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Molecular Detection and Association of *QnrA*, *QnrB*, *QnrS* and *BlaCMY* Resistance Genes among Clinical Isolates of *Salmonella* spp. in Iran

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ABSTRACT

Prevalence of three plasmid-mediated quinolone resistance determinant *qnrA, qnrB, qnrS* and extended spectrum Cephalosporins determinant *blaCMY*, among eighty-five isolates of *Salmonella* spp. collected in the community between 2008 and 2010 was determined by PCR. Not only *qnr* genes but also *bla* genes were positive in twenty-four different isolates. PCR assay detected that 22 of 85 (25.8%) *Salmonella* spp. carried the *qnrA*, 1 (1.17%) of 85 isolates harbored the *qnrB*, 1 (1.17%) of them contained the *qnrS*, 1 (1.17%) isolate carried all the three *qnrA*, *qnrB*, *qnrS* genes, 24 of 85 (28.2%) *Salmonella* carried *blaCMY* and 5 (5.88%) isolates carried *qnrA* and *blaCMY*. Antimicrobial susceptibility patterns of isolates were as follows: 49 (57.6%) exhibited resistance to Nalidixic acid and none of them to Ciprofloxacin. 33 (38.82%) isolates exhibited resistance to Cephalosporins and 2 (2.35%) of them exhibited ESBL phenotype and 12 (14.1%) isolates resistance to Ampicilin. These results were confirmed by MIC determination test as well. Having detected *qnr* and *bla* genes suggested that these genes spread antibiotic resistance among pathogenic bacteria.

KEYWORDS

QnrA*; *QnrB*; *QnrS*; *ESBL* (*Extended-Spectrum Beta-Lactamase*); *BlaCMY

1. Introduction

Salmonella spp. is an important cause of human infection worldwide. Resistance to flouroquinolone, quinolone and extended spectrum Cephalosporins (ESR) that are potentially lifesaving treatment is of great concern [1-3]. Resistance to extended-spectrum Cephalosporins was the result of *blaCMY*, a classC plasmid-encoded *ampC* gene [4]. Plasmid-mediated *β*-lactamases were classified into six genetic clusters and *blaCMY* was the most prevalent one. The *blaCMY* has been found on plasmid of sizes

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variable for 7 to 180 kb [5,6].

Plasmid-mediated quinolone resistance (PMQR) was first discovered in a clinical isolate of *Kelebsiella penemoniae* from Birmingham, Albama collected in 1998 by Martinez-Martinez. Since then, three major groups of *qnr* determinant were introduced. *QnrA* with 6 variants, *QnrB* with 19 variants and *QnrS* with 3 variants, differ from each other by 40% or more in nucleotide sequences [7]. Resistance to quinolone and flouroquinolone arises with mutation within the DNA gyrase (topoisomerase II) and topoisomerase IV gene, especially DNA gyrase and often with decreased expression of outer membrane proteins and overexpression of efflux pump. The *qnr* gene encodes a pentapeptide repeat protein that protects DNA gyrase against inhibition by quinolone and flouroquinolone [8-13].

Resistance to quinolone and flouroquinolone, is often associated with *ESBL*-producing organisms as well as association with integron carrying *qnr*. Sequencing is a good way to identify *qnr* and *ESBL* producing genes [8,14].

ESBL was first explained in 1983. *β*-lactamases are bacterial enzymes that are inactive *β*-lactame antibiotic. These enzymes have the ability to hydrolyse oxyimino Cephalosporin but not Cephamycines or Carbapenems. From different parts of the world, ESBL has been introduced in Enterobacteriaceae and Pesedomonadaceaesis. These enzymes belong to the Ambler class A and D, *β*-lactamases. The activity of class A of *β*-lactamases is inhibited by *β*-lactamases inhibitor such as Clavulonic Acid, Sulbactam and tazobactam [15].

2. Material and Methods

2.1. Bacterial Isolates

Eighty-five isolates of *Salmonella* spp. were obtained from blood, stool, synovial fluid, abscess, urine and bone marrow during the years 2008 to 2010 in different provinces of Iran. Each strain obtained from a unique patient in different hospital. Stool isolates were the highest one of all the others. Biochemical and serological methods were used to identify each isolates [16].

2.2. Antimicrobial Susceptibility Test

Disk diffusion method was used to select resistant bacteria. All isolates tested and inoculated with nalidixic acid 30 µgml, Ciprofloxacin 30 µgml, Ceftazidim 30 µgml, Ceftriaxon 30 µgml, Cefepime 30 µgml, Cefpodoxime 30 µgml, Ampicilin 30 µgml by clinical and laboratory standard institute (CLSI). Disk prepared by Mast company [14,17]. Broth microdilution method [Sensitite, West Lake, OH, USA] was used to determine the minimum inhibitory concentration (MIC), confirmed by E test [AB Biodisk, Piscataway NJ, USA]. *Kelebsiella pneumonia* ATCC 700603 was used as quality control strain.

2.3. PCR Detection

The *qnrA*, *qnrB*, *qnrS* and *blaCMY* were detected by PCR in clinical isolates using the following primers. For *qnrA*_ up (GATAAAGTTTTTCAGCAAGAGG) and *qnrA*_ dw (ATCCAGATCCGCAAAGGTTA) to give a 700 bp product (**Figure 1**). Primer for *qnrB*_ up (ATGACGCCATTACTGTATAA) were *qnrB*_ dw (GATCGCAATGTGTGAAGTTT) generating a 120 bp fragment (**Figure 2**). Primer for *qnrS*_ up (ATGGAAACCTACAATCATAC) and *qnrS*_ dw (AAAAACACCTCGACTTAAGT) and amplicon size of *qnrS* was 280 bp and primers (**Figure 3**) for *blaCMY*_ up (ATGATGAAAAAATCGTTATGC) and *blaCMY*_ dw (TTGCAGCTTTTCAAGAATTCGC) with 800bp amplicon size (**Figure 4**) [4].

2.4. DNA Sequencing

The *qnrA*, *qnrB*, *qnrS* and *blaCMY* were sequenced by specific primers according to previous study. Analysis was performed using Bio Edit (www.Mbio.ncsu.edu/Bioedit/bioedit.html). The BLAST

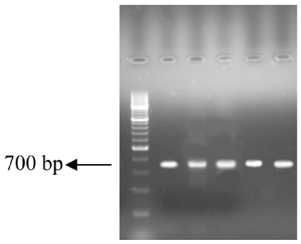

Figure 1. PCR products of *qnrA* and 50 bp DNA ladder.

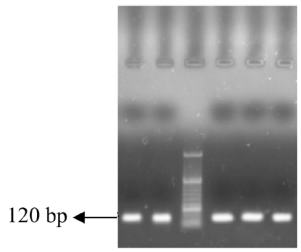

Figure 2. PCR products of *qnrB* and 1 kb DNA ladder.

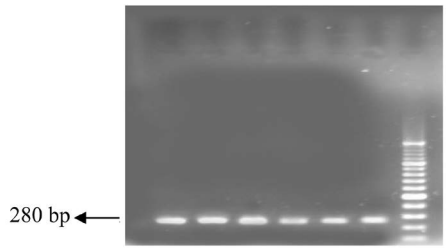

Figure 3. PCR products of *qnrS* and 100 bp DNA ladder.

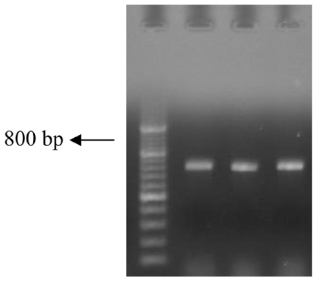

Figure 4. **PCR products of *blaCMY*-2 100 bp DNA ladder.**

algorithm used to screen the gene bank database at the National Center of Biotechnology information website (http://www.ncbi.nlm. nih.gov/BLAST).

3. Results

3.1. Prevalence of Quinolone and Flouroquinolone and Ampicilin, Cephalosporins Resistance

Antimicrobial susceptibility pattern of 85 isolates of *Salmonella* were as a fallow: 9 (10.5%) isolates exhibited resistance to Ceftazidim. MIC range of these antibiotics were 0.25 - 128 µgml, 6 (7.05%) isolates to Ceftriaxone, 9 (10.5%) isolates to Cefotaxime, 6 (7.05%) isolates to Cefexime, 5 (5.88%) isolates to Cefepime, 4 (4.7%) isolates to Cefpodoxime, 12 (14.1%) isolates to ampicilin with MIC range of <4 - >2048 µgml. 49 (57.6%) isolates were resistance to Nalidixic acid and none of them were resistant to Ciprofloxacin with the MIC range of 0.64 - 0.125 µgml (**Table 1**). Among all the isolates only 2 isolates were resistant to all Cephalosporins that we tested. We showed that these two isolates were exhibited *ESBL* phenotype, but only one isolate had a *blaCMY* gene indicating that it may have the other *β*-lactamase gene.

Table 1. **Information about antimicrobial agents, MIC range and antimicrobial resistance percentage for 85 samples of *Salmonella* isolates obtained from stool, blood, bone marrow, synovial fluid, abscess, urine.**

Antimicrobial agent	MIC range in isolates (µg/ml)	No. of isolates resistant to antimicrobial agents (%)
Ceftazidim	0.25 - 128 µgml	9 (10.5%)
Ceftriaxone	ND*	6 (7.05%)
Cefexime	ND	6 (7.05%)
Cefotaxime	ND	9 (10.5%)
Cefepime	ND	5 (5.88%)
Cefpodoxime	ND	4 (4.7%)
ampicilin	<4 - >2048 µgml	12 (14.1%)
Nalidixic	8 - >1024 µgml	49 (57.6%)
Ciprofloxacin	0.64 - 0.125 µgml	0

*Not determined.

3.2. Screening of *Qnr* and *Bla* Genes by PCR

Twenty two (25.8%) isolates were found to be *qnrA*-positive among all of the quinolone resistance isolates, *qnrS* and *qnrB* genes were detected in one (1.17%) of them. *blaCMY* gene were identified in twenty four (28.23%) among all of the Cephalosporins resistance isolates. Moreover, 5 isolates were found to have *blaCMY* and *qnrA* genes (**Table 2**). Some isolates carrying *qnrA*, *qnrB*, *qnrS* and *blaCMY* genes were selected for sequencing. The comparison between sequence results and Gene bank databases revealed identity to sequence of the *qnrA*, *qnrB* and *qnrS* with the accession numbers No. AY906856.1, DQ303919.1, FJ4181530.1 respectively and sequence of *blaCMY*-2 with the accession number Nos. EU1132222.1, EU1132220.1, AB3658670.1.

4. Discussion

Three major plasmid-mediated quinolone resistance and expended spectrum Cephalosporins were studied. Although resistance to quinolone and flouroquinolone with *qnr* gene is rare, most of the *qnrA* positive clinical isolates were found to have high level quinolone resistance in the present study which was usually detected by resistance to Nalidixic acid. The ability of these genes to supplement resistance is due to mutation in DNA gyrase and topoisomerase IV, porin or efflux mutations and *qnrB* seems to be even more potent than *QnrA* in blocking the action of Ciprofloxacin. These data show that *qnrA* presents a potential problem for the spread of quinolone resistance. Spread of these gene and *bla* gene is depending on the geographical region. The high prevalence of *qnr* among *Salmonella* spp. isolates and extended spectrum Cephalosporin-resistant or *ESBL* producing isolates has also been described in several countries. For example, the *qnrB* gene has been found in Senegal, USA and Korea, while the *qnrS* gene has been found in Enterobacteriacea in Germany, USA, Taiwan, Vietnam, France, Sothern and eastern Asia, North and south American, Europe and *blaCMY* has been reported in France, Turkey, Greek, USA, Mexico and United Kingdom [8,12,18,19]. Cephalosporins resistance is quite important due to its rapid spread not only among clinical isolates but also among Community in other countries [20]. The frequent association of *qnr* resistance gene with *bla* has been noted in several studies. It is Noteworthy that perception of *qnrA*, *qnrB*, *qnrS* in the overall quinolone and flouroquinolone was noticeable. Nonetheless, association between *qnr* and *blaCMY* was more important [10]. In both India and United States *qnrB* has been found on plasmid that encoding *blaESBL*, *CTX-M*-15 in India and *SHV*-12 in United States [13]. The presence of *qnr* and

Table 2. The information of *BlaCMY*-2 and *qnrA*, *qnrB*, *qnrS* positive in *Salmonella* spp.

Isolate name	Isolation data	Isolation source	MIC (µg·ml) CP	MIC (µg·ml) CAZ	MIC (µg·ml) AM	*qnrA*	*qnrB*	*qnrS*	*blaCMY*
Sal188	2008	Stool	0.094	0.5	<4	+	−	−	−
Sal1215	2009	Blood	0.064	1	<4	+	−	−	−
Sal2309	2010	Stool	0.074	1	<4	+	−	−	−
Sal193	2008	Stool	0.125	1	<4	+	−	−	−
Sal1571	2009	Stool	0.125	1	<4	+	−	−	−
Sal1153	2009	Stool	0.125	2	<4	+	−	−	−
Sal1562	2009	Stool	0.064	0.25	<4	+	−	−	−
Sal1947	2009	Abscess	0.074	2	<4	+	−	−	+
Sal2312	2010	Stool	0.094	0.25	<4	+	−	−	+
Sal1567	2009	Stool	0.125	0.25	<4	+	+	+	−
Sal1263	2009	Stool	0.064	1	<4	+	−	−	−
Sal1264	2009	Stool	0.094	1	<4	+	−	−	−
Sal1572	2009	Stool	0.125	1	<4	+	−	−	−
Sal2073	2010	Blood	0.125	1	<4	+	−	−	−
Sal2089	2010	Synovial fluid	0.094	2	8	+	−	−	+
Sal1259	2009	Blood	0.064	2	<4	+	−	−	−
Sal 184	2008	Blood	0.074	0.5	<4	+	−	−	+
Sal563	2008	Stool	0.074	0.5	<4	+	−	−	+
Sal2310	2010	Stool	0.094	2	<4	+	−	−	−
Sal1886	2009	Blood	0.094	1	<4	+	−	−	−
Sal2087	2010	Stool	0.074	0.5	<4	+	−	−	−
Sal1147	2009	Stool	0.094	0.5	<4	+	−	−	−
Sal186	2008	Stool	0.125	1	<4	−	−	−	+
Sal188	2008	Stool	0.094	0.5	<4	−	−	−	+
Sal189	2008	Stool	0.125	0.5	<4	−	−	−	+
Sal190	2008	Blood	0.008	1	8	−	−	−	+
Sal262	2008	Stool	0.006	0.5	<4	−	−	−	+
Sal1149	2009	Stool	0.125	1	<4	−	−	−	+
Sal1152	2009	Stool	0.012	128	<4	−	−	−	+
Sal1215	2009	Blood	0.064	1	<4	−	−	−	+
Sal1565	2009	Stool	0.074	0.5	<4	−	−	−	+
Sal1255	2009	Bone marrow	0.008	1	<4	−	−	−	+
Sal1146	2009	Stool	0.008	0.5	<4	−	−	−	+
Sal1097	2009	Stool	0.008	2	<4	−	−	−	+
Sal1098	2009	Stool	0.008	0.5	<4	−	−	−	+
Sal2124	2010	Stool	0.064	1	<4	−	−	−	+
Sal2312	2010	Stool	0.064	0.5	<4	−	−	−	+
Sal1944	2009	Stool	0.074	1	<4	−	−	−	+
Sal1885	2009	Stool	0.074	0.5	>2048	−	−	−	+

Continued

Sal175	2008	Bone marrow	0.064	2	2048	−	−	−	
Sal2311	2010	Stool	0.064	0.25	8	−	−	−	−
Sal2313	2008	Stool	0.094	0.25	8	−	−	−	−
Sal1891	2008	Stool	0.074	0.25	8	−	−	−	−
Sal1888	2008	Stool	0.064	0.5	8	−	−	−	−
Sal192	2008	Stool	0.008	1	8	−	−	−	−
Sal564	2008	Stool	0.008	1	>2048	−	−	−	−
Sal1256	2010	Stool	0.008	1	>2048	−	−	−	−
Sal2345	2008	Stool	0.074	0.25	8	−	−	−	−
Sal259	2008	Blood	0.064	2	<4	−	−	−	+
Sal184	2008	Stool	0.074	0.5	<4	−	−	−	+

bla genes on the same plasmid is one of the several possible explanations. In this study, *qnrA* gene coresistance with *blaCMY* in some isolates may be on the same plasmid. *qnrA* was as common as *blaCMY* in these community but there is no *qnr* gene with *ESBL* in the same isolates. The similar results were acquired at Turkey in 2008 [20]. All our *qnr* positive isolates couldn't carry *blaCMY*-2. The *qnrA* gene was more prevalent than the *qnrB* and *qnrs*. In 85 clinical isolates of *Salmonella*, 57.6% of isolates were resistant to quinolone, 38.82% to Cephalosporins and 14.1% to ampicillin. If the plasmids carrying both the *qnrA* and *blaCMY* genes spread rapidly, an important ally like the quinolone could be lost in the near future. Exploration about the events leads to co resistance of quinolone and extended spectrum Cephalosporins. In another study, we have shown that class 2 integron carrying gene cassettes which confer resistance to different classes of antibiotics such as Aminoglycosides and Trimethoprim are prevalent in *Salmonella* serovars isolated in Iran [21]. Presence of plasmid-mediated resistance, as well as association of class 2 integron, in *Salmonella* serovars demonstrates that antimicrobial selection pressure is widespread in our clinical settings. Regarding this issue and to avoid distribution of multidrug resistance, the limited use of antibiotics in clinics would be recommended.

Authors' Contributions

This work was carried out in collaboration between all authors. Authors Raheleh Saboohi and Seyed Davar Siadat with the same contributions, designed the study, managed the literature searches, performed the analysis of data and wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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