The Journal of Microbiology (2012) Vol. 50, No. 1, pp. 166–169
DOI 10.1007/s12275-012-1350-z
Copyright © 2012, The Microbiological Society of Korea

NOTE

Comparison of the Genetic Structures Surrounding *qnrA1* in Korean *Enterobacter cloacae* and Chinese *Escherichia coli* Strains Isolated in the Early 2000s: Evidence for *qnrA* Mobilization via Inc HI2 Type Plasmid

Sang Hoon Han[1], Young Ah Kim[2], Minggui Wang[3], Yangsoon Lee[4], Hae-Sun Chung[4], Jong Hwa Yum[5], Dongeun Yong[4]*, Kyungwon Lee[4], and June Myung Kim[1]

[1]*Department of Internal Medicine and AIDS Research Institute, Yonsei University College of Medicine, Seoul 120-752, Republic of Korea*
[2]*Department of Laboratory Medicine, National Health Insurance Corporation Ilsan Hospital, Gyeonggi-do 410-719, Republic of Korea*
[3]*Institute of Antibiotics, Huashan Hospital, Fudan University, Shanghai 200040, P. R. China*
[4]*Department of Laboratory Medicine and Research Institute of Bacterial Resistance, Yonsei University College of Medicine, Seoul 120-752, Republic of Korea*
[5]*Department of Clinical Laboratory Science, Dong-eui University, Busan 614-714, Republic of Korea*

(Received July 18, 2011 / Accepted October 25, 2011)

The flanking genetic structure of *qnrA1* in Korean *Enterobacter cloacae* was identical to that of the Chinese *Escherichia coli* strain, the first *qnrA1*-carrying strain reported in Asia. Analysis of restriction enzyme sites and Southern blot hybridization results showed that *qnrA1* was transferred between *E. cloacae* and *E. coli* via Inc HI2 type plasmid.

Keywords: *qnrA1*, plasmid, plasmid-mediated quinolone resistance, gene mobilization

Fluoroquinolones are one of the most widely used antimicrobials in the hospital and community settings, acting by directly inhibiting DNA synthesis. The resistances to fluoroquinolones are mostly through chromosomal mutations of topoisomerase genes, *gyrA* or *parC*. However, since the first report of a plasmid-mediated fluoroquinolone resistance gene from *Klebsiella pneumoniae* in 1994 in the USA, *qnrA* has been increasingly found in *Enterobacteriaceae* worldwide (Robicsek *et al.*, 2006). In 2000, a first Asian *qnrA1*-carrying *Escherichia coli* strain was reported in Shanghai, China (Wang *et al.*, 2003). In Korea, *qnrA1* was detected in two *E. coli* strains (#56 and #97) in 2001 (Jeong *et al.*, 2005), and the prevalence of *qnrA1* was 14.5% among expended-spectrum β-lactamase-producing *Enterobacter cloacae* strains (Park *et al.*, 2007). Interestingly, we found that the *qnrA1*-carrying *E. cloacae* index strain isolated in 2001 from Severance Hospital in Seoul shared an identical genetic structure surrounding the *qnrA1* locus with that of In36 from the *qnrA1*-carrying *E. coli* strain isolated in Shanghai, China in 2000, a long way of about 900 km from Seoul (Fig. 1).

The genetic structure flanking *qnrA* in *K. pneumoniae* has been demonstrated (Shen *et al.*, 2008), but the comparison of genetic structure surrounding *qnrA* in the isolates from East Asia has never been reported. In this study, we investigated the genetic relatedness of *qnrA1*-containing plasmids between the Korean *E. cloacae* index strain and Chinese *E. coli* strain.

A *qnrA1*-carrying *E. cloacae* strain (YMC01/10/R768) isolated at a Korean teaching hospital in 2001 was included as an index strain. Also, we used *E. coli* #4 strain isolated in 2000 from Shanghai, China, the first Asian strain carrying *qnrA1* (Wang *et al.*, 2003). *E. coli* Lo was kindly provided by Dr. Patrice Nordmann in Faculté de Médecine et Université Paris-Sud and was used as a comparator strain.

In this study, *qnrA1* and its surrounding structure were examined using PCR amplification and nucleotide sequencing (Table 1 and Fig. 1). The genomic DNA in agarose plugs was digested with the restriction enzyme S1 (Sigma-Aldrich GmbH, Germany), and its DNA fragments were separated by CHEF-DR II (Bio-Rad, USA) for 16 h at 6 V/cm at 4°C with the initial and final pulse times of 5.3 sec and 34.9 sec, respectively. The exact size of the qnrA1-carrying plasmids could be measured by using pulsed field gel electrophoresis (PFGE) technique. The DNA in the gel was transferred onto a nylon membrane (Bio-Rad) and was hybridized with a *qnrA1* probe. DIG DNA labeling and detection kits (Roche Diagnostics, USA) were used for probe labeling and Southern blot hybridization (SBH) (Fig. 2A).

To compare the genetic structures of the strains, the plasmid DNA was digested at 37°C overnight with *Sma*I alone and with *Sma*I plus *Sac*I, *Sfi*I, or *Xho*I (TaKaRa, Japan) followed by SBH with a *qnrA1* probe (Fig. 2B). These enzymes, with the exception of *Sma*I, do not cut *qnrA*1; therefore we could

*For correspondence. E-mail: deyong@yuhs.ac; Tel.: +82-2-2228-2442; Fax: +82-2-364-1583

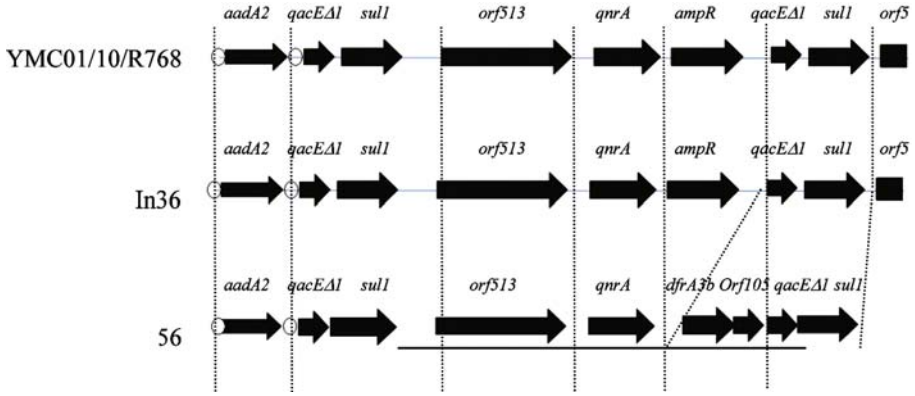

Fig. 1. Comparison of the nucleotide sequences surrounding *qnrA1*: *E. cloacae* index strain (YMC01/10/R768), In36 of Chinese *E. coli* #4 strain from Shanghai (Wang *et al.*, 2003), and integron from Korean *E. coli* strain #56 (Jeong *et al.*, 2005).

compare the genetic environment surrounding *qnrA1* (Fig. 2C). DNA fragments were separated by CHEF-DR II (Bio-Rad) for 2 h at 9 V/cm at 4°C with the initial and final pulse times of 1 sec and 25 sec, respectively. SBH was performed as described above. Plasmid replicon typing using multiplex PCR (Carattoli *et al.*, 2005) and SBH was performed with the plasmids on the S1-treated PFGE gel (Fig. 3). In this study, all tests were performed repeatedly to rule out the possibility of incomplete digestion by the restriction enzymes. We also tried several times to transfer the plasmid carrying *qnrA* gene from Chinese and Korean strains to *E. coli* J53 (azide R) using plate-mating method.

From PFGE and SBH, we found that the sizes of *qnrA1*-carrying plasmids in the test strains were similar, ranging from 250 to 300 kb, except in the *E. coli* Lo strain (Fig. 2A). The probe did not hybridize with the chromosomal bands, and hence with the plasmid location of *qnrA1*. Interestingly, all strains possessed the second *qnrA1*-positive plasmid that was twice the size of the smaller ones, suggesting that plasmid existed as monomers and dimers (Fig. 2A). The analysis of restriction enzyme sites revealed that *qnrA1*-carrying DNA fragments had variable sizes that were less than 50 kb (Fig. 2B). In particular, the *qnrA1*-positive DNA band sizes of the Chinese *E. coli* strain were very similar to those of the index strain. However, more *qnrA1*-carrying DNA fragments were observed in the index strain than in the Chinese strain (Fig. 2B). Based on these results, we proposed a plasmid model carrying 2 copies of *qnrA1* to explain the additional *qnrA* fragments (Fig. 2C). An open reading frame (orf) present in the upstream of the *qnrA1* gene (orf513) belongs to *IS*CR1 and might be responsible for the mobility of *qnrA* (Toleman *et al.*, 2006).

Replicon typing with multiplex-PCR and SBH showed that the *qnrA1*-carrying plasmids in both the index strain and the Chinese *E. coli* #4 strain were the HI2 replicon type (Fig. 3). Interestingly, HI2 type plasmid carrying *qnrA1* was previously reported in *K. pneumonia* from Australia and in *E. cloacae* from France (Poirel *et al.*, 2007), indicating the global dissemination of *qnrA*-carrying HI2 type plasmid among *Enterobacteriaceae*.

We tried several times to transfer the plasmid carrying *qnrA* gene. However, the transconjugant was not isolated on MacConkey agar containing azide (100 μg/ml) and levofloxacin (0.12 μg/ml). This result reveals that the frequency of transcojugation must be low, supporting that the prevalence of *qnrA*-carrying *E. coli* strains was very low in Korea (Jeong *et al.*, 2005).

Many studies have reported that *qnrA1* plasmids are vari-

Table 1. Primers used in this study for PCR amplification and nucleotide sequencing

Primer	Target DNA	Sequence (5′-3′)	PCR product size	Reference
AADA2-F	AADA2, qacEΔ1	GTGCTAAGCGTCATTGAGC	1141	In this study
10 qacE delta1-R		AACCAGGCAATGGCTGTAAT		In this study
qacED1	qacEΔ1, sul1	TCGCAACATCCGCATTAAAA	1302	Eckert *et al.* (2006)
Orf5 13 rev		GCGTTTTATCGGTAGTCGTC		Eckert *et al.* (2006)
Sul2	sul1, orf513	CCGTTGGCCTTCCTGTAAAG	1248	Heuer and Smalla (2007)
CRF qnr r		TCATCGTAAGTGGGTGCCTTGACA		In this study
ORF5132-F	orf513	ATGGTTTCATGCGGGTT	1321	Arduino *et al.* (2003)
ISCR1-R		CGACTCTGTGATGGATCGAA		In this study
RECR qnr r1	qnrA1	CGTCAAAGTGATTGCCAGCAT	1046	In this study
QnrAm-R		TGCCAGGCACAGATCTTGAC		Cattoir *et al.* (2007)
QnrF	qnrA1, ampR	AGAGGATTTCTCACGCCAGG	1377	Cattoir *et al.* (2007)
DHA-AmpR-mR		CAGGGTAAAGCGGTGAACAT		Roche *et al.* (2008)
DHA-AmpR-mF	ampR, qacEΔ1, sul1	GATTTTCAGTGCACCACACG	1576	In this study
3CS2-R		TTTGAAGGTTCGACAGC		Arduino *et al.* (2002)
Y-qacED1F	qacEΔ1, sul1, orf5	CTTCATGGGCAAAAGCTTGA	1298	In this study
5.2F2		ACAAGGTGCAACAGCCGACG		In this study

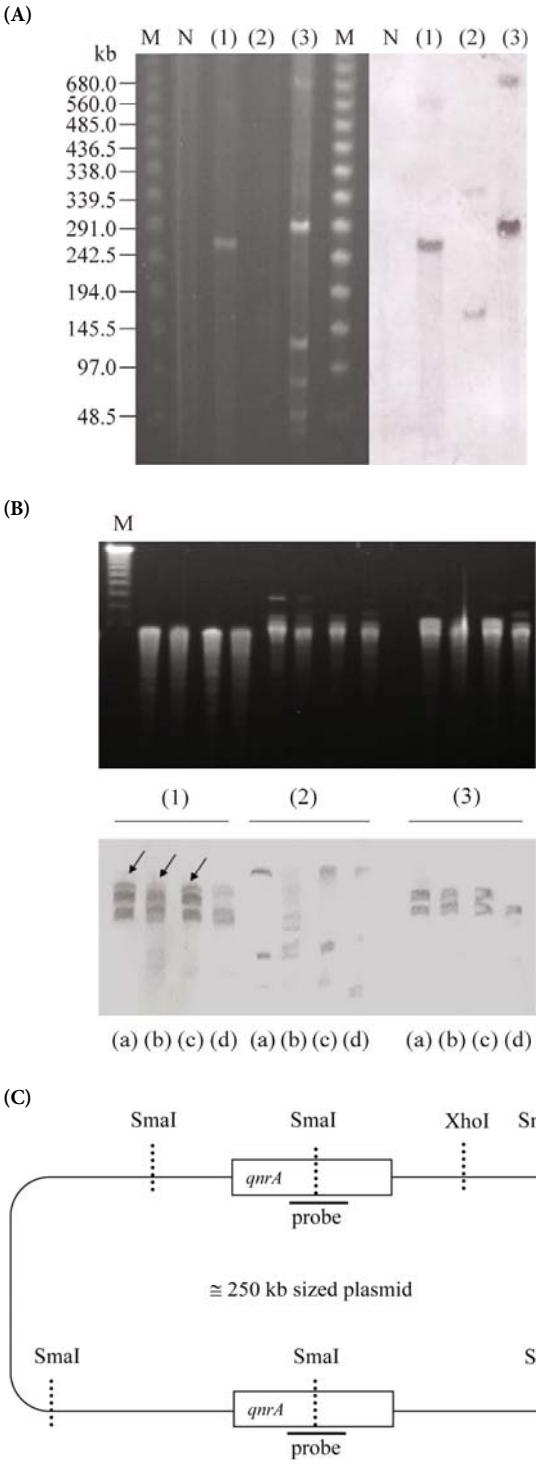

Fig. 2. (A) PFGE findings of S1-digested genomic DNA and SBH with a *qnrA1* probe, M, marker; N, negative control; (1) YMC01/10/R768 (index strain, *E. cloacae*); (2) *E. coli* Lo; and (3) Chinese *E. coli* #4.
(B) Double digestion with (a) SmaI alone, (b) SmaI plus SacI, (c) SmaI plus SfiI, or (d) *Sma*I plus *Xho*I in SBH with a *qnrA1* probe. The arrows indicate the extra *qnrA1*-carrying bands compared with the sequence of the Chinese *E. coli* #4 strain.
(C) Modeling of the *qnrA*-carrying plasmid of YMC01/10/R768 (index strain, *E. cloacae*) to explain the band difference of double digestion and SBH study. Dotted lines indicate the enzyme cutting sites. The white boxes and bars represent *qnrA* and probe binding sites, respectively.

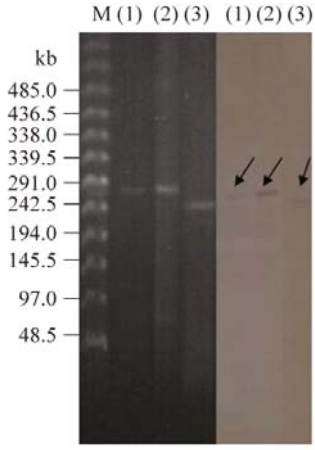

Fig. 3. SBH with a HI2 probe. Lanes: M, marker; (1, 2) Chinese *E. coli* #4 and transconjugant; (3) YMC01/10/R768 (index strain, *E. cloacae*). The bands with arrows indicate positive signals by SHB with a HI2 probe.

able in size ranging from 54 to >180 kb, and all have multiple resistance determinants conferring resistance to antibiotics such as β-lactams, aminoglycosides, rifampin, or tetracycline (Mammeri *et al.*, 2005; Nordmann and Poirel, 2005; Robicsek *et al.*, 2006; Cattoir *et al.*, 2007; Park *et al.*, 2007; Ma *et al.*, 2009). In this study, we could measure the sizes of *qnrA1*-carrying plasmids quite precisely ranging from 250 to 300 kb using PFGE and SBH.

In summary, our results showed that the genetic structure surrounding *qnrA1* was identical in the Korean *E. cloacae* index strain and Chinese *E. coli* strain, although there was no epidemiological association between the strains, indicating the HI2 type plasmids played a substantial role in *qnrA1* dissemination between the two species in East Asia.

The nucleotide sequence reported in the present study has been assigned with an EMBL nucleotide accession number HQ184955. This study was supported by a faculty research grant from Yonsei University College of Medicine for 2008 (6-2008-0267).

References

Arduino, S.M., Catalano, M., Orman, B.E., Roy, P.H., and Centron, D. 2003. Molecular epidemiology of orf513-bearing class 1 integrons in multiresistant clinical isolates from argentinean hospitals. *Antimicrob. Agents Chemother.* **47**, 3945–3949.

Arduino, S.M., Roy, P.H., Jacoby, G.A., Orman, B.E., Pineiro, S.A., and Centron, D. 2002. $bla_{CTX-M-2}$ is located in an unusual class 1 Integron (In35) which includes Orf513. *Antimicrob. Agents Chemother.* **46**, 2303–2306.

Carattoli, A., Bertini, A., Villa, L., Falbo, V., Hopkins, K.L., and Threlfall, E.J. 2005. Identification of plasmids by PCR-based replicon typing. *J. Microbiol. Methods* **63**, 219–228.

Cattoir, V., Poirel, L., Rotimi, V., Soussy, C.J., and Nordmann, P. 2007. Multiplex PCR for detection of plasmid-mediated quinolone resistance *qnr* genes in ESBL-producing enterobacterial isolates. *J. Antimicrob. Chemother.* **60**, 394–397.

Eckert, C., Gautier, V., and Arlet, G. 2006. DNA sequence analysis of

the genetic environment of various bla_{CTX-M} genes. *J. Antimicrob. Chemother.* **57**, 14–23.

Heuer, H. and Smalla, K. 2007. Manure and sulfadiazine synergistically increased bacterial antibiotic resistance in soil over at least two months. *Environ. Microbiol.* **9**, 657–666.

Jeong, J.Y., Yoon, H.J., Kim, E.S., Lee, Y., Choi, S.H., Kim, N.J., Woo, J.H., and Kim, Y.S. 2005. Detection of *qnr* in clinical isolates of *Escherichia coli* from Korea. *Antimicrob. Agents Chemother.* **49**, 2522–2524.

Ma, J., Zeng, Z., Chen, Z., Xu, X., Wang, X., Deng, Y., Lu, D., Huang, L., Zhang, Y., Liu, J., and *et al.* 2009. High prevalence of plasmid-mediated quinolone resistance determinants *qnr*, *aac(6')-Ib-cr*, and *qepA* among ceftiofur-resistant *Enterobacteriaceae* isolates from companion and food-producing animals. *Antimicrob. Agents Chemother.* **53**, 519–524.

Mammeri, H., Van De Loo, M., Poirel, L., Martinez-Martinez, L., and Nordmann, P. 2005. Emergence of plasmid-mediated quinolone resistance in *Escherichia coli* in Europe. *Antimicrob. Agents Chemother.* **49**, 71–76.

Nordmann, P. and Poirel, L. 2005. Emergence of plasmid-mediated resistance to quinolones in *Enterobacteriaceae*. *J. Antimicrob. Chemother.* **56**, 463–469.

Park, Y.J., Yu, J.K., Lee, S., Oh, E.J., and Woo, G.J. 2007. Prevalence and diversity of *qnr* alleles in AmpC-producing *Enterobacter cloacae*, *Enterobacter aerogenes*, *Citrobacter freundii* and *Serratia marcescens*: a multicentre study from Korea. *J. Antimicrob. Chemother.* **60**, 868–871.

Poirel, L., Villa, L., Bertini, A., Pitout, J.D., Nordmann, P., and Carattoli, A. 2007. Expanded-spectrum β-lactamase and plasmid-mediated quinolone resistance. *Emerg. Infect. Dis.* **13**, 803–805.

Robicsek, A., Jacoby, G.A., and Hooper, D.C. 2006. The worldwide emergence of plasmid-mediated quinolone resistance. *Lancet Infect. Dis.* **6**, 629–640.

Roche, C., Boo, T.W., Walsh, F., and Crowley, B. 2008. Detection and molecular characterisation of plasmidic AmpC β-lactamases in *Klebsiella pneumoniae* isolates from a tertiary-care hospital in Dublin, Ireland. *Clin. Microbiol. Infect.* **14**, 616–618.

Shen, P., Jiang, Y., Zhou, Z., Zhang, J., Yu, Y., and Li, L. 2008. Complete nucleotide sequence of pKP96, a 67,850 bp multiresistance plasmid encoding *qnrA1*, *aac(6')-Ib-cr* and $bla_{CTX-M-24}$ from *Klebsiella pneumoniae*. *J. Antimicrob. Chemother.* **62**, 1252–1256.

Toleman, M.A., Bennett, P.M., and Walsh, T.R. 2006. Common regions e.g. orf*513* and antibiotic resistance: Is*91*-like elements evolving complex class 1 integrons. *J. Antimicrob. Chemother.* **58**, 1–6.

Wang, M., Tran, J.H., Jacoby, G.A., Zhang, Y., Wang, F., and Hooper, D.C. 2003. Plasmid-mediated quinolone resistance in clinical isolates of *Escherichia coli* from Shanghai, China. *Antimicrob. Agents Chemother.* **47**, 2242–2248.