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qnrA in CTX-M-Producing *Escherichia coli* Isolates from France▽

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By PCR, we screened for *qnr* genes 112 clinical isolates of extended-spectrum β-lactamase-producing *Escherichia coli* collected from hospitals in France during 2004. For the first time, 7.7% of CTX-M-producing *E. coli* isolates presented a plasmid-mediated resistance to quinolones. All strains harbored a *qnrA* gene located on a *sul1*-type class 1 integron with similar structure to the In*36* integron.

Recently, several studies revealed that CTX-M-type enzymes are becoming the most prevalent extended-spectrum β-lactamases (ESBL) in *Escherichia coli* isolates in different geographic areas (5, 29, 32, 35, 36, 38). Localized outbreaks of CTX-M-producing *E. coli* infection have been reported in the north of France (12, 21, 22). However, the link between the increase in ESBL-producing *E coli* prevalence and the emergence of CTX-M enzyme is not yet established. Moreover ESBL-producing strains generally displayed a high level of resistance to several other non-β-lactam antibiotic families, in particular, to quinolones. A plasmid-mediated quinolone resistance based on the Qnr production has been successively identified in the United States, in China, and recently in France. Interestingly, the *qnr*-containing plasmid was frequently associated with plasmid-mediated ESBLs (7, 16, 26, 31), but no search was focused on Qnr determinant detection in CTX-M-producing *E. coli* strains.

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To investigate the relationship between CTX-M-producing *E. coli* and *qnr* genes, we undertook a prospective study of ESBL-producing *E. coli* isolates from 1 January 2004 until 31 December 2004 in three university hospitals (Clermont-Ferrand, Montpellier, and Nîmes) and one community hospital (Perpignan) in the south and center of France. Only one ESBL-producing isolate per sampling type and patient was included in the study. Patients were deemed to have community disease if the first culture found positive for ESBL-producing *E. coli* was obtained within 48 h of admission. The genus and species were determined biochemically either with the Vitek 2-ID-GNB identification card or the API 20E system (bioMérieux, Marcy-l'Etoile, France). Susceptibility to antimicrobial agents was tested by using the agar disk diffusion assay on Mueller-Hinton agar. Strains were classified as susceptible, intermediate resistant, or resistant to the antibiotics tested according to the recommendations of the Antibiotic Susceptibility Testing Committee of the French Society for Microbiology (40). ESBL production was confirmed by the double-disk synergy test using not only ceftazidime and cefotaxime but also cefpodoxime disks (39). Isoelectric focusing was performed with polyacrylamide gels as previously described (10). The genes bla_{TEM}, bla_{SHV}, and $bla_{\text{CTX-M}}$ were detected by PCR using specific primers as previously reported (3, 10, 11, 24) and further identified by sequencing the PCR products. A macrorestriction analysis of chromosomal DNA was performed using pulsed-field gel electrophoresis (PFGE) according to a previous published procedure after XbaI restriction (New England Biolabs, Inc.) by using the contour-clamped homogeneous electric field system (Bio-Rad SA, Ivry-sur-Seine, France) (20). The PFGE patterns were analyzed with Gel compar computer software (Applied Math, Kortrijk, Belgium) and an unweighted-pair group method with the Dice coefficient of similarity. Isolates were considered to be within a cluster if the coefficient of similarity was >80% (42). Phylogenetic grouping of the *E. coli* isolates was determined by a PCR-based method developed by Clermont et al. (8). The fingerprinting analysis of ESBL-carrying plasmid DNA was performed with the clinical isolates and their electroporants, after digestion with HindIII endonuclease (New England Biolabs, Inc.) and electrophoresis on 0.8% (wt/vol) agarose gels at 100 V for 2 h. The *qnrA*, *qnrB*, and *qnrS* genes were screened by PCR as previously described (15, 16, 34, 43, 44) in all quinolone-resistant strains and electroporants. For PCR mapping of the integrons that contained the $bla_{\text{CTX-M}}$ and the *qnr* genes, PCR primers were used in combination and studied by sequencing the PCR products (26). A search for additional chromosome-encoded quinolone resistance determinants (*gyrA*, *gyrB*, *parC*, and *parE* genes) was

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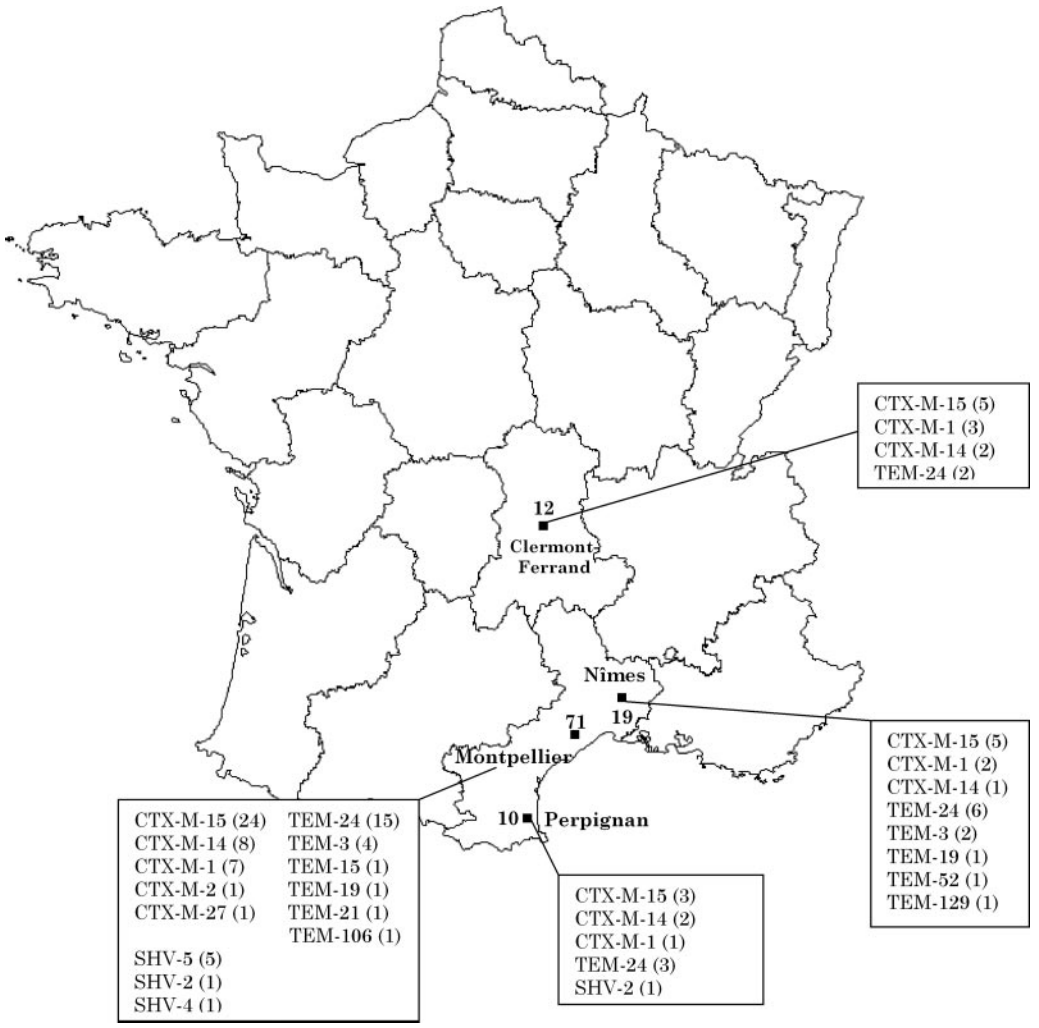

FIG. 1. Distribution of ESBL-producing *Escherichia coli* strains found in four hospitals in France.

performed by PCR (26). PCR products were sequenced to detect mutations.

During the period studied, 112 ESBL-producing *E. coli* strains were isolated from 111 patients. The prevalence of the ESBL production in the *E. coli* isolates was <3% in the four centers. The distribution of the different types of ESBL according to the geographical origin of the corresponding strains is shown in Fig. 1. *E. coli* produced mainly CTX-M-ESBLs (58.0%), followed by TEM-type (34.8%) and SHV-type (7.2%) β-lactamases. CTX-M-15 was the most prevalent ESBL in our study (57.0% of CTX-M β-lactamases). We observed that 33.8% of the CTX-M-producing strains were isolated in outpatients compared to strains producing other ESBLs (6.4%), as previously noted by other authors (1, 30, 32, 35, 37, 45). PFGE revealed three independent clonal propagations of CTX-M-15-producing isolates in hospitals in Montpellier (19 isolates), Nîmes (4 isolates), and Perpignan (2 isolates) (Fig. 2). Twelve of these strains were shown to be of community origin, and seven patients infected by these strains had never been hospitalized. The clonal strains belonged to the B2 phylotyping group. No epidemiological link between patients could be demonstrated. The study on the CTX-M-15-producing plasmids revealed that all isolates had one or more plasmids. Electroporation of plasmid DNA from the clinical strains into *E. coli* DH5α successfully transferred the ESBL phenotype. Analysis of the resulting electroporants revealed the presence of large plasmids (>80 kb) (data not shown) and the same ESBL resistance pattern. Plasmids corresponding to the clonal CTX-M-15-producing *E. coli* strains yielded similar restriction patterns after digestion with HindIII, whereas those corresponding to the unrelated CTX-M-15-producing *E. coli* strains harbored different restriction patterns (data not shown).

In the selected ESBL-producing *E. coli* strains, 64.3% of the isolates were resistant to nalidixic acid and 56.3% were resistant to ciprofloxacin. The comparison of susceptibility profiles observed for CTX-M- and other ESBL-producing strains indicates that CTX-M-producing strains had more associated resistances than the other ESBL-producing strains. A total of 73.8% of CTX-M-producing *E. coli* strains were resistant to quinolones versus 51.1% of TEM- and SHV-producing *E. coli* strains ($P < 0.01$). Similarly, 66.2% of CTX-M-producing isolates were resistant to ciprofloxacin versus 42.6% for TEM-

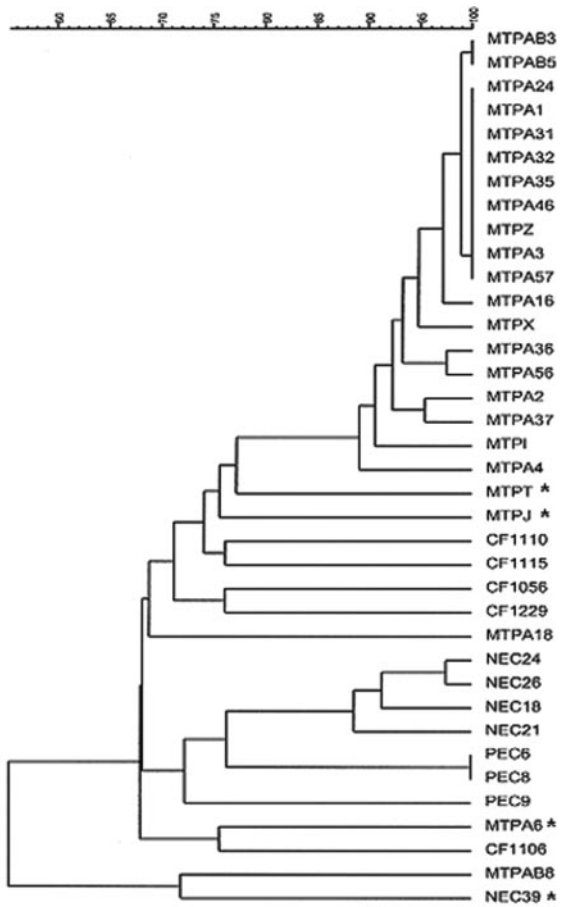

FIG. 2. Dendrogram of XbaI-digested genomic DNAs from all of the CTX-M-15-producing *E. coli* strains isolated in four hospitals in France. MTP, Montpellier; CF, Clermont-Ferrand; NEC, Nîmes; and PEC, Perpignan. Strains were clustered by the unweighted-pair group method using arithmetic averages (UPGMA). The scale indicates the percentage of genetic similarity. Max. tol., percent maximum tolerance of the curve that matches the bands; Min. surf., percent minimum surface area of a band. The four strains harboring the *qnrA* gene are noted with asterisks.

and SHV-producing isolates ($P < 0.01$). With the aim of investigating an association between CTX-M production and resistance to quinolones, we screened for *qnr* genes and transfer of quinolone resistance. We detected the *qnrA* gene in 7.7% of CTX-M-producing *E. coli* strains, including four epidemiologically unrelated isolates (MECJ, MECT, MECA6, and NEC39) producing CTX-M-15 and strain NEC34 producing CTX-M-1. No TEM- or SHV-producing strains had *qnr* genes. In these five *qnr*-positive strains, MICs of nalidixic acid and ciprofloxacin varied between 64 to 256 μg/ml and 4 to 16 μg/ml, respectively. Except for strain NEC39, the resistance to nalidixic acid was cotransferred with the ESBL-type resistance phenotype for the *qnrA*-containing strains and the *qnrA* gene was detected by PCR in the transformants (Table 1). The clinical strains and electroporants harbored a similar *qnrA* gene to that originally identified in a *Klebsiella pneumoniae* isolate in United States (43) with a single functionally silent nucleotide change, CTA→CTG, at position 537. The *qnrA* gene was located on a *sul1*-type class 1 integron. The structures of the integrons were identical in the five strains and were homologous to the In*36* integron (*orf513 qnr ampR qacEΔ1 sul1*) identified in *E. coli* isolates from Shanghai (44). The detection of an associated chromosomal quinolone resistance revealed the presence of GyrA in all five strains, with a mutation at codon 83 (Ser→Tyr) and a *parC* mutation in one strain (NEC39) at codon 80 (Ser→Ile).

The aim of this study was to establish the dissemination of and a link between ESBL-producing *E. coli* isolates and quinolone plasmid resistance. The selected strains represented an actual evolution of *E. coli* in most parts of the world: increasing prevalence of ESBL-producing *E. coli*, emergence and diffusion of CTX-M-producing strains (4, 6, 9, 14, 18, 27–29, 38, 41), diffusion of CTX-M-15 (2, 5, 13, 17, 19, 20, 22, 23, 27, 29), and community outbreak of clonally CTX-M isolates (32). The strains were particularly interesting because the majority of CTX-M-producing strains displayed a high level of resistance to quinolones. This high level of resistance associated with CTX-M production was previously described (14), but the actual causes of this association remain not well known. Recently, a plasmid-mediated quinolone resistance determinant named Qnr has been described as leading to a low level of resistance to quinolones (43). Different studies throughout the world showed that the Qnr determinant occurred in between 0.3 and 48% of the strains (15, 26, 33, 43, 44). We found a high rate of *qnrA* genes (7.7%) associated with CTX-M-type ESBL, in comparison to 0.3% found in a first French study (26). However, we found that four out five strains transferred the two resistances in electroporant strains, suggesting colocalization of *qnrA* and $bla_{\text{CTX-M}}$ genes on the same plasmid. The majority of *qnr*-positive strains were associated with multidrug

TABLE 1. Characteristics of *qnr*-positive strains[a]

Strain	MIC (mg/liter)				Mutation(s) in topoisomerase genes	Associated ESBL	Phylotyping group
	Clinical strain		Electroporant				
	Nalidixic acid	Ciprofloxacin	Nalidixic acid	Ciprofloxacin			
MECJ	>256	4	8	0.25	*gyrA* (Ser83→Tyr)	CTX-M-15	A
MECT	>256	8	8	0.125	*gyrA* (Ser83→Tyr)	CTX-M-15	D_2
MECA6	64	8	4	0.125	*gyrA* (Ser83→Tyr)	CTX-M-15	D_1
NEC39	>256	16			*gyrA* (Ser83→Tyr) *parC* (Ser80→Ile)	CTX-M-15	D_1
NEC34	>256	16	8	0.5	*gyrA* (Ser83→Tyr)	CTX-M-1	A

[a] The *qnrA* gene (mutation position 537) was found in each strain shown, and the integron structures were homologous to In*36*. The accession number for the *qnrA* gene and In*36* is AY259085 (44). The PFGE results showed that each strain was nonclonal.

resistance and ESBL- or cephalosporinase-producing strains (15, 43). Interestingly, we have described how in the five strains, the *qnrA* gene is embedded in a complex *sul1*-type class 1 integron known to integrate numerous multidrug resistance genes as previously suggested (44). The integrons are frequent in strains of *Enterobacteriaceae* and in strains with multidrug resistance (25). This genetic support induces the transfer between bacteria of the same plasmid or integron and then the transmission and dissemination of these strains. The origin of the plasmid supporting the Qnr region has been detected in a waterborne species, *Shewanella algae* (31). Our study suggests that the plasmid-mediated Qnr-based mechanism of quinolone resistance could be emerging in France in CTX-M-producing strains. To date, no genetic link has been found between the two emerging mechanisms of resistance. However, recently *qnrB* has been detected on plasmids also encoding CTX-M-15 in *Klebsiella pneumoniae* isolates in India (16). Interestingly, an associated chromosomal quinolone resistance was detected in all our strains. This observation explains the higher level of resistance to quinolones in our isolates compared with those observed for *qnr*-positive transconjugants (Table 1).

In conclusion, CTX-M β-lactamases were associated with quinolone/fluoroquinolone resistance, and in some cases, this association was linked to the Qnr determinant. The frequency of CTX-M strains in weakened patients and their community character invite examination of the epidemiological evolution of these strains and the necessity to inform the medical profession of these results.

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