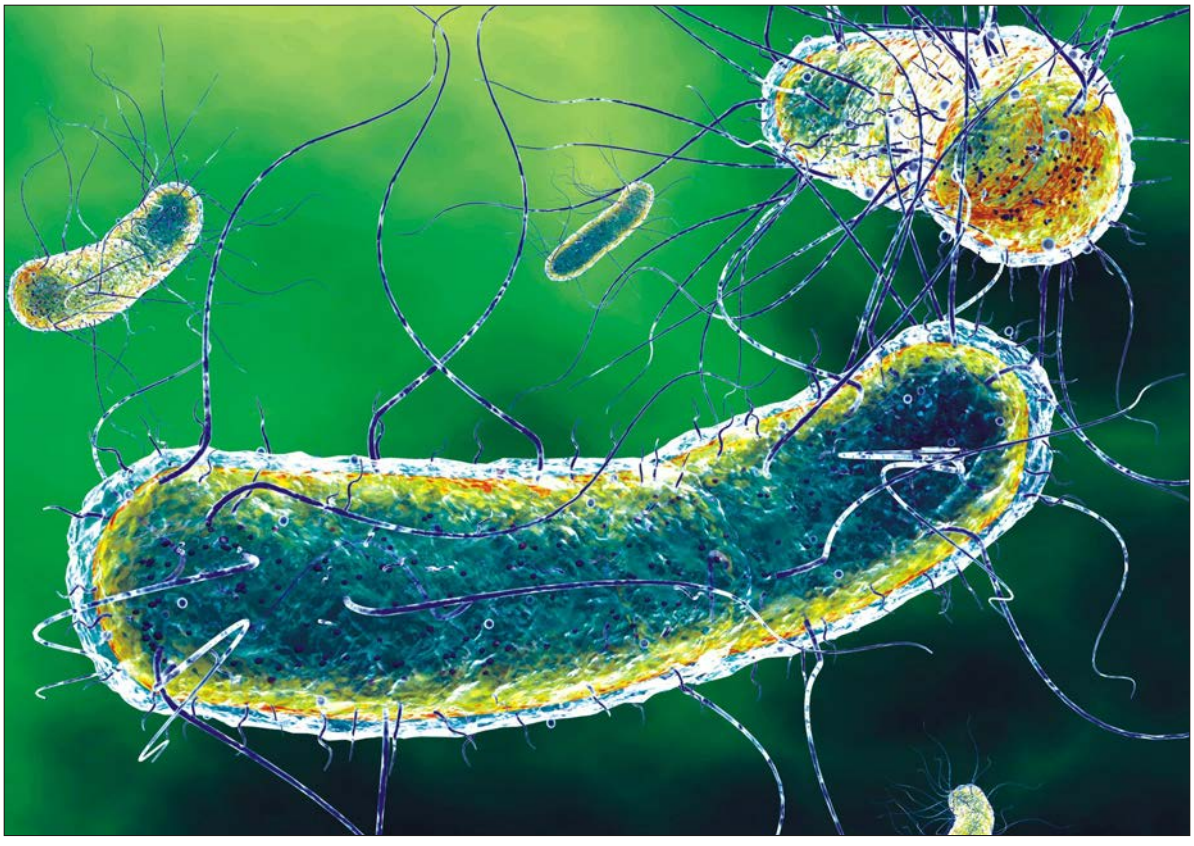

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Molecular Detection of *qnrA*, *qnrB* and *qnrS* Resistance Genes among *Salmonella* spp. in Iran

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ABSTRACT

Eighty five isolates of *Salmonella* spp. collected from different provinces of Iran during the years 2008 to 2010, were screened for the *qnrA*, *qnrB* and *qnrS* genes by PCR. PCR assays detected 22 of 85 (25.8%) *Salmonella* spp. isolates carried the *qnrA* gene, 1 (1.17%) of 85 isolates harbored the *qnrB* and 1 (1.17%) of 85 isolates contained the *qnrS* gene and 1 isolate carried all the three *qnrA*, *qnrB* and *qnrS* genes. Resistance to quinolones and fluoroquinolones by these genes has been confirmed. Antimicrobial susceptibility patterns of isolates were as follow: 49 (57.6%) isolates exhibited resistance to nalidixic acid and none to ciprofloxacin. As expected, MIC assay confirmed these results. Having detected the *qnr* genes and consequently resistance to quinolones and fluoroquinolones in *Salmonella* isolates indicated that *qnr* genes are disseminating among *Salmonella* serovars.

Key words: Quinolones, fluoroquinolones, *qnrA*, *qnrB*, *qnrS*, *Salmonella* spp., plasmid mediate quinolones resistance

INTRODUCTION

Salmonella resistance to antimicrobial agents such as quinolones and fluoroquinolones has emerged worldwide in recent years (Shahina *et al*., 2011; Mansouri *et al*., 2011; Farivar *et al*., 2006). The molecular epidemiology of resistance plasmids indicates the importance and role of plasmids in the spread of antimicrobial drug resistance (Roy *et al*., 2002; Belaluddin and Shahjahan, 2003; Yekhan *et al*., 2000). The first plasmid-mediated with low-level quinolone resistance (PMQR), *qnr* (later termed *qnrA*), was described in a *Klebsiella pneumoniae* strain from

the USA by Soto *et al.* (2003), Cheung *et al.* (2005), Jacoby *et al.* (2003), Jiang *et al.* (2008) and Wu *et al.* (2007). Recently the *qnrA* gene has been found in Enterobacteriaceae worldwide and it is more detected in Asian isolates. New *qnr* genes, the *qnrB* and the *qnrS* genes have been identified in *klebsiella pneumonia* strains isolated in USA and India as well as in *Shigella flexneri* isolated in Japan (Hopkins *et al.*, 2007). Quinolone resistance mostly caused by chromosomal mutations. In recent years, plasmid-mediated quinolone resistance, *qnrA*, *qnrB* and *qnrS*, has been reported in several parts of the world and differing from each other 40% or more in nucleotide sequence (Vasilaki *et al.*, 2008). This gene, encoding a pentapeptide repeat protein which protects DNA gyrase and topoisomerase IV from quinolone inhibition (Hopkins *et al.*, 2005; Cheung *et al.*, 2005; Park *et al.*, 2006; Jacoby *et al.*, 2006; Jiang *et al.*, 2008). In Gram-negative bacteria, topoisomerase IV is a secondary target for quinolone agents (Hopkins *et al.*, 2005; Kilmartin *et al.*, 2007). The serious problem is that pentapeptide has been shown to block the action of ciprofloxacin, a member of the large and widely group of antimicrobial drug. The *qnrB* and *qnrS* genes appear to be more widespread; The *qnrB* gene having been found in Senegal, USA and Korea while the *qnrS* genes have been found in Enterobacteriacea in Germany, USA, Taiwan, Vietnam and France. Pentapeptide repeat family amino acid in the *qnrB* gene indicates 39.5% identity with the *qnrA* gene and 37.4% amino acid identity with the *qnrS* gene (Jacoby *et al.*, 2006; Hopkins *et al.*, 2007). The plasmid increased resistance to both nalidixic acid and ciprofloxacin had a widespread range and that are not belong to plasmid group IncC (Jacoby *et al.*, 2003). The *qnr* genes corroborate resistance to nalidixic acid as well as reducing susceptibility to ciprofloxacin but the main level of quinolone resistance by the *qnr* genes is low (Crump *et al.*, 2003). Some research detected that ESBL gene and integron have been often associated with qnr gene, sequencing was performed to identify *qnr* and association ESBL gene (Hopkins *et al.*, 2007; Sharma *et al.*, 2009). In this study, Molecular epidemiology of the *qnrA*, *qnrB*, *qnrS* genes and decreased susceptibility to quinolones and fluoroquinolones in eighty five isolates of *Salmonella* spp. were studied.

MATERIALS AND METHODS

Bacterial isolates: A total of 85 isolates of *Salmonella* spp. were collected from several hospitals in different provinces of Iran, during the years 2008 to 2010. Each isolate was obtain from stool, blood, Synovial fluid, abscess, urine and bone marrow specimen and each strain came from a unique patient in different hospital. Bacterial isolates were mainly recovered from stool. All isolates were identified by biochemical and serological methods as previously described (Ahmed *et al.*, 2009).

Antimicrobial susceptibility test: Antimicrobial susceptibility of clinical isolates were studied by disk diffusion method. All isolates tested and inoculated by clinical and laboratory standard institute (CLSI) (CLSI, 2009; Sharma *et al.*, 2009). Disks prepared by MAST company (Mast Co, Merseyside, UK) were used to determine the susceptibility of isolates to nalidixic acid 30 μg mL^{-1} and ciprofloxacin 30 μg mL^{-1}.

Minimum Inhibitory Concentration (MIC) of selected antimicrobial agents were determined by broth microdilution method (Sensitite, West lake, OH, USA) and then were confirmed by E test (AB Biodisk, Piscataway NJ, USA). *Klebsiella pneumoniae* ATCC 700603 was used as quality control strain (Table 1).

PCR detection: Plasmid DNA was extracted according to the previous study (Sambrook *et al.*, 1989). The *qnrA*, *qnrB* and qnrS genes were detected by PCR (Ependrof Master Cycler®, MA). The

Table 1: The information of bla_{CMY-2} positive *Salmonella* sp.

Isolate name	Isolation date	Isolation source	MIC (μg mL^{-1}) CP	*qnrA*	*qnrB*	*qnrS*
Sal188	2008	Stool	0.094	+	-	-
Sa11215	2008	Blood	0.064	+	-	-
Sal2309	2008	Stool	0.074	+	-	-
Sal193	2008	Stool	0.125	+	-	-
Sal1571	2008	Stool	0.125	+	-	-
Sal1153	2008	Stool	0.125	+	-	-
Sal1562	2008	Stool	0.064	+	-	-
Sal1947	2008	Abscess	0.074	+	-	-
Sal2312	2008	Stool	0.094	+	-	-
Sa1567	2009	Stool	0.125	+	+	+
Sal263	2009	Stool	0.064	+	-	-
Sal264	2009	Stool	0.094	+	-	-
Sal1572	2009	Stool	0.125	+	-	-
Sal2073	2009	Blood	0.125	+	-	-
Sal2089	2009	Synovial fluid	0.094	+	-	-
Sal259	2008	Blood	0.064	+	-	-
Sal184	2008	Blood	0.074	+	-	-
Sal563	2008	Stool	0.074	+	-	-
Sal2310	2008	Stool	0.094	+	-	-
Sal1886	2008	Blood	0.094	+	-	-
Sal2087	2008	Stool	0.074	+	-	-
Sal1147	2010	Stool	0.094	+	-	-

CP: Ciprofloxacin (=.0.64 μg mL^{-1}), +: Present, -: Absent, MIC: Minimum inhibitory concentration

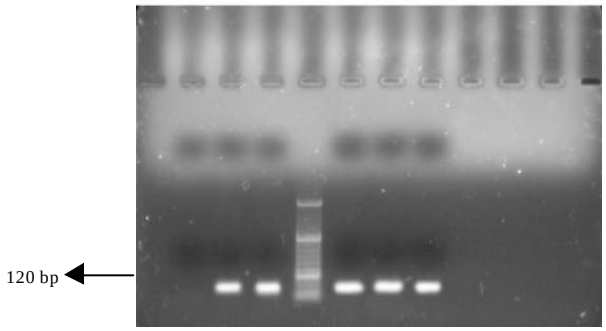

Fig. 1: PCR products of *qnrB* and 50 bp DNA ladder

primer used for amplification of the *qnr* genes was described by Whichard *et al.* (2007). Programs used for these genes are as follow: The *qnrA* gene, 35 cycles of 1 at 94°C, 40 sec at 50°C, 40 at 72°C, 10 at 72°C, *qnrB*, 35 cycles of 1 at 94°C 30 at 40°C, 20 at 72°C, 10 at 72°C, The *qnrS* gene, 35 cycles of 1 at 94°C 30 at 40°C, 30 at 72°C, 10 at 72°C. The amplicon size of *qnrA, qnrB, qnrS* were 700, 120 and 280 bp, respectively (Fig. 1-3). *K. Pneumoniae* UAB1 Strain containing PMG252 plasmid (*qnrA1*) or PMG298 (*qnrB1*) and *S. enterica* serotype Bovis morbificans strain AM12888 (*qnrS*) were used as a positive control for presence of the *qnr* gene (Jacoby *et al.*, 2003).

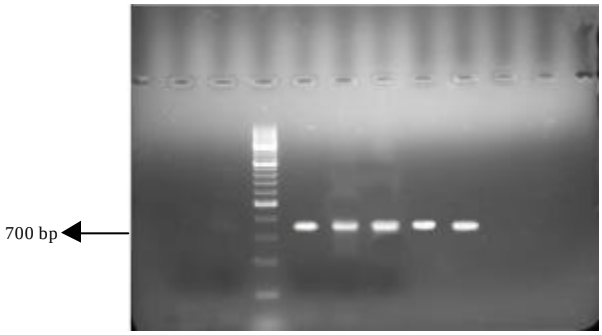

Fig. 2: PCR products of *qnrA* and 1 kb DNA ladder

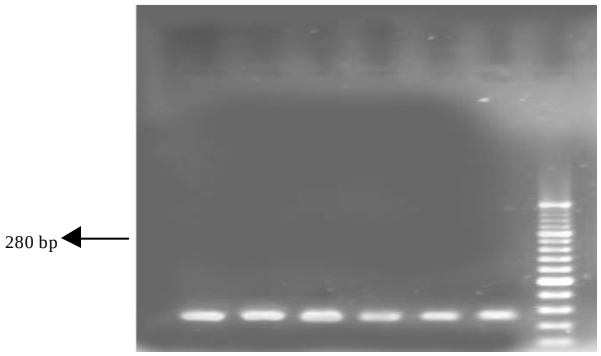

Fig. 3: PCR products of *qnrS* and 100 bp DNA ladder

DNA sequencing: The *qnrA, qnrB* and *qnrS* genes were amplified and sequenced by using specific primers. Analysis was performed by using Bio Edit (www. Mbio.ncsu. edu/Bioedit/bioedit. html). DNA sequences were determined and used to screen the GenBank database using the BLAST algorithm available at the National Center of Biotechnology information web site (http://www.ncbi.nlm.nih.gov/BLAST; Veldman *et al*., 2008; Aschbacher *et al*., 2008).

RESULTS

Antimicrobial susceptibility test: Antimicrobial susceptibility patterns according to the disk diffusion method were as follow: 49 (57.6%) isolates exhibited resistance to nalidixic acid and none isolates to ciprofloxacin. Isolates indicated ciprofloxacin MIC range of 0.064-0.125 μg mL^{-1} (Table 1).

PCR detection of the *qnr* genes: Detection of the *qnr* genes in 85 *Salmonella* isolates was carried out by PCR amplification. The 700 bp amplicons of the *qnrA* gene were identified in 22 (25.8%) of 85 clinical isolates. The *qnrB* and the *qnrS* genes with the amplicon size of 120 and 280 bp, respectively were each found in 1 (1/17%) isolate of 85 clinical isolates.

Some isolates with the *qnrA* and only isolate with the *qnrA, qnrB* and *qnrS* were selected for sequencing. The comparison between sequence results and GenBank revealed identity to the

sequence of the *qnrA*, *qnrB* and *qnrS* with the accession numbers No.AY906856.1 and No.DQ30391901 and No.FJ4181530.1, respectively (Fig. 1-3).

DISCUSSION

Quinolone resistance has been described previously by chromosomal mutation in bacterial topoisomerase gene and genes regulating expression of efflux pump or both. In recent years, plasmid-mediated quinolone resistance has been reported in several parts of the world. The *qnr* genes responsible for plasmid-born quinolone resistance have been found in clinical isolates of Enterobacteriaceae such as *Salmonella* and also resistance to different classes of antimicrobial agents is associated with the *qnr* gene (Oktem *et al*., 2008). This gene has been reported in different countries all over the world such as Senegal, USA, Korea, Germany, Taiwan, Vietnam and France (Hopkins *et al*., 2007).

A plasmid-mediated quinolone resistance conferring low resistance to quinolone, have been widely associated with clinical isolates of Enterobacteriaceae such as *Salmonella* (Cheung *et al*., 2005; Jiang *et al*., 2008; Jacoby *et al*., 2003; Wu *et al*., 2007). In fact, some reports suggested that the *qnr* gene confers only low-level resistance and its main contribution is to facilitate the development of QRDR mutations.

The present study demonstrates decreased quinolone and fluoroquinolone susceptibility as well as resistance to nalidixic acid and ciprofloxacin. It is very important to know that ciprofloxacin, a member of the large and widely used fluoroquinolone group of antimicrobial drugs, is considered the empirical choice treatment of infections in adults (Casin *et al*., 2003).

In this study, the isolates were of different serovars. Some of the isolates carried the *qnrA* and one isolate contained the qnrB and qnr*S* genes on their plasmids. All isolates were examined for the presence of the *qnr* resistance genes by PCR. Of 85 isolates one isolate harbored the three *qnrA, qnrB, qnrS* genes. The *qnrA* gene was more prevalent than the *qnrB* and *qnrS* gene among 85 *Salmonella* clinical isolates. Almost similar results are obtained in the United States in 2006 (Jacoby *et al*., 2006). Thus; the *qnrA* gene was more common among *Salmonella* clinical isolates in Iran. Despite possessing the gene encoding resistance to quinolone and fluoroquinolone in *Salmonella*, most isolates were susceptible to ciprofloxacin. Perhaps absence of an efficient promoter region is a compelling reason for this contradiction or perhaps these genes confer resistance or decreased susceptibility to nalidixic acid more than ciprofloxacin. Antibiotic resistance results to nalidixic acid and ciprofloxacin were almost the same for some countries, for example other parts of Iran (Tabriz) (Ghorashi *et al*., 2010; Manikandan *et al*., 2011).

The presence of *qnr* genes has been associated with an increase in the quinolone MIC values and therefore leads to incomplete treatment when quinolones are used as selective therapeutic drugs. Since plasmids carrying *qnr* resistance determinants have high prevalence and a worldwide distribution, care should be taken when quinolone are used to treat infections.

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