New Microbiologica, **34**, 225-228, 2011

Co-expression of plasmid-mediated quinolone resistance-*qnrA1* and *bla*$_{VEB-1}$ gene in a *Providencia stuartii* strain

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SUMMARY

An extended-spectrum β-lactamase (ESBL)-producing *Providencia stuartii* isolate was studied. A *qnrA1* gene co-expressing *bla*$_{VEB-1}$ gene was detected. Both genes were transferred to the recipient strain. The ciprofloxacin MIC of recipient strain increased tenfold. The *bla*$_{VEB-1}$ gene persisted in microorganisms in Turkey but it also spread with PMQR genes to other species. The combination of PMQR with multidrug resistant isolates producing ESBLs may compromise the use of valuable antibiotics. Serious efforts are necessary to detect PMQR determinants not only with common β-lactamases in widespread pathogens but also with uncommon forms that are encountered infrequently.

KEY WORDS: Plasmid, Quinolone, VEB-1, *Providencia stuartii*

Received October 07, 2010 *Accepted* February 02, 2011

Today, an increase in bacterial resistance against antibiotics has become a major problem worldwide (Robin *et al.,* 2010; Shahcheraghi *et al.,* 2010; Poirel *et al.,* 2008). The use of many antibiotics such as β-lactams and fluoroquinolones widely prescribed by clinicians in the treatment of infections is limited. Then production of ESBLs and plasmid-mediated quinolone resistance (PMQR) proteins are matters of concern. After the detection of *qnr* determinants which protect target enzymes against quinolone inhibition, two other mechanisms have recently been identified: the *qepA* gene encodes an efflux pump which confers reduced susceptibility to hydrophobic fluoroquinolones such as norfloxacin and ciprofloxacin; and the *aac(6')-Ib-cr* gene which encodes modified aminoglycoside-acetylating enzymes can both inactivate aminoglycosides and fluoroquinolones (Martinez-Martinez *et al.,* 1998; Robicsek *et al.,* 2006; Yamane *et al.,* 2007). This study describes a ceftazidime-resistant, ciprofloxacin-susceptible *P.stuartii* isolate co-expressing *qnrA1* and *bla*$_{VEB-1}$ gene.

The clinical *P.stuartii* isolate 78 was derived in 2009 from a patient with diabetes mellitus in the intensive care unit (ICU) of Gulhane Military Medical Academy (GMMA) Haydarpasa Training Hospital (1000 beds). He was a 55-year-old man who suffered from extensive severe burns intubated for mechanical ventilation. After being stabilized, a wound infection with purulent secretion emerged. Culture of samples from wound secretions yielded multidrug-resistant *P.stuartii* isolates. The isolates were identified by the VITEK 2 automated system (bioMérieux, Marcy I'Etoile, France). Individual strains were tested based on the recommendations of the Clinical and Laboratory Standards Institute (CLSI), by the Kirby-Bauer disc diffusion method for susceptibility (CLSI, 2009). The double-disc synergy test with cefotaxime and ceftazidime was used for screening the ESBL production. For the *P. stuartii* isolate and its transconjugant; minimal in-

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hibitor concentration (MIC) values were determined by VITEK 2 automated system. MIC values of ciprofloxacin were determined by E-test method (AB, Biodisk, Solna, Sweden). Conjugation experiments using an azide-resistant *E.coli* J53 (AzR) as the recipient were performed in liquid culture media as described previously (Wang *et al.,* 2004). Transconjugants were selected on trypticase soy agar plates containing sodium azide (100 µg/L) for counter selection and amoxicillin (100 µg/L), cefotaxime (8 µg/L), ceftazidime (8 µg/L), nalidixic acid (16 µg/L). The High Pure Plasmid Isolation Plasmid DNA Kit (Roche, Mannheim, Germany) was used for the extraction of plasmid DNA. *E.coli* V517 was used as the size marker for the plasmids. The presence of transferred PMQR genes and related ESBLs was confirmed by PCR. The $bla_{TEM,}$ $bla_{SHV,}$ $bla_{CTX\text{-}M,}$ and bla_{VEB} genes were investigated by PCR as described previously (Pallecchi *et al.,* 2007; Poirel *et al.,* 2005). A multiplex PCR was performed to detect *qnrA*, *qnrB* and *qnrS* as described previously by Cattoir *et al.* (Cattoir *et al.,* 2007). PCR amplification of *qnrC*, *qnrD*, and *qepA* and *aac (6')-Ib* was carried out with specific primers and conditions (Cavaco *et al.,* 2009; Robicsek *et al.,* 2006; Yamane *et al.*, 2007). The DNA(s) for controls of each specific gene regions were included with each group of tested strains. The amplification products of PMQR and related β-lactamase gene were sequenced with an Applied Biosystem sequencer (ABI PRISM 310 Genetic Analyzer; Applied Biosystems, Foster City, CA, USA). The nucleotide and amino acid sequences were analyzed and compared with the BLAST computer program available over the Internet at the National Center for Biotechnology Information website (www.ncbi.nlm.nih.gov).

The routine antibiogram revealed that *P. stuartii* was resistant to ampicillin, cefazolin, ceftazidime, gentamicin, nitrofurantoin, chloramphenicol, rifampin and was susceptible to carbapenems, cefotaxime, cefepime trimethoprim-sulfamethoxazole and ciprofloxacin. The double-disc synergy test between cefotaxime and amoxicillin-clavulanic acid disks on Mueller-Hinton agar plates suggested the presence of an ESBL. A marked synergy pattern between cefepime, aztreonam, and cefoxitin or imipenem containing disks demonstrated the presence of a VEB-type β-lactamase. *P. stuartii* and its transconjugant were positive for $bla_{VEB\text{-}1}$. In addition to $bla_{VEB\text{-}1}$, a *qnrA1* gene was detected in the same strain, however, the isolate lacked other known PMQR determinants and bla_{TEM}, bla_{SHV}, $bla_{CTX\text{-}M}$ genes (Figure 1). Agarose gel electrophoresis of plasmid DNA preparations from transconjugant of *P.stuartii* indicated transfer of a single plasmid of ~50 kb. The susceptibility pattern of transconjugants to β-lactams showed the expression of a clavulanic acid-inhibited ESBL. The conjugated plasmid conferred resistance to ampicillin, cefazolin, rifampin and intermediated susceptibility to ceftazidime. The resistance to β-lactams were reduced by tazobactam and clavulanic acid. In the transconjugant of *P. stuartii*, the MICs were increased tenfold for ciprofloxacin (Table 1).

Over the last decade, PMQR, especially among the various species of the *Enterobacteriaceae,* has been increasingly reported from many regions of the world (Martinez-Martinez *et al.*, 2008). Plasmid carrying genes could contribute to the development of higher level fluoroquinolone resistance and may pose a threat allowing the rapid spread of resistance among organisms. Although these PMQR genes have been associated with low level quinolone resistance, it may cause high level quinolone resistance by facilitating the selection of chromosomal mutations.

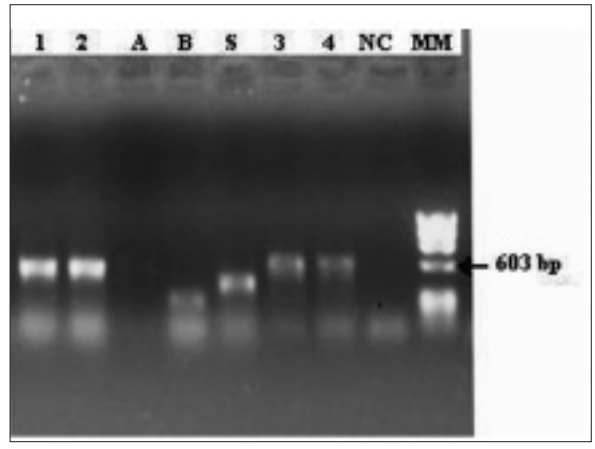

FIGURE 1 - *PCR gel showing qnrA1 and* $bla_{VEB\text{-}1}$ *from Providencia stuartii strain. Line 1/Line 2 (qnrA1, 580 bp) and Line 3/Line 4 (VEB-1, 627 bp) from Providencia stuartii 78 and 78T respectively. A, B, S are positive control for qnrA, qnrB, and qnrS respectively; NC - negative control; MM - molecular marker (Φ174).*

TABLE 1 - *The resistance genes and antibiotic susceptibilities of Providencia stuartii clinical isolate and its transconjugants.*

	MIC values (mg/L) for		
Antibiotics	*Providencia stuartii 78 qnrA1-positive, bla_{VEB-1}*	*Escherichia coli J53 (p78) (qnrA1-positive bla_{VEB-1})*	*E.coli J53*
Ampicillin	≥32	≥32	≤2
Amoxicillin-clavulanic acid	8	8	4
Piperacillin-tazobactam	≤4	≤4	≤4
Cefazolin	≥64	32	≤4
Cefoxitin	≤4	≤4	4
Ceftazidime	≥64	16	≤1
Ceftriaxone	≤1	≤1	≤1
Cefepime	≤1	≤1	≤1
Ertapenem	≤0,5	≤0,5	≤0,5
Imipenem	2	≤1	≤1
Meropenem	≤0,25	≤0,25	≤0,25
Gentamicin	8	4	≤1
Ciprofloxacin	0.128	0.064	0.006
Levofloxacin	1	0.5	≤0,12
Tigecycline	2	≤0,5	≤0,5
Trimethoprim/sulfamethoxazole	≤20	≤20	≤20

MIC: minimal inhibitor concentration

E.coli carrying *qnrA*, *qnrB*, *qnrS* and *aac(6')-Ib-cr* genes have been reported from Turkey (2006; Nazik *et al.,* 2009; Nazik *et al.,* 2008; Oktem *et al.,* 2008). *qepA* was first identified in 2007 in two *E.coli* clinical isolates from Japan and Belgium (Yamane *et al.,* 2008) and a new variant (*qepA2*) has already been detected in France (Cattoir *et al.,* 2008). However, recently, a *qepA* producing *E.coli* strain also possessing *qnrB2* and *aac(6')-Ib-cr* gene was reported from our country (Gulay *et al.,* 2010). Many studies demonstrated the association between TEM-SHV-CTX-M type β-lactamases and PMQR in *Enterobacteriaceae* (Pitout, 2008). The association of *qnrA* with VEB-1 type β-lactamases was investigated first in a single *Enterobacter cloacae* isolate from France and in 11 out of 23 bla_{VEB-1} positive enterobacterial isolates from Thailand by Poirel *et al.* (Poirel *et al.,* 2005) in a previous study. In addition, a *qnrA*-positive-*Citrobacter freundii* isolate producing bla_{VEB-1} and bla_{OXA-48} has been reported from Turkey (Mammeri *et al.,* 2006). Here again, five years later, in the same hospital, *qnrA1* co-expressing with bla_{VEB-1} gene was presented but differently in a ciprofloxacin-susceptible *P.stuartii* isolate. This finding also showed that VEB-1 type β-lactamase persists in microorganisms in Turkey.

The combination of PMQR with multidrug resistant isolates producing ESBLs compromises the use of valuable antibiotics worldwide. These resistance genes may be spreading rapidly in various species in countries such as Turkey where the antimicrobials are consumed in large quantities. Considerable efforts are needed to detect of PMQR determinants, not only common β-lactamases in widespread pathogens but also those encountered infrequently.

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