Expanded-spectrum β-Lactamase and Plasmid-mediated Quinolone Resistance

To the Editor: The emergence of plasmid-mediated, and thus transferable, quinolone resistance determinants has been recently discovered (*1*) and shown to involve the pentapeptide repeat protein Qnr, which interacts with DNA gyrase and topoisomerase IV to prevent quinolone inhibition (*2*,*3*). Qnr determinants confer resistance to nalidixic acid and reduced susceptibility to fluoroquinolones (*3*). They have been identified worldwide in a variety of enterobacterial species and were often associated to expanded-spectrum β-lactamases (ESBLs) (*2*). The association between the ESBL VEB-1 and the QnrA1 determinants was reported (*4*). Because plasmid co-localization of QnrA and VEB-1 encoding genes has been reported repeatedly from scattered clonally-unrelated enterobacterial isolates, our objective was to use replicon typing to trace a possible dissemination of a common plasmid worldwide.

The bla_{VEB-1}- and/or *qnrA*-positive plasmids that have been included in the study were from 17 isolates previously described in detail (*3*–*8*) (Table). *Escherichia coli* transconjugants (Tc) were obtained for 14 of 17 clinical isolates, allowing an accurate replicon typing since original clinical isolates might harbor several plasmids. They were collected from 1999 to 2005, from patients hospitalized in different parts of the world (Table). The 13 bla_{VEB-1}-positive isolates were from 5 countries (France, Turkey, Algeria, Thailand, and Canada), scattered on 4 continents. Among them, the *Providencia stuartii* and *Proteus mirabilis* isolates from Algeria were negative for *qnrA1*. In addition, 4 bla_{VEB-1}-negative but *qnrA1*-positive isolates recovered from France and Australia were also included in the study.

PCR-based replicon typing (PBRT), which recognizes FIA, FIB, FIC, HI1, HI2, I1-Iγ, L/M, N, P, W, T, A/C, K, B/O, X, Y, and FII replicons (*9*), was applied to type the resistance plasmids from all the strains.

Table. Features of the VEB-1– or QnrA-positive isolates used in this study*

Strain† (Ref.)	Sp. of origin	Country	Year of isolation	Plasmid size (kb)	ESBL	QnrA1	Replicon	Resistance markers‡
Escherichia coli TcE1 (*5*)	*E. coli*	Thailand	1999	160	VEB-1	+	A/C_2	NAL, K, SSS, C, RA
E. coli TcE4 (*5*)	*E. coli*	Thailand	1999	150	VEB-1	+	A/C_2	NAL, K, TM, SSS, C, RA
E. coli TcE5 (*5*)	*E. coli*	Thailand	1999	150	VEB-1	+	A/C_2	NAL, K, TM, SSS, SXT
E. coli TcE7 (*5*)	*E. coli*	Thailand	1999	150	VEB-1	+	A/C_2	NAL, K, TM, SSS, C, RA
E. coli TcE8 (*5*)	*E. coli*	Thailand	1999	150	VEB-1	+	A/C_2	NAL, K, TM, SSS, TE
E. coli TcE16 (*5*)	*E. coli*	Thailand	1999	140	VEB-1	+	A/C_2	NAL, K, TM, SSS, RA
E. coli TcE18 (*5*)	*E. coli*	Thailand	1999	180	VEB-1	+	A/C_2	NAL, K, SSS, C, RA
E. coli Tc(p1) (*5*)	*E. coli*	Canada	2000	180	VEB-1	+	A/C_2	NAL, K, SSS, C, RA
E. coli Tc(pQR1) (*4*)	*E. coli*	France	2003	180	VEB-1	+	A/C_2	NAL, K, SSS, C, RA, SXT
E. coli Tc(GOC) (*4*)	*Enterobacter cloacae*	France	2003	190	VEB-1	+	A/C_2, FIB	NAL, K, TM, SSS, C
Citrobacter freundii LUT (*3*)	*C. freundii*	Turkey	2004	ND	VEB-1	+	A/C_2, FIB, K	NA
Providencia stuartii 15 (this study)	*P. stuartii*	Algeria	2004	ND	VEB-1	–	A/C_2	NA
E. coli TcMAA (this study)	*Proteus mirabilis*	Algeria	2004	190	VEB-1	–	A/C_2	K, TM, SSS, C, SXT
E. coli TcK147 (*7*)	*Klebsiella pneumoniae*	Australia	2002	160	SHV-12	+	HI2, A/C_1, P	NAL, K, TM, C, TE, SXT
E. cloacae A1 (*8*)	*E. cloacae*	France	2004	75	SHV-12	+	HI2	NA
E. coli TcA2 (*8*)	*Enterobacter aerogenes*	France	2005	150	SHV-12	+	FII	NAL, K, TM, TE
E. coli TcA3 (*8*)	*K. pneumoniae*	France	2005	40	–	+	I1, K	NAL, K, TM, C, TE

*Ref., reference; ESBL, expanded-spectrum β-lactamase; NAL, nalidixic acid; K, kanamycin; SSS, sulfonamides; C, chloramphenicol; RA, rifampin; TM, tobramycin; SXT, trimethoprim-sulfamethoxazole; TE, tetracycline; ND, not determinable; NA, not applicable.
†Tc indicates that this is a transconjugant or a transformant.
‡Non–β-lactam–associated markers.

Amplicons were confirmed by DNA sequencing and used as probes in hybridization experiments on purified plasmids (data not shown).

PBRT results showed that the 13 bla_{VEB-1}-positive plasmids (including 11 *qnrA1*-positive) belonged to the IncA/C incompatibility group. DNA sequencing identified the A/C_2 replicon variant (European Molecular Biology Laboratory no. AM087198) in all these plasmids (Table). Plasmids of this type were recently identified in the United States and in Italy carrying the AmpC-type cephalosporinase CMY-2–encoding gene (*10*). In 2 strains (*E. coli* TcGOC and *Citrobacter freundii* LUT), the IncA/C_2 plasmids were associated with additional replicons, which suggests the presence of multiple plasmids or fusions between plasmids of different backbones. By contrast, all the 4 bla_{VEB-1}-negative isolates but *qnrA1*-positive were negative for the A/C replicon, except transconjugant TcK147; however, sequencing identified an A/C_1-type replicon in that strain. These results indicated that the genes encoding QnrA1 and VEB-1, when identified concomitantly in a given isolate, were always located on plasmids belonging to the same IncA/C_2-incompatibility group that may vary in size and digestion pattern (Table; unpub. data). In addition, we showed that plasmids carrying the bla_{VEB-1} gene but lacking *qnrA1* were also of the IncA/C_2 type (Table). Plasmids that were bla_{VEB-1}-negative but *qnrA1*-positive were of distinct replicon types, thus suggesting independent acquisition of the *qnrA1* gene on different plasmids. It is remarkable that since VEB-1 is apparently always encoded by IncA/C_2 plasmids, when genes for QnrA1 and VEB-1 are found together, they also occur on IncA/C_2 plasmids.

Thus, evidence here shows that the IncA/C_2 plasmid is the main vehicle of the bla_{VEB-1} gene worldwide, on which the *qnrA1* gene may be added. The possibility that both bla_{VEB-1} and *qnrA1* genes may be identified on a single genetic structure in several isolates has been recently shown with their identification within the same *sul1*-type integron (*6*).

Because results of these experiments provided a good marker for tracing bla_{VEB-1}-positive plasmids, and taking in account the property of A/C-type plasmids to have a broad range of hosts (note: this has not been demonstrated for the specific A/C_2 subgroup), we tried to amplify the A/C_2 replicon in a collection of 15 bla_{VEB-1}-positive and clonally unrelated *Pseudomonas aeruginosa* isolates from France, Thailand, India, and Kuwait. The bla_{VEB-1} gene was supposed to be chromosome-encoded in those isolates. PCR failed to give any positive results, confirming the absence of an IncA/C-type plasmid and also ruling out the hypothesis of IncA/C_2-type plasmid co-integration at the origin of bla_{VEB-1} acquisition in *P. aeruginosa*.

The spread of plasmids carrying a large array of resistance genes among *Enterobacteriaceae* is of concern since this provides a convenient genetic mechanism for a given strain to become panresistant to antimicrobial drugs. In particular, the recent identification of the Qnr determinants has shown that plasmids may provide resistance (or at least reduced susceptibility) to quinolones and fluoroquinolones, whereas they are already known to carry resistance to β-lactams, aminoglycosides, chloramphenicol, tetracycline, rifampin, sulfonamides, and disinfectants. pQR1 (*4*) or p1 (*6*) are examples of well-characterized plasmids that mediate multidrug resistance by carrying bla_{VEB-1} and *qnrA1*, together with aminoglycoside resistance genes *aadB*, *aacA1*, and *aadA1*, chloramphenicol resistance gene *cmlA*, rifampin resistance gene *arr2*, disinfectant resistance gene *qacI*, and sulfonamides resistance gene *sul1*.

Our study showed that the IncA/C_2-type plasmids may be the source of such worldwide dissemination. It means that 1 plasmid scaffold has brought the same (or at least very similar) multidrug resistance to multiple enterobacterial species in different continents.

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Viral Load and Crimean-Congo Hemorrhagic Fever

To the Editor: Crimean-Congo hemorrhagic fever (CCHF) is a severe viral disease transmitted to humans by tick bite or contact with blood, excreta, or tissues of infected patients or livestock. The disease is endemic in many African, Asian, and European countries. Sporadic cases or outbreaks have been observed in the Balkan Peninsula (*1*–*5*). Prompt diagnosis of the disease is essential for preventing human-to-human transmission. Reverse transcription–PCR (RT-PCR) is the detection method of choice in first days of illness and in severe cases with no antibody production. In recent years, real-time RT-PCR approaches have been described for detection and quantification of CCHF virus (*6*–*8*). However, no information is available on viral RNA concentration in patients. We describe a real-time RT-PCR for detection and quantification of CCHF virus, present the results of its use with clinical samples, and report the relationship between viral load and severity and outcome of CCHF.

We tested 29 serum samples from Albanian patients with suspected CCHF or their contacts who were living in a CCHF-endemic area of Albania. Serum samples were collected during 2003–2006 and categorized into 3 groups. Group A contained samples from 11 patients with CCHF confirmed by a conventional RT-nested PCR (*9*). Group B contained samples from 5 patients who had negative RT-nested PCR results and positive serologic results. Group C contained samples from 15 persons who were from the same region as the CCHF patients but who did not have any clinical symptoms of CCHF and had negative PCR or serologic results.

One set of primers and 1 probe were designed to amplify an 84-bp genome region of the S RNA segment of CCHF virus on the basis of European sequences (Balkan and Russian strains available in GenBank): primers CCEuS 5′-TGACAGCATTTCTTTAACAGACATCA-3′ and CCEuAs 5′-AAACACGGCAGCCTTAAGCA-3′, and probe 5′-TCGCCAGGGACTTTATATTCTGCAAGG-3′. A 25-μL reaction was conducted in a LightCycler (Roche, Indianapolis, IN, USA) with 10 mmol/L of each deoxynucleotide triphosphate, 600 nmol/L of each primer, 200 nmol/L of probe, and 3 μL of RNA. Cycling conditions were 50°C for 30 min and 95°C for 15 min, followed by 45 cycles at 95°C for 15 s and 58°C for 30 s. A quantification curve was constructed with 10-fold serial dilutions of in vitro–transcribed CCHF virus RNA. Positive results were obtained up to a dilution of 10^{-12}, which corresponds to ≈45 virus genome equivalents (geqs) per reaction.

Twelve samples had positive results: all 11 samples in group A and 1 in group B (Table). Results for the remaining samples in groups B and C were negative. Levels of tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), IL-10, and a 60-kDa soluble receptor of TNF were previously measured in most of the samples in this study (*10*), and their values are shown in the Table.

Viral loads ranged from 14×10^6 to 28.99×10^6 geqs/reaction. The highest level was observed in the patient who died (23/03). High loads were observed in all primary case-patients (23/03, 82/03, 178/04, 252/06) except for patient 154/04, from whom a sample was obtained 18 days after onset of disease. All primary case-patients had severe disease with high fever and clinically apparent hemorrhage. All secondary case-patients, except patient 34/03, were contacts of the patient who died (24b/03, husband; 25b/03, brother-in-law; 50/03 and 52/03, cousins; 56/03, sister-in-law; 40/03, son of sister) and had symptoms of disease ≈1 week after the death of patient 23/03.

Viral load of secondary case-patients was <250 geqs/reaction, which was much lower than that of primary case-patients. This finding suggests that the disease is more severe in primary case-patients and becomes a milder form in secondary case-patients. Samples of secondary case-patients 24b/03 and 25b/03 were obtained on day 9 of illness, and patient 24b/03 had a 4× higher viral load than patient 25b/03. A possible explanation might be that because patient 24b/03 had closer contact with the person who died, he received a higher dose of virus, which might affect severity of the disease. Other secondary case-patients had milder symptoms with no clinically apparent hemorrhage and were not hospitalized. All hospitalized patients had leukopenia, except for the patient whose sample was taken 18 days after the onset of disease. No correlation was observed between viral load and cytokine levels or platelet counts, which suggests that other factors are involved in pathogenicity and immune response.