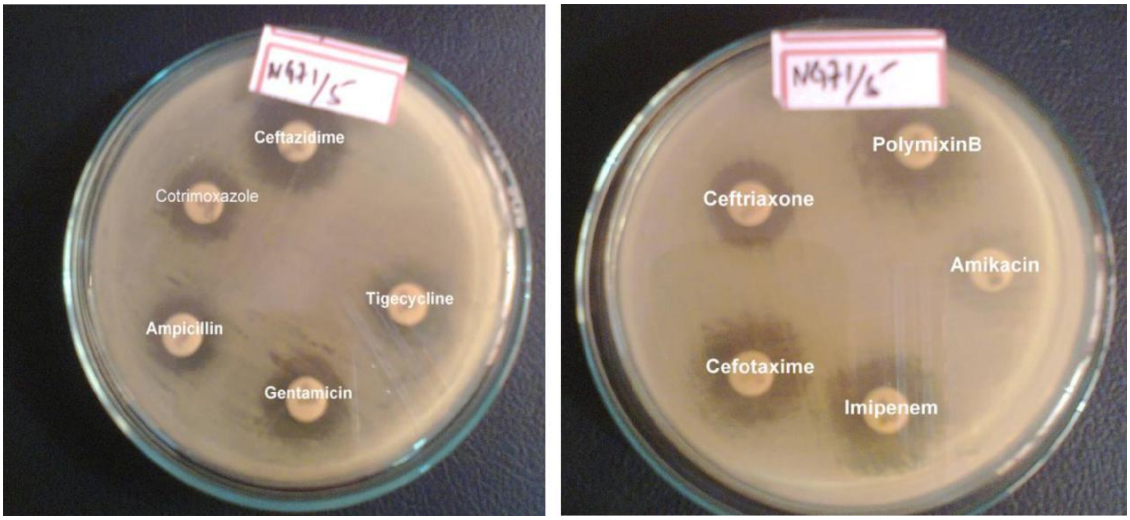


Figure 30: Antibiotic susceptibility of quinolone resistant isolates

Table 52: Antibiogram profile of quinolone resistant isolates**:**

Types of isolates	***E. coli* n=139**		***Klebsiella pneumoniae* n=53**		***Klebsiella oxytoca* n=7**		***Proteus vulgaris* n=8**		***Proteus mirabilis* n=2**	
Antibiotics	N	%	N	%	N	%	N	%	N	%
Ampicillin	42	30.22	22	33.33	_	_	1	_	_	_
Cotrimoxazole	33	23.74	21	35	_	_	3	_	1	_
Gentamicin	64	40.04	25	41.66	2	_	3	_	_	_
Amikacin	97	69.78	31	51.66	3	_	6	_	_	_
PolymixinB	111	79.86	51	85	3	_	_	_	_	_
Tigecycline	40	28.77	32	53.33	2	_	1	_	_	_
Imipenem	99	71.22	47	88.67	2	_	4	_	2	_
Cefotaxime	101	72.66	29	48.33	4	_	5	_	1	_
Ceftazidime	99	71.22	49	81.66	4	_	7	_	1	_
Ceftriaxone	97	69.78	53	88.33	5	_	5	_	2	_

n=Total no.of isolate, N=No. of sensitve isoloate,%=Percentage