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Detection of Quinolone Resistance Qnr Genes and its Association with Extended Spectrum B-Lactamase and Carbapenemase Genes in Qnr Positive Enterobacteriaceae in a Tertiary Hospital in Bangladesh

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Abstract **Original Research Article**

Background: Multidrug resistance in Enterobacteriaceae including resistance to quinolone is dramatically increasing day by day. Widespread use of quinolones against Enterobacteriaceae has contributed to the rise of drug resistance. Resistance to quinolone is generally due to chromosomal mutations in genes coding for the target molecules of these agents, namely the DNA gyrase and topoisomerase IV enzymes. Recently, a multiresistance plasmid is discovered that encodes transferable resistance to quinolones; its prevalence is increasing gradually throughout the world. Plasmids have a crucial role in the dissemination of drug resistance genes like plasmid mediated quinolone resistant (PMQR) genes, extended-spectrum β-lactamase (ESBL) genes and carbapenemase genes. ***Objective*:** To detect the prevalence of quinolone resistance Qnr genes and its association with extended-spectrum β-lactamase and carbapenemase genes in Qnr positive Enterobacteriaceae. ***Methods*:** A prospective cross sectional study was conducted from January 2015 to December 2015 at Dhaka Medical College hospital, Bangladesh. A total of 270 Enterobacteriaceae were isolated from 340 samples collecting from inpatient and outpatient departments of DMCH irrespective of age, sex and antibiotic intake. Qnr determinant screening among ciprofloxacin resistant strains were conducted using PCR amplification. Searching of Extended-spectrum β-lactamase and carbapenemase genes in Qnr positive Enterobacteriaceae were conducted also by using PCR. ***Results:*** The Qnr genes were detected in 141 (62.67%) of the 225 quinolone resistant isolates by using PCR. Highest proportion of QnrS (n =84, 59.57%) were detected followed by QnrB (n =70, 49.64%) and QnrA (n =33, 23.40%). In the present study, 48 ESBL producers were identified among 141 Qnr positive strains. Highest proportion of CTX-M-15 (n= 38, 69.09%) were detected followed by TEM (n= 20, 36.36%) and OXA-1 (n= 16, 29.09%). In the present study, 22 carbapenemase producers were identified among 141 Qnr positive strains. Highest proportion of NDM-1 (n= 15, 68.18%) were detected followed by KPC (n=5, 22.73%), VIM (n=4, 18.18%), IMP (n=3, 13.64%). CTX-M-15 was highly prevalent in QnrA gene. QnrB genes were co-existed with TEM, CTX-M15, NDM-1, VIM, IMP and KPC genes. QnrS genes were also associated with TEM, CTX-M-15, NDM-1, VIM, IMP and KPC genes. Antimicrobial-resistance rates of Enterobacteriaceae to ciprofloxacin was 83.33%. ***Conclusion:*** The Qnr genes were highly prevalent in Enterobacteriaceae. They were closely associated with Extended-spectrum β-lactamase and carbapenemase genes.

Keywords: Bangladesh, Carbapenemase genes, ESBL genes, Qnr genes.

INTRODUCTION

Quinolones are synthetic antimicrobial agents that are extensively used in medical applications throughout the world [1]. The most commonly used fluoroquinolone named ciprofloxacin, which was introduced in 1987 [2]. Ciprofloxacin is mainly used against gram-negative bacteria such as Enterobacteriaceae, *Pseudomonas aeruginosa*, *Haemophilus influenzae* and *Moraxella catarrhalis*; however, it displays less activity against gram-positive

infections [3]. However, owing to the widespread use of fluoroquinolones in human, the resistance rate of these antimicrobial agents has risen in all bacterial species. In the case of UTI patients, the rate of fluoroquinolone resistance was reported in the range of 6.3% to 62% in gram-negative strains [4]. Resistance to quinolone is generally due to chromosomal mutation [5]. Recently, a multiresistance plasmid is discovered that encodes transferable resistance to quinolones; its prevalence has been increasingly described among Enterobacteriaceae in recent decades [6]. Plasmids have a crucial role in the dissemination of drug resistance genes like plasmid mediated quinolone resistance (PMQR) genes, extended spectrum β-lactamase (ESBL) genes and carbapenemase genes [7]. Plasmid-mediated quinolone resistance (PMQR) determinant, Qnr genes have been mostly identified in clinical isolates of Enterobacteriaceae and have been shown to play not only an important role in quinolone resistance but also this drug resistance gene can disseminate from one bacterium to another. So presence of PMQR genes among Enterobacteriaceae provides a wider reservoir for the spread of these organisms [8]. All types of Qnr determinants have been reported in various Enterobacteriaceae, and were frequently found in *E. coli*, followed by *Klebsiella spp*., *Enterobacter spp*. and *Salmonella spp.* from clinical isolates around the world [9]. In addition, they have been detected less frequently in non-fermenters such as *P. aeruginosa*, *Stenotrophomonas maltophilia* and *Acinetobacter baumannii* [10]. Simultaneous presence of Qnr and other drug resistance genes such as extended-spectrum β-lactamase and carbapenemase on the same plasmid causing multidrug failure [7]. QnrB has been co-existed with IMP and KPC genes [11]. QnrS has been coexisted with TEM gene and is associated with IMP gene [12, 13]. Both QnrA and QnrB are associated with CTX-M gene [14]. Few studies have been carried out in detecting Qnr genes among *Shigella flexneri* from clinical samples and *Esch. coli* from water samples in Bangladesh [15, 16]. Therefore, this study was designed to explore quinolone resistant Enterobacteriaceae from different specimens followed by searching the prevalence of three groups of quinolone resistance genes (QnrA, QnrB & QnrS) by PCR. Then genes for extended spectrum β-lactamase and carbapenemase were deteted in Qnr positive strains to observe the association of these genes with Qnr genes in developing multiple drug resistance.

MATERIALS AND METHODS

Bacterial Strains

This study was approved by the Ethical Review Committee of Dhaka Medical College and informed written consent was taken from each participant. Sampling locations included urine (N=96), wound swab (N=62), sputum (N=58) and blood (N=54). All the wound swab, sputum and urine samples were inoculated in blood agar and MacConkey agar media and incubated at 37ºC aerobically for 24 hours. Incubated plates were then examined for the presence of colonies of bacteria. Primary blood culture was done in Trypticase soya broth then subculture on blood agar and MacConkey agar media. Smear was prepared from sample and culture plate and stained by Gram's stain as per standard procedure and were examined under microscope for the presence of gram positive or gram negative organisms. All the isolated organisms were identified by their colonial morphology, Gram stain characteristic and relevant biochemical tests [17]. *Escherichia coli* grew as smooth, glossy, pink colonies on MacConkey agar media, gram negative rod, motile and characteristic sugar fermentation tests, indole positive, citrate non-utilizer. *Klebsiella* spp. were lactose fermenting mucoid pink colony in MacConkey agar media, acidic (yellow) slant and butt with gas production but no H_2S in TSI agar, urease positive, non-motile. *Citrobacter* spp. are late lactose fermenter, motile with citrate positive. *Proteus* spp. were pale colony on MacConkey agar media, characteristic swarming growth on blood agar media and 'fishy' odour, rapid urea hydrolysis in MIU media, motile, slant (red) butt (yellow) with H_2S production. *Enterobacter* spp. were pink colony in MacConkey agar media, motile, oxidase negative, citrate utilizer, indole negative, urease negative, gas producing, no hydrogen sulphide production in TSI agar media.

Antimicrobial susceptibility test

Susceptibility to antimicrobial agents of all isolates was done by Kirby Bauer modified disk diffusion technique using Muller Hinton agar (MHA) plates and zones of inhibition were interpreted according to CLSI guidelines (CLSI, 2014) [18]. Antibiotic disks such as ciprofloxacin (5 µg), cefepime (30 µg), ceftazidime (30 µg), cefotaxime (30 µg), ceftriaxone (30 µg), amoxiclav (amoxicillin 20 µg & clavulanic acid 10 µg), amikacin (30 µg), piperacillin-tazobactam (100/10 µg), imipenem (10 µg), cefoxitin (30 µg) and cotrimoxazoe (1.25/23.75 µg). Screening of quinolone resistant Enterobacteriaceae was done during disk-diffusion method using ciprofloxacin disk. The minimum inhibitory concentration (MIC) of ciprofloxacin was determined by agar dilution method. *Esch.coli* ATCC 25922 was used as control strain to assess the performance of the method. Drug resistant organisms were classified as multidrug- resistant, extensively drug-resistant and pandrug-resistant [19].

Screening for ESBL producers among Qnr positive Enterobacteriaceae by double disk synergy (DDS) test

For detection of ESBL producers, screening of ESBL producers was done during disk-diffusion method using ceftazidime, cefotaxime and ceftriaxone disks [20]. If the isolates were resistant to any of these drugs, these were considered as suspected ESBL producer (CDC, 2011). Using sterile cotton swab, test inoculums (compared with McFarland standard) were inoculated in Muller-Hinton agar plate. Third generation

cephalosporins (ceftriaxone, ceftazidime and cefotaxime) were placed 20 mm apart from center of the amoxiclav disk. Inoculated plate was incubated at 37°C for 24 hours. A clear extension of the edge of the inhibition zone of cephalosporin disk towards amoxiclav disk was interpreted as positive for ESBL production.

Phenotypic detection of carbapenemase producers

All the isolates showing reduced susceptibility to imipenem (zone diameter < 19mm) were tested for carbapenemase production using the Modified Hodge Test. Briefly, a lawn culture of 1:10 dilution of 0.5 McFarland's standard *Esch. coli* ATCC 25922 broth was done on a Mueller-Hinton agar plate. A 10 µg imipenem disk was placed in the centre of the plate. Then, imipenem resistant test strain were streaked from the edge of the disk to the periphery of the plate in three different directions. After overnight incubation, the plates were observed for the presence of a clover leaf shaped zone of inhibition and the plates with such zones were interpreted as Modified Hodge test positive [21]. Double –disk synergy test (DDS) test and combined disk (CD) assays were also performed to screen MBLs producers [22, 23].

PCR for detection of Qnr and other genes in Qnr positive quinolone resistant strains

Genomic DNA of the ciprofloxacin resistant bacterial strains was amplified by PCR for the detection of three groups of Qnr determinants (QnrA, QnrB and QnrS). Briefly, colonies were suspended in 50 µL of sterile distilled water in a microcentrifuge tube and boiled to prepare DNA templates. PCR reaction consisted of preheat at 94^0C for 10 minutes followed by 36 cycles of denaturation at 94^0C for 45 seconds, annealing at 53^0C for 45 seconds, extension at 72^0C for one minute with a final extension at 72^0C for 10 minutes. Reaction mixtures without a DNA template served as negative controls. PCR was used for identification of ESBLs encoding genes (CTX-M-15, TEM and OXA-1), carbapenemase encoding genes (NDM-1, KPC, IMP, VIM) among Qnr positive strains (Table-1).

Table I: Primers for genes of Qnr, ESBL and carbapenemase used in this study

Genes	Sequence (5′-3′)	Bp
QnrA-F QnrA-R	ATTTCTCACGCCAGGATTTG GATCGGCAAAGGTTAGGTCA	516
QnrB-F QnrB-R	GATCGTGAAAGCCAGAAAGG ACGATGCCTGGTAGTTGTCC	469
QnrS-F QnrS-R	ACGACATTCGTCAACTGCAA TAAATTGGCACCCTGTAGGC	417
TEM-F TEM-R	GTCGCCCTTATTCCCTTTTTTG TAGTGTATGCGGCGACCGAG	231
CTX-M-15-F CTX-M-15-R	CACACGTGGAATTTAGGGACT GCCGTCTAAGGCGATAAACA	996
OXA-F OXA-R	ACCAGATTCCAACTTTCAA TCTTGGCTTTTATGCTTG	598
NDM_1-F NDM_1-R	ACCGCCTGGACCGATGACCA GCCAAAGTTGGGCGCGGTTG	264
IMP-F IMP-R	GGAATA GAGTGGCTTAAYTCTC CCAAACYACTASGTTATCT	188
VIM-F VIM-R	GATGGTGTTTGGTCGCATA CGAATGCGCAGCACCAG	390
KPC-F KPC-R	CGTCTAGTTCTGCTGTCTTG CTTGTCATCCTTGTTAGGCG	498

RESULTS

Of 270 (71.05%) Enterobacteriaceae, *Esch. coli* was the predominant strains (n =127, 47.04%) (Table II). Total 225 (83.33%) Enterobacteriaceae showed ciprofloxacin resistance. The Qnr gene was detected in 141 (62.67%) of the 225 quinolone resistant isolates by using PCR. Total 187 Qnr genes were isolated. Highest proportion of QnrS (n =84, 59.57%) were detected followed by QnrB (n =70, 49.64%) and QnrA (n =33, 23.40%) from quinolone resistant strains. QnrA and QnrS were highly prevalent in *Esch. coli*. On the other hand, QnrB was highly prevalent in *Klebsiella* spp. (Table III). More than one type of Qnr genes were present in many organisms (Figure I).

In the present study, 48 ESBL producers were identified among 141 Qnr positive strains. 47 ESBL producers were positive for ESBL encoding genes detected by PCR. Total 74 ESBL encoding genes were isolated. Highest proportion of CTX-M-15 (n= 38, 69.09%) were detected followed by TEM (n= 20, 36.36%) and OXA-1 (n= 16, 29.09%). All the genes were highly prevalent in *Esch. coli.* CTX-M-15 were highly prevalent among QnrA and QnrS genes, TEM were commonly identified among QnrB gene of ESBL producers (Table IV).

In the present study, 22 carbapenemase producers were identified among 141 Qnr positive strains. 18 carbapenemase producers were positive for carbapenemase encoding genes detected by PCR. Total 27 carbapenemase encoding genes were isolated. Highest proportion of NDM-1 (n= 15, 68.18%) were detected followed by KPC (n=5, 22.73%), VIM (n=4, 18.18%), IMP (n=3, 13.64%). All the genes were highly prevalent in *Esch.coli*.

QnrB and QnrS had been coexisted with genes for carbapenemase such as NDM-1, KPC, IMP and VIM genes. No carbapenemase encoding genes were found with QnrA. In the present study, 22 carbapenemase producers were identified among 141 Qnr positive strains. Eighteen carbapenemase producers were positive for carbapenemase encoding genes detected by PCR. Total 27 carbapenemase encoding genes were isolated. Highest proportion of NDM-1 (n= 15, 68.18%) were detected followed by KPC (n=5, 22.73%), VIM (n=4, 18.18%), IMP (n=3, 13.64%). All the genes were highly prevalent in *Esch.coli*.

QnrB and QnrS had been coexisted with genes for carbapenemase such as NDM-1, IMP and VIM genes. No carbapenemase encoding genes were found with QnrA (Table V). The DDS test, CD assay and MHT detected 13(59.09%), 15(68.18%) and 7 (31.82%) carbapenemase producers respectively, among the 22 imipenem- resistant isolates. Out of the four negative amplified PCR products, two were positive by the DDS test and all were positive by CD assay and MHT. Considering the PCR as the gold standard, the sensitivity of the DDS test, CD assay and MHT were 61.11%, 61.11% and 16.67% respectively.

Table II: Distribution of Enterobacteriaceae isolated from different samples (N=270)

Enterobacteriaceae	Urine	Blood	Sputum	Wound	Total
swab					
n (%)		n (%)	n (%)	n (%)	n (%)
Esch. Coli	62 (64.58)	25 (46.30)	21 (36.21)	19 (30.64)	127 (47.04)
Klebsiella spp.	12 (12.50)	24 (44.44)	31 (53.45)	20 (32.26)	87 (32.22)
Citrobacter spp.	8 (8.34)	3 (5.56)	3 (5.17)	4 (6.45)	18 (6.67)
Proteus spp.	6 (6.25)	1 (1.85)	1 (1.72)	9 (14.52)	17 (6.29)
Enterobacter spp.	8 (8.33)	1 (1.85)	2 (3.45)	10 (16.13)	21 (7.78)
Total	96 (100)	54 (100)	58 (100)	62 (100)	270 (100)

Table III: Prevalence of different Qnr genes among Qnr positive Enterobacteriaceae (N=141)

Enterobacteriaceae	QnrA n (%)	QnrB n (%)	QnrS n (%)	Percentage of Qnr positive strains
***Esch.coli* (N=71)**	17+2* (26.76)	20+2* (30.98)	44+2* (64.79)	50.36
***Klebsiella* spp. (N=50)**	6+4* (20.00)	38+4* (84.00)	24+4* (56.00)	35.46
***Citrobacter* spp. (N=10)**	1 (10.00)	2 (20.00)	7 (70.00)	7.09
***Proteus* spp. (N=2)**	0 (00.00)	1 (50.00)	1 (50.00)	1.42
***Enterobacter* spp. (N=8)**	3 (37.50)	3 (37.50)	2 (25.00)	5.67
Total	33 (23.40)	70 (49.64)	84 (59.57)	100.00

Note: N= Total number of Qnr positive Enterobacteriaceae, n = Number of Qnr genes.
'*' denotes positive for all varieties of Qnr genes.

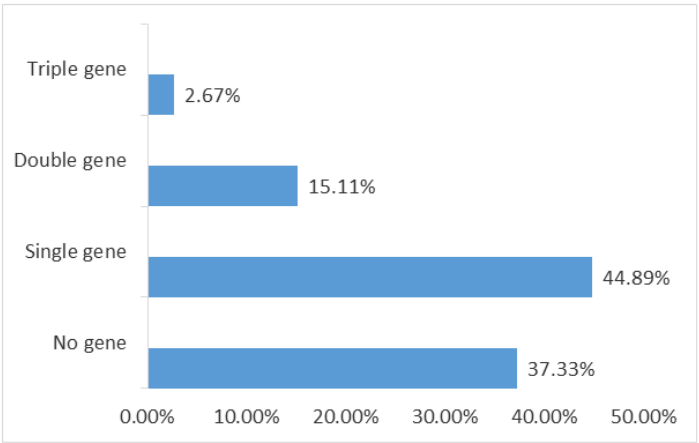

Figure I: Distribution of Qnr encoding genes in quinolone resistant Enterobacteriaceae (N=225)

Table IV: Distribution of plasmid mediated ESBLs encoding genes among different Qnr genes of ESBL producing Enterobacteriaceae (N=55)

Qnr genes	TEM n (%)	CTX-M-15 n (%)	OXA-1 n (%)
***QnrA* (N=10)**	0 (00.00)	7 (70.00)	0 (00.00)
***QnrB* (N=15)**	11 (73.33)	9 (60.00)	5 (33.33)
***QnrS* (N=30)**	9 (30.00)	22 (73.33)	11 (36.67)
Total	20 (36.36)	38 (69.09)	16 (29.09)

Note: N= Total number of Qnr genes in ESBL producing Enterobacteriaceae, n = Number of ESBLs encoding genes among Qnr genes.

Table V: Prevalence of carbapenemase encoding genes among different species of imipenem resistant Qnr positive Enterobacteriaceae (N=22)

Imipenem resistant Enterobacteriaceae	Carbapenemase encoding genes			
	NDM-1 n (%)	VIM n (%)	IMP n (%)	KPC n (%)
***Esch. coli* (N=13)**	9 (69.23)	2 (15.38)	3 (23.08)	3 (23.08)
***Klebsiella* spp. (N=7)**	6 (85.71)	2 (28.57)	0 (0.00)	2 (28.57)
***Citrobacter* spp. (N=1)**	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
***Enterobacter* spp.(N=1)**	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Total	**15 (68.18)**	**4 (18.18)**	**3 (13.64)**	**5 (22.73)**

Note: N= Total number of imipenem resistant Qnr positive Enterobacteriaceae, n = Number of carbapenemase encoding genes

Table VI: Distribution of carbapenemase encoding genes among different Qnr genes of carbapenemase producers (N=20)

Qnr Genes	NDM-1 n (%)	VIM n (%)	IMP n (%)	KPC n (%)
***Qnr A* (N=3)**	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
***QnrB* (N=5)**	4 (80.00)	2 (40.00)	2 (40.00)	3 (60.00)
***QnrS* (N=12)**	11 (91.67)	2 (16.67)	1 (8.33)	2 (16.67)
Total	15 (75.00)	4 (20.00)	3 (15.00)	5 (25.00)

Note: N = Total number of Qnr genes in carbapenemase producers, n = Number of carbapenemase encoding genes among Qnr genes

DISCUSSION

There is an increasing trend of quinolone resistant organisms all over the word. In consistent with other report of DMCH [24] quinolone resistant Enterobacteriaceae were 83.33% in this study. In contrast to the present findings, 27.66% quinolone resistant organisms were reported from Korea [11]. Factors such as widespread indiscriminate use of drugs, truncated antimicrobial therapy, inadequate access to effective drugs and sometimes drugs of questionable quality are likely to be contributing factors for high resistance rate to ciprofloxacin in the present study [25, 26]. For this reason, high proportion of (62.67%) Qnr positive Enterobacteriaceae were observed in this study. High MIC values (8 to ≥128 µg/ml) were also found for ciprofloxacin in this study which might be due to the same reason [27].

Qnr gene was absent in 84 (37.33%) of the quinolone resistant isolates. This might be due to possibility of presence of other variety of Qnr genes such as, QnrC and QnrD genes or presence of another genes such as AAC (6′)-Ib gene that causing drug modification [28] or genes for quinolone efflux pumps (QepA and OqxAB) that enhanced efflux of drugs [29]. QnrA and QnrS were highly prevalent in *Esch. coli*. and QnrB was highly prevalent in *Klebsiella* spp. in the present study and these results were consistent with other report [11].

Total 48 ESBL producers were identified among 141 Qnr positive strains. In 2008, ESBL producing organisms isolated from gram negative bacteria were 30.98% in 2013 [30] followed by 32.61% in 2015 [24] and 34.04% in the present study. The gradual increase of ESBL producing strains in Bangladesh might be due to widespread use of drugs which leads to plasmid mediated ESBL genes transmission and this mobile elements accelerates the transfer of various antibiotic resistance genes between organisms [31]. The high prevalence of CTX-M-15 type ESBL (93.54%) among *Esch. coli* identified in the present study. In a study in DMCH; it was reported that 91.18% *Esch. coli* were positive for CTX-M-15 [24]. This suggests the high prevalence of CTX-M gene among Enterobacteriaceae of DMCH. Several studies have reported that dissemination of this gene is associated with highly efficient mobile genetic

elements, including the ISEcp1, ISCR1 or phage-related sequences [32]. Using specific primers for CTX-M-15, TEM and OXA-1, present study could not detect any ESBL genes in one (2.09%) of the phenotypically positive ESBL producers. The reason might be due to the presence of other variants of ESBL genes. CTX-M-15 was highly prevalent among QnrA and QnrS genes in the present study. TEM was highly prevalent among QnrB gene. In a study in Hong Kong it was reported that QnrA and QnrB had been identified with CTX-M-15 [33]. In the case of QnrS, the gene had been co-existed with TEM [12].

Total 22 carbapenemase producers were identified among 141 Qnr positive strains. Carbapenemase producing organisms isolated from imipenem resistant Qnr positive Enterobacteriaceae were 84.21% in 2015 [24] followed by 81.82 % in the present study. High prevalence of carbapenemase producing strains are found among Enterobacteriaceae because of the presence of carbapenemase- encoding genes on plasmid along with other resistance genes such as Qnr, resulting in multidrug-resistant ,extremely drug-resistant and pandrug-resistant bacteria [34].

In the present study, highest 68.18% isolates were positive for NDM-1 among 22 imipenem resistant strains. The prevalence of NDM-1 producers is increasing in Bangladesh which is reflected by another study conducted in DMCH which was 55% [24]. NDM-1 producing organisms are now alarmingly rising worldwide and pose therapeutic failure [35]. The high prevalence of NDM-1 type carbapenemase (85.78%) among Klebsiella was identified in the present study. In a study in DMCH, it was reported that 80% Klebsiella were positive for NDM-1 [24]. Another study in India it was reported that *K. pneumoniae* and *Esch. coli* were the most frequent bacteria for NDM-1 production [36]. Rapid dissemination of NDM-1 producing organisms might be facilitated by the conditions like overcrowding, over-the-counter availability of antibiotics, low level of hygiene, and weak hospital antibiotic policies [37].

In this study, the high prevalence of KPC type carbapenemase (22.73%) were identified among 22 imipenem resistant strains. In a study in DMCH, it was reported that 20% *Klebsiella spp.* were positive for KPC [38]. This suggests the high prevalence of KPC genes among Enterobacteriaceae in Bangladesh. The presence of this gene suggests the possibility of horizontal transmission, as this carbapenemase has been associated with mobile genetic elements (transposons) which can be transferred from one bacterium to another [39].

In consistent with other report from DMCH [24], 4 (18.18%) isolates were positive for VIM and 3 (13.64%) isolates were positive for IMP among 22 imipenem resistant strains in this study. In contrast to the present findings, 100% VIM-1 producers were identified from Greece [40]. The proportion of MBL producers from different studies including the present one suggests that the prevalence of MBL producers varies with geographical areas and time.

In this study, 4 (18.19%) carbapenemase reistant isolates had no carbapenemase encoding genes .This may be due to presence of other carbapenemase encoding genes such as OXA-23, OXA-48, OXA-181 types that was not included in the current study [41, 42]. Enterobacteriaceae have the capacity to elude the action of carbapenems through modification of outer membrane permeability such as porin loss, up-regulation of efflux systems, production of carbapenem hydrolyzing β-lactamases (i.e. hyperproduction of AmpC β-lactamases, certain ESBLs with increased capacity to hydrolyze carbapenems) and production of carbapenemases (either serine based carbapenemases or MBLs) [43].

In this study, QnrB and QnrS had been coexisted with genes for carbapenemase such as NDM-1, VIM, IMP & KPC. NDM-1 were highly prevalent among QnrS and QnrB genes, No carbapenemase encoding genes were found with QnrA. In a study in Hong Kong it was found that QnrB had been also co-existed with KPC and IMP [44].

In this study, MIC of imipenem among imipenem resistant Qnr positive Enterobacteriaceae ranged from 4 to ≥128 μg/ml. The variation in the MIC values for carbapenems may be influenced by type and expression of carbapenemase enzymes, the bacterial species, the presence of other resistance mechanism (such as, Extended spectrum and AmpC β-lactamase), reduced permeability and or efflux pump which is similar to the present study [45].

CONCLUSION

The presence of significant proportion of ESBL and carbapenemase producing Enterobacteriaceae among Qnr positive strains highlights the emerging therapeutic challenge in Bangladesh. So detection of multiple genes in an organism will be helpful in selection of appropriate antimicrobial strategics which will reduce the emergence and spread of MDR Enterobacteriaceae.

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