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reported in China [5]. Both scenarios could lead to the rapid spread, to and among animals, of MRSA strains that are capable of causing severe skin and soft tissue infection in healthy human populations.

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Transparency Declaration

None of the authors has any associations that could pose a possible conflict of interest.

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Prevalence of plasmid-mediated quinolone resistance determinants in *Enterobacteriaceae* from Tunisia

S. Dahmen[1,2], L. Poirel[1], W. Mansour[2], O. Bouallègue[2] and P. Nordmann[1]

1) *Service De Bactériologie-Virologie, INSERM U914 'Emerging Resistance to Antibiotics', Hôpital de Bicêtre, Assistance Publique/Hôpitaux de Paris, Faculté de Médecine et Université Paris-Sud, France and* 2) *Unité des Infections à Bactéries Multirésistantes aux Antibiotiques, UR/29/04, Laboratoire de Microbiologie, CHU Sahloul, Sousse, Tunisia*

Abstract

The spread of plasmid-mediated quinolone resistance determinants (*qnr*-like determinants, *aac(6')-Ib-cr* and *qepA* genes) was evaluated in a collection of 281 nalidixic acid-resistant enterobacterial isolates recovered between September 2005 and December 2007 at the Sahloul Hospital, Sousse, Tunisia. Sixteen percent

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of those isolates carried *qnr* genes encoding the QnrB1, QnrB2, QnrA6 or QnrS1 determinants. Most *qnr*-positive isolates were extended-spectrum ß-lactamase (ESBL) producers, being predominantly of the CTX-M-15 type, but also of the SHV-28 and SHV-12 types. The *qnr* genes were located on plasmids with a size in the range 55–150 kb. The *qnrB2* gene was associated with *sul1*-type integron structures and the *qnrB1* gene was associated with *orf1005*, whereas the genetic environment of *qnrA6* was unknown. In two isolates, the *qnrS1* gene was located downstream of an IS*Ecl2* element on plasmids that often carried the narrow-spectrum ß-lactamase gene bla_{LAP-2}; *qepA* and *aac(6')-Ib-cr* were not detected. The present study highlights the wide spread of Qnr-like determinants in Tunisia, with an association with the ESBL CTX-M-15 in human clinical isolates.

Keywords: Extended-spectrum ß-lactamase, plasmid, *qnr*, quinolone, Tunisia

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Corresponding author and reprint requests: L. Poirel, Service de Bactériologie-Virologie, Hôpital de Bicêtre, 78 rue du Général Leclerc, 94275 Le Kremlin-Bicêtre cedex, France
E-mail: laurent.poirel@bct.aphp.fr

Recently, three transferable quinolone resistance mechanisms have been described: (i) a target-protective mechanism encoded by the *qnr* genes [1]; (ii) a dual antibiotic-modifying enzyme that acetylates aminoglycosides, but also ciprofloxacin and norfloxacin encoded by the *aac(6')-Ib-cr* gene [2]; and (iii) an efflux pump encoded by the *qepA* gene [3]. The *qnr* genes encode pentapeptide repeat proteins that protect the DNA gyrase and topoisomerases IV from quinolone binding, thereby reducing the susceptibility to nalidixic acid and fluoroquinolones [4]. Five types of Qnr determinants have been described in *Enterobacteriaceae*, which are encoded by the *qnrA*, *qnrB*, *qnrC*, *qnrD* and *qnrS* genes [5–8]. The *qnrA* genes are usually located on complex *sul1*-type class 1 integrons [9]. The *qnrB* genes are either associated with *sul1*-type integron structures, a specific *orf1005* or the insertion sequence IS*Ecp1* [10,11]. The *qnrS1* gene has always been identified in association with IS*Ecl2*, whereas *qnrS2* has been identified in very peculiar mobile insertion cassette structures [12,13].

In the present study, we investigated the prevalence of plasmid-mediated quinolone resistance determinants among a collection of extended-spectrum ß-lactamase (ESBL)- and non-ESBL-producing enterobacterial isolates, which are all resistant to nalidixic acid, and which were isolated at Sahloul Hospital, Sousse, Tunisia, from September 2005 to December 2007. A total of 1705 nonrepetitive enterobacterial isolates were recovered during the study period. Among them, 580 (34%) isolates were resistant to nalidixic acid, of which 281 (approximately half of that collection) were available and retained for study, and among which 196 (70%) were producing an ESBL. The species distribution comprised: *Klebsiella pneumoniae* (*n* = 130), *Escherichia coli* (*n* = 85), *Enterobacter cloacae* (*n* = 36), *Morganella morganii* (*n* = 10), *Citrobacter freundii* (*n* = 8), *Proteus mirabilis* (*n* = 6), *Klebsiella oxytoca* (*n* = 5) and *Providencia stuartii* (*n* = 3). The *qnrA*, *qnrB* and *qnrS* genes (the *qnrC* and *qnrD* genes were not known at the time of the study) were screened for by a multiplex PCR-based technique as described previously [14]. The search for the *qepA* gene was performed using primers QepA-F (5′-CGTGTTGCTGGAGTTCTTC-3′) and QepA-R (5′-CTG CAGGTACTGCGTCATG-3′), amplifying a 403-bp fragment. The presence of the *ac(6')-Ib* gene was detected only among the *qnr*-positive isolates by using primers AAC6′-A (5′-TTGCGATGTCTATGAGTGGTA-3′) and AAC6′-B (5′-CTCGAATGCCTGGCGTGTTT-3′) to produce a 482-bp product [13]. The corresponding amplicons were subsequently sequenced to identify the *aac(6')-Ib-cr* variant. The bla_{TEM}, bla_{SHV} and bla_{CTX-M} genes were searched for by PCR among all *qnr*-positive isolates using specific primers, as previously reported [9,15], and were further identified by sequencing. Among 281 nalidixic acid-resistant isolates, 45 were found to be *qnr*-positive (16%). Seven out of these 45 isolates (15%) were *qnrS*-positive, 12 (27%) were *qnrA*-positive and 26 (58%) were *qnrB*-positive. Sequencing of the two former led to the identification of only *qnrS1* and *qnrA6* alleles. Among the 26 *qnrB*-positive isolates, 15 were *qnrB1* and 11 were *qnrB2*. The *qnrB* gene was more prevalent among *E. cloacae* isolates, whereas *qnrA* was more prevalent among *K. pneumoniae* isolates. Overall, the *qnrB* gene was the most prevalent compared to the *qnrA* and *qnrS* genes. This result agrees with the most recent studies indicating that QnrA, although representing the Qnr determinant identified initially, is not the most prevalent Qnr determinant worldwide [5,14,15]. This is also in accordance with the only published study of the Qnr prevalence in the Maghreb, which reported QnrB1, QnrB4 and QnrS1 in *E. cloacae* from Algeria [16].

TABLE 1. Features of the QnrA-, QnrB- and QnrS-positive enterobacterial isolates

Isolate	Date of isolation (mm/dd/yy)	Specimen	Hospitalization unit	Qnr Determinant/ ß-lactamase(s)	Plasmid size (kb)[a]	Associated non ß-lactam antibiotic resistance markers[b]
Klebsiella pneumoniae 13	05/20/06	Blood	ICU	QnrA6 SHV-28	120	GEN AMK NET TOB KAN TMP RIF CHL TET
Klebsiella pneumoniae 59	06/25/06	Urine	Urology	QnrA6 SHV-28, TEM-1	120	GEN AMK NET TOB KAN TMP RIF TET
Klebsiella pneumoniae 3	07/04/06	Urine	ICU	QnrA 6 CTX-M-15	120	GEN AMK NET TOB KAN TMP RIF CHL
Klebsiella pneumoniae 9	02/12/07	Urine	ICU	QnrA6 CTX-M-15, TEM-1	120	TOB KAN TET
Klebsiella pneumoniae 21	06/19/07	Urine	ICU	QnrA6 SHV-28	120	GEN AMK NET TOB KAN TMP RIF CHL
Klebsiella pneumoniae 36	11/17/07	Blood	Urology	QnrA6 SHV-28, TEM-1	120	GEN AMK NET TOB KAN TMP RIF CHL
Citrobacter freundii 2	06/02/06	Urine	ICU	QnrA6	120	GEN AMK TOB KAN TMP RIF CHL TET
Proteus mirabilis 4	10/13/06	Urine	Urology	QnrA6	55	GEN AMK NET TOB KAN TMP RIF CHL TET
Proteus mirabilis 1	01/16/07	Blood	ICU	QnrA6 TEM-1	120	AMK NET TOB KAN TMP RIF CHL TET
Proteus mirabilis 3	09/22/07	Blood	ICU	QnrA6	120	GEN AMK NET TOB KAN TMP RIF CHLTET
Providencia stuartii 3	09/12/06	Blood	ICU	QnrA6	120	GEN AMK NET TOB KAN TMP RIF CHL TET
Providencia stuartii 2	09/27/06	Blood	ICU	QnrA6	55	NET TOB KAN TMP RIF CHL TET
Klebsiella pneumoniae 39	07/27/06	Blood	ICU	QnrB1 CTX-M-15, SHV-28, TEM-1	125	GEN AMK NET TOB KAN TMP RIF TET
Klebsiella pneumoniae 4	09/02/06	Urine	Urology	QnrB1	60	AMK TMP RIF CHL
Escherichia coli 17	02/06/06	Urine	Urology	QnrB1 CTX-M-15, TEM-1	66	GEN NET TOB KAN TMP RIF CHL TET
Escherichia coli 11	07/07/06	Urine	Paediatric	QnrB1 CTX-M-15, TEM-1	80	GEN TOB KAN TMP RIF CHL TET
Escherichia coli 6	07/27/06	Urine	Paediatric	QnrB1 CTX-M-15, TEM-1	80	NET TOB KAN TMP RIF CHL TET
Escherichia coli 2	08/07/06	Urine	Paediatric	QnrB1 CTX-M-15, TEM-1	80	GEN TOB KAN TMP CHL TET
Enterobacter cloacae 31 (PII)	12/29/05	Urine	Urology	QnrB1 CTX-M-15, TEM-1	150	GEN TOB KAN TMP RIF CHL TET
Enterobacter cloacae 33 (PI)	05/02/06	Urine	ICU	QnrB1 CTX-M-15	120	GEN AMK NET TOB KAN TMP RIF CHL TET
Enterobacter cloacae 10 (PI)	10/09/06	Urine	Urology	QnrB1 CTX-M-15, TEM-1	60	GEN TOB KAN NET TMP CHL TET RIF
Enterobacter cloacae 8 (PI)	01/27/07	Urine	Urology	QnrB1 CTX-M-15, TEM-1	60	GEN TOB KAN NET TMP CHL TET RIF
Enterobacter cloacae 6 (PI)	01/29/07	Blood	Urology	QnrB1 CTX-M-15, TEM-1	60	GEN TOB KAN NET TMP CHL TET RIF
Enterobacter cloacae 2 (PI)	05/21/07	Urine	ICU	QnrB1 CTX-M-15, TEM-1	60	GEN TOB KAN NET TMP CHL TET RIF
Enterobacter cloacae 14 (PI)	09/21/07	Blood	ICU	QnrB1 CTX-M-15, TEM-1	60	GEN TOB KAN NET TMP CHL TET RIF
Enterobacter cloacae 13 (PI)	10/08/07	Blood	ICU	QnrB1 CTX-M-15, TEM-1	60	GEN TOB KAN TMP RIF CHL TET
Citrobacter freundii 1	10/07/05	Urine	Urology	QnrB1	100	GEN NET TOB KAN TMP RIF CHL TET
Klebsiella pneumoniae 7	05/31/07	Blood	ICU	QnrB2 CTXM-15, SHV-28, TEM-1	120	GEN NET TOB KAN TMP RIF L TET
Klebsiella oxytoca 1	04/08/06	Urine	Urology	QnrB2	100	GEN NET TOB KAN TMP RIF L TET
Escherichia coli 5	01/16/06	Urine	Urology	QnrB2 TEM-1	60	KAN TMP RIF TET NET
Escherichia coli 1	02/06/06	Urine	Urology	QnrB2 CTX-M-15, TEM-1	120	GEN TOB KAN TMP RIF CHL TET
Escherichia coli 3	03/22/07	Urine	Urology	QnrB2	55	GEN AMK NET TOB KAN RIF CHL TET
Enterobacter cloacae 9 (PII)	12/03/05	Urine	Urology	QnrB2 SHV-12, TEM-1	150	GEN TOB KAN TMP RIF CHL TET
Enterobacter cloacae 1 (PIII)	07/07/06	Urine	Paediatric	QnrB2 SHV-12 TEM-1	150	GEN TOB KAN TMP RIF
Enterobacter cloacae 11 (PI)	04/12/07	Blood	Paediatric	QnrB2 CTX-M-15, TEM-1	65	GEN NET TOB KAN TMP RIF CHL TET
Enterobacter cloacae 15 (PI)	11/08/07	Blood	ICU	QnrB2 CTX-M-15, TEM-1	65	GEN NET TOB KAN TMP RIF C TET
Citrobacter freundii 4	12/17/05	Urine	Urology	QnrB2 CTX-M-15, TEM-1	120	TOB KAN TMP RIF TET
Citrobacter freundii 3	12/29/06	Urine	ICU	QnrB2 SHV-2a, TEM-1	150	GEN NET TOB KAN TMP RIF CHL TET
Klebsiella pneumoniae 1	12/20/06	Blood	Paediatric	QnrS1 LAP-2	100	NET RIF CHL TET
Klebsiella pneumoniae 6	05/07/07	Urine	Urology	QnrS1 TEM-1	100	TMP RIF
Escherichia coli 38	10/30/05	Urine	Urology	QnrS1 CTX-M-15, TEM-1	80	GEN NET TOB KAN TMP RIF
Escherichia coli 39	01/09/06	Urine	Urology	QnrS1 CTX-M-15, TEM-1	80	TOB KAN TET
Enterobacter cloacae 19	05/22/06	Blood	Urology	QnrS1 CTX-M-15, LAP-2	120	TMP RIF TET
Enterobacter cloacae 4	11/01/06	Blood	ICU	QnrS1 CTX-M-15, TEM-1	80	GEN AMK NET TOB KAN TMP RIF
Enterobacter cloacae 22	11/14/07	Urine	Urology	QnrS1 CTX-M-15, LAP-2	120	NET RIF TET

Sizes of the plasmids harbouring the *qnrA6*, *qnrB1*, *qnrB2* or *qnrS1* genes and associated non ß-lactam antibiotic resistance markers.

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Twenty-eight *qnr*-positive, ESBL-producing isolates were detected, further underlining the frequent association between *qnr* and ESBL genes, as previously demonstrated [8,17]. The CTX-M-15 determinant was identified in 19 *qnr*-positive isolates, whereas six isolates produced SHV-28, two produced SHV-12 and one produced SHV-2a. The gene encoding the narrow-spectrum β-lactamase TEM-1 was detected in 64% of the *qnr*-positive isolates. The clinical features of the *qnr*-positive isolates and their distribution among species are indicated in Table 1. All *qnr*-positive isolates harboured the *aac(6′)-Ib* gene, but sequencing did not reveal the *aac(6′)-Ib-cr* gene. The search for *qepA* remained negative for all isolates.

Clonal relatedness among the *qnr*-positive isolates was evaluated by Rep-PCR for *E. coli* and ERIC-2 for non *E. coli* isolates, as described previously [18]. Many isolates exhibited different DNA patterns, indicating that they were not clonally related. However, *E. cloacae* isolates carrying *qnrB* genes showed three major patterns, namely PI (nine isolates), PII (two isolates) and PIII (one isolate). In addition, the six *K. pneumoniae* isolates harbouring the *qnrA6* gene were clonally related (data not shown). These results demonstrated that the occurrence of *qnr* genes was related to the dissemination, at the same time, of some specific isolates and of clonally unrelated isolates.

Electrophoresis of the plasmid extract, followed by Southern hybridization, as described previously [9] showed that the *qnr* genes were located on plasmids with sizes in the range of 55–150 kb. All *qnrA6*-positive plasmids identified among the clonally related *K. pneumoniae* isolates, but also among the other *qnrA6*-positive enterobacterial isolates were 120 kb in size, suggesting the diffusion of a single plasmid. The clonally related *qnrB1*-positive *E. cloacae* isolates exhibiting pattern PI carried a 60-kb plasmid and expressed the ESBL CTX-M-15 together with β-lactamase TEM-1, with the exception of *E. cloacae* 33, which carried a 120-kb *qnrB1*-positive plasmid encoding CTX-M-15 only. The *qnrS1*-carrying plasmids differed in size, in the range of 80–120 kb. The corresponding isolates all produced the ESBL CTX-M-15, whereas some produced additionally the narrow-spectrum ß-lactamase LAP-2 [19].

Genetic structures surrounding the *qnrA*, *qnrB* and *qnrS* genes were detected by PCR mapping using different primer sets, as described previously [9,12]. The *qnrB2* gene was systematically associated with the IS*CR1* element embedded in a *sul1*-type integron. The *qnrB1* gene was always found downstream of *orf1005*, as described previously [10]. The IS*CR1* element was not identified in the *qnrA6* vicinity, although it is usually identified in association with *qnrA*. The bla_{LAP-2} gene was identified upstream of *qnrS1* in one *K. pneumoniae* (isolate 1) and in two *E. cloacae* (isolates 19 and 22), with both genes being separated by the insertion sequence IS*Ecl2* [20]. PCR mapping revealed that IS*Ecl2* and bla_{LAP-2} were absent upstream of *qnrS1* in *K. pneumoniae* 1, *K. pneumoniae* 6, *E. coli* 38, *E. coli* 39, and *E. cloacae* 4 isolates.

The present study revealed quite a high prevalence of plasmid-borne quinolone resistance determinants among nalidixic acid-resistant enterobacterial isolates recovered in a Tunisian hospital. It allowed us to conclude that small-scale outbreaks had occurred in this hospital and it is the first study to focus on the epidemiology of plasmid-borne quinolone resistance determinants among clinical isolates in Tunisia.

Finally, the present study further indicates that the most prevalent ESBL in this region is CTX-M-15, as is the case in many parts of the world.

Transparency Declaration

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Highly structured genetic diversity of the *Mycobacterium tuberculosis* population in Djibouti

S. Godreuil[1,2], F. Renaud[2], M. Choisy[2], J. J. Depina[3,4], E. Garnotel[3,4], M. Morillon[3], P. Van de Perre[1] and A. L. Bañuls[2]

1) *Université Montpellier 1, EA 4205 'Transmission, Pathogenèse et Prévention de l'Infection par le VIH', and CHU Montpellier, Laboratoire de Bactériologie-Virologie Arnaud de Villeneuve, Montpellier,* 2) *GEMI, UMR CNRS-IRD 2724, Centre IRD de Montpellier,* 3) *Laboratoire de Biologie Médicale, Service de Biologie, HIA Laveran, Marseille, France and* 4) *Laboratoire de Biologie, Hôpital Paul Faure, Djibouti Ville, Djibouti*

Abstract

Djibouti is an East African country with a high tuberculosis incidence. This study was conducted over a 2-month period in Djibouti, during which 62 consecutive patients with pulmonary tuberculosis (TB) were included. Genetic characterization of *Mycobacterium tuberculosis*, using mycobacterial interspersed repetitive-unit variable-number tandem-repeat typing and spoligotyping, was performed. The genetic and phylogenetic analysis revealed only three major families (Central Asian, East African Indian and T). The high diversity and linkage disequilibrium within each family suggest a long period of clonal evolution. A Bayesian approach shows that the phylogenetic structure observed in our sample of 62 isolates is very likely to be representative of the phylogenetic structure of the *M. tuberculosis* population in the total number of TB cases.

Keywords: Djibouti, genetic diversity, *Mycobacterium tuberculosis*, population structure, spoligotyping/MIRU-VNTR

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Corresponding author and reprint requests: S. Godreuil, CHU Montpellier, Laboratoire de Bactériologie-Virologie Arnaud de Villeneuve, 371 Avenue du Doyen Gaston Giraud, Montpellier, France
E-mail: godreuil@yahoo.fr

Djibouti is an East African country with a total population of over 819 000. In 2004, the estimated tuberculosis (TB) incidence was 951 cases per 100 000 inhabitants, which is one of the highest incidences in the world [1]. The objectives of this study were to identify the *Mycobacterium tuberculosis* families responsible for the TB cases, and to analyse their genetic diversity and the structure of the *M. tuberculosis* population in an area with this high TB incidence.

The study was conducted over a 2-month period at Paul Faure Hospital in Djibouti City. During this period, 62 consecutive patients with symptomatic disease and sputum culture positive for *M. tuberculosis* complex were included. Spoligotyping [2] and mycobacterial interspersed repetitive-unit variable-number tandem-repeat (MIRU-VNTR) typing [3] was performed with DNA from each isolate. To study the genetic variability, a set of diversity indices, including genotypic diversity and mean genetic diversity (*H*), was evaluated using F-STAT version 2.9.3 [4]. The population structure was

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