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Differential Phenotypic and Genotypic Characteristics of *qnrS1*-Harboring Plasmids Carried by Hospital and Community Commensal Enterobacteria▽†

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The *qnrS1* gene induces reduced susceptibility to fluoroquinolones in enterobacteria. We investigated the structure, antimicrobial susceptibility phenotype, and antimicrobial resistance gene characteristics of *qnrS1* plasmids from hospitalized patients and community controls in southern Vietnam. We found that the antimicrobial susceptibilities, resistance gene characteristics, and plasmid structures of *qnrS1* plasmids from the hospital differed from those from the community. Our data imply that the characteristics of the two plasmid groups are indicative of distinct selective pressures in the differing environments.

Quinolone resistance and reduced susceptibility to fluoroquinolones in members of the *Enterobacteriaceae* are related to mutations in the DNA gyrase and topoisomerase genes (2, 22). However, resistance can be increased by the activity of plasmid-mediated quinolone resistance (PMQR) genes, which can be transferred horizontally (18). *qnrS1* is an example of a PMQR gene and encodes a protein which protects the DNA gyrase from quinolone activity (20). Transfer of a *qnrS1* gene into a naive laboratory strain confers an increase in the MIC of some fluoroquinolones, including ciprofloxacin (13).

The majority of studies of PMQR plasmids have been performed on strains isolated from infection sites (11, 15, 16, 19, 21). Consequently, little is known about such plasmids in commensal *Enterobacteriaceae* species and the role they may play in disseminating other resistance genes. In Vietnam, fluoroquinolones are among the most commonly used antimicrobials and are available without prescription. Our previous work demonstrated that a high proportion of "healthy" members of the Vietnamese population carry *Enterobacteriaceae* species harboring *qnrS1* in the gastrointestinal tract (12). We aimed to investigate the cotransfer of antimicrobial resistance genes with *qnrS1* from plasmids that originated from hospitalized patients and from community controls in Ho Chi Minh City, Vietnam. We isolated plasmid DNA from 32 different *qnrS1* PCR amplicon-positive strains from the hospital and the community, as previously described (12) (Table 1). These strains were selected from a larger collection (12) as a result of our inability to genetically define the region surrounding the *qnrS1* gene, thus maximizing potential plasmid diversity. To ensure that we assessed only *qnrS1* plasmids, an *Escherichia coli* strain was transformed with plasmid DNA and selected on media supplemented with ciprofloxacin. All organisms were subjected to *qnrS1* PCR amplification and sized by a plasmid extraction method to ensure transformation of a single appropriate plasmid (5, 12). The plasmids were found to be of various sizes, ranging from 9 to 140 kbp, with a median of 84 kbp (Table 1). The plasmids carrying *qnrS1* that originated from the hospital ranged from 48 to 105 kbp in size (median, 58 kbp), and those originating from the community ranged from 9 to 140 kbp in size (median, 103 kbp).

In order to compare plasmid structures, plasmid DNA was digested with EcoRI and plasmids were assigned to incompatibility groups by PCR-based replicon typing, as previously described (4, 7). We identified 11 different incompatibility groups, of which *incN* ($n = 4$) and *incR* ($n = 4$) were the most common members (Table 1). An association between *qnr* genes and *incN* plasmids was previously identified and is likely due to integration and dissemination of a *qnr* gene into this prevalent plasmid group (7). EcoRI restriction fragments were compared to identify common banding patterns (Fig. 1). Thirty strains demonstrated less than 67% identity, and the restriction patterns could be divided into groups 1 and 2. The digestion patterns of the *qnrS1* plasmids originating in the community and the hospital were significantly associated with group 1 and 2, respectively ($P = 0.0017$; two-tailed Fisher's exact test).

The 32 strains were subjected to antimicrobial susceptibility profiling and extended-spectrum β-lactamase (ESBL) testing (Table 1) (14). There was no significant disparity (apart from chloramphenicol) between the resistance profiles of plasmids originally isolated from *E. coli* and those isolated from *Kleb-*

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TABLE 1. The origins and characteristics of 32 *qnrS1*-bearing plasmids

Strain or plasmid identification no.	Plasmid name	Source[a]	Bacterial species[b]	Incompatibility group(s)[c]	Size (kbp)	Presence of ESBL	MIC (μg/ml)		Resistance pattern[d]
							NAL	CIP	
Strain									
Top10			*E. coli*			−	1.5	0.006	
Plasmids									
LTMV1	pEW62RMAN	Community	*K. pneumoniae*	A/C	9	−	6	0.38	CIP_L
LTMV2	p033CA22	Community	*E. coli*	FIA; A/C	101	−	4	0.25	CHL, TET, SXT, CIP_L
LTMV3	p036CN2	Community	*E. coli*	repF; HI1; FIA; A/C	80	−	12	0.38	CHL, GEN, CIP_L
LTMV4	pA003IaI	Community	*K. pneumoniae*	FIA; A/C; R	132	−	4	0.25	CHL, GEN, TET, SXT, CIP_L
LTMV5	pK261An	Hospital	*K. pneumoniae*	R	70	−	4	0.25	AMP, CHL, GEN, TET, SXT, CIP_L
LTMV6	p023CN2	Community	*E. coli*	repF; FIB	140	−	4	0.25	AMP, CHL, CIP_L
LTMV7	p038CN2	Community	*K. pneumoniae*	R	64	−	4	0.25	AMP, CHL, GEN, TET, SXT, CIP_L
LTMV8	p008CN1	Community	*E. coli*	repF; FIB	140	−	8	0.38	CHL, GEN, CIP_L
LTMV9	p001CN2	Community	*E. coli*	Unknown	96	−	4	0.38	AMP, CHL, GEN, TET, CIP_L
LTMV11	p024CAZZ	Community	*E. coli*	FIA	44	−	6	0.38	AMP, TET, SXT, CIP_L
LTMV12	p0022IbI	Community	*K. pneumoniae*	Unknown	98	−	4	0.25	AMP, TET, SXT, CIP_L
LTMV13	p045CN2	Community	*E. coli*	repF	107	−	6	0.25	AMP, CHL, CIP_L
LTMV14	pK233Ca	Hospital	*K. pneumoniae*	Unknown	51	+	4	0.12	AMP, GEN, CRO, CIP_L
LTMV15	p025CN1	Community	*E. coli*	Unknown	135	−	6	0.38	AMP, CHL, TET, SXT, CIP_L
LTMV16	p065CN2	Community	*K. pneumoniae*	Unknown	71	−	4	0.25	AMP, CHL, GEN, TET, CIP_L
LTMV17	p048CN2	Community	*E. coli*	R	26	−	6	0.38	CHL, CIP_L
LTMV18	pE18An	Hospital	*E. coli*	Unknown	58	−	4	0.25	AMP, CIP_L
LTMV19	pD025IaI	Community	*E. coli*	Unknown	130	−	6	0.38	AMP, CHL, GEN, TET, SXT, CIP_L
LTMV20	p051CN	Community	*E. coli*	R; ColE	108	−	4	0.25	AMP, CHL, GEN, TET, SXT, KAN, CIP_L
LTMV21	pK300N	Hospital	*K. pneumoniae*	R	57	+	6	0.25	AMP, FEP, GEN, TIC, CRO, CIP_L
LTMV23	p039CN2	Community	*E. coli*	Unknown	109	−	4	0.25	AMP, CHL, GEN, TET, TIC, SXT, KAN, CIP_L
LTMV24	p008Na2	Community	*E. coli*	Unknown	43	−	6	0.12	AMP, CIP_L
LTMV25	pA0001IaI	Community	*E. coli*	Unknown	118	−	3	0.25	AMP, CHL, GEN, SXT, CIP_L
LTMV26	pB011IaI	Community	*E. coli*	Y	103	−	3	0.25	AMP, CHL, GEN, CIP_L
LTMV27	pEW20NMAG	Community	*K. pneumoniae*	Unknown	132	−	4	0.25	AMP, GEN, TET, SXT, KAN, CIP_L
LTMV28	pK18An	Hospital	*K. pneumoniae*	N	58	−	4	0.25	GEN, CIP_L
LTMV29	pE66An	Hospital	*E. coli*	N	87	+	4	0.25	AMP, FEP, GEN, TET, SXT, CRO, CIP_L
LTMV30	pK218Ca	Hospital	*K. pneumoniae*	A/C	48	−	4	0.12	GEN, CIP_L
LTMV31	pK218Ca	Hospital	*K. pneumoniae*	N	60	+	6	0.38	AMP, FEP, GEN, CRO, CIP_L
LTMV32	pK79N	Hospital	*K. pneumoniae*	N	74	+	6	0.38	AMP, FEP, GEN, TET, SXT, CRO, CIP_L
LTMV33	pK263Ax	Hospital	*K. pneumoniae*	Unknown	49	−	6	0.19	GEN, CIP_L
LTMV34	pK279N	Hospital	*K. pneumoniae*	Unknown	105	+	4	0.25	AMP, FEP, GEN, CRO, CIP_L

[a] Location of isolation of the original bacterial isolate containing *qnrS1*-carrying plasmid (12).
[b] Original bacterial species from which the *qnrS1*-carrying plasmid was isolated.
[c] Incompatibility group determined by PCR (4).
[d] NAL, nalidixic acid; CIP_L, reduced susceptibility to ciprofloxacin; CHL, chloramphenicol; TET, tetracycline; SXT, trimethoprim-sulfamethoxazole; GEN, gentamicin; AMP, ampicillin; CRO, ceftriaxone; KAN, kanamycin; FEP, cefepime; TIC, ticarcillin.

siella pneumoniae. However, the profiles of the plasmids isolated in the community demonstrated several differences from the profiles of the plasmids isolated from the hospital. The *qnrS1* plasmids isolated in the hospital were frequently more resistant to gentamicin ($P = 0.0273$; all compared by two-tailed Fisher's exact test), ceftriaxone ($P = 0.0004$), and cefepime ($P = 0.0019$), all of which are parenteral and not commonly used in the community. Only chloramphenicol resistance was associated with plasmids isolated from the community ($P = 0.0118$).

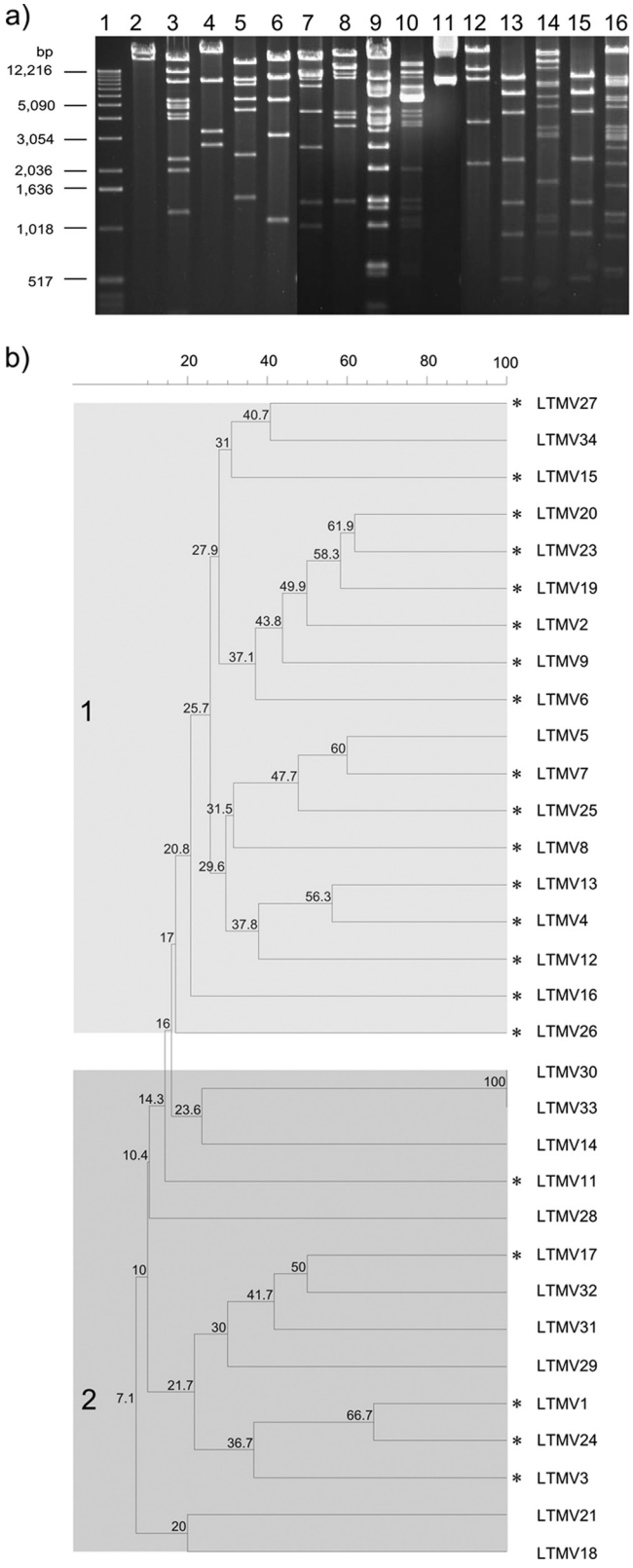

FIG. 1. EcoRI restriction digestions of plasmids carrying *qnrS1*. (a) An example of EcoRI digestion of 15 plasmids carrying *qnrS1*. Lanes: 1, 1-kbp ladder (Invitrogen); 2, plasmid LTMV21; 3, LTMV16; 4, LTMV28; 5, LTMV14; 6, LTMV11; 7, LTMV25; 8, LTMV5; 9, LTMV2; 10, LTMV20; 11, LTMV1; 12, LTMV18; 13, LTMV33; 14, LTMV6; 15, LTMV30; 16, LTMV19. (b) Neighbor-joining tree of 32 plasmids carrying *qnrS1* derived from digestion with EcoRI. Plasmids that were isolated from strains originating in the community are indicated by asterisks. Numbers within the shaded boxes correspond to the percent relationship between the individual nodes. The shaded boxes labeled 1 and 2 correspond to the two major groups of digested plasmids.

To assess the antimicrobial resistance gene characteristics, DNA from the 32 strains was hybridized to a miniaturized oligonucleotide antimicrobial resistance gene microarray containing probes for 54 antimicrobial resistance genes of clinical importance, and data were analyzed as previously described (1, 3). There were clear differences in hybridization patterns between plasmids that originated in the hospital and those that originated in the community (Fig. 2), thus partitioning the plasmids into groups A and B, corresponding to community and hospital plasmids ($P < 0.001$; Fisher's exact test). Grouping results also correlated with the plasmid digestion patterns, with identification of 14 and 2 community and hospital group 1A plasmids, respectively, and 8 and 1 hospital and community group 2B plasmids, respectively ($P < 0.001$; Fisher's exact test). The average number of genes detected by the assay in isolates from the community (6.3 per plasmid) differed significantly from the number detected in plasmids from the hospital (3.7 per plasmid) ($P = 0.013$; 2-tailed *t* test). Of the 32 plasmids, 25 demonstrated hybridization to probes for β-lactam resistance genes, with 19/32 (59.4%) and 4/32 (12.5%) producing a positive signal for *tem1* and *ctxM9*, respectively. Hybridization to the *oxa1*, *ctxM9*, or *shv1* gene was detected only in the hospital plasmids, and hybridization to *tem1* was more common in community plasmids. Correspondingly, only the hospital plasmids exhibited an ESBL phenotype (Table 1).

There has been some previous characterization of plasmids which can carry and transfer PMQR genes (6, 7, 17). Work conducted in Asia investigated the genetic properties of two plasmids carrying *qnrS1* and *qnrB4* isolated from an infectious *K. pneumoniae* strain in China. Hu et al. found that the *qnrB4* and *qnrS1* genes were located on two plasmids that demonstrate significant genetic variations (8). A sentinel study concerning *E. coli* and *K. pneumoniae* in China found that 8% of the investigated ESBL-producing strains were also PCR amplicon positive for a *qnr* gene. The $bla_{\text{CTX-M}}$ gene was the most commonly identified in 27 of 29 *qnr*-positive isolates, and TEM-1-type β-lactamase was detected in 16 *qnr*-positive isolates (9).

Here, we demonstrate that the sizes, incompatibility groups, resistance gene characteristics, and antimicrobial resistance phenotypes of plasmids carrying *qnrS1* are highly divergent. The concept that *qnr* genes circulate on unrelated plasmids is supported by recent work that characterized PMQR plasmids in *Salmonella* strains isolated from patients and poultry meat in the Netherlands (7). The spread of antimicrobial resistance is of global relevance and yet is particularly significant for many developing countries with improving economic prospects and unregulated use of antimicrobials. The recent report of a novel mechanism of resistance to carbapenems conferred by a metallo-β-lactamase in India highlights the origins and potential transcontinental spread of such determinants (10). Our

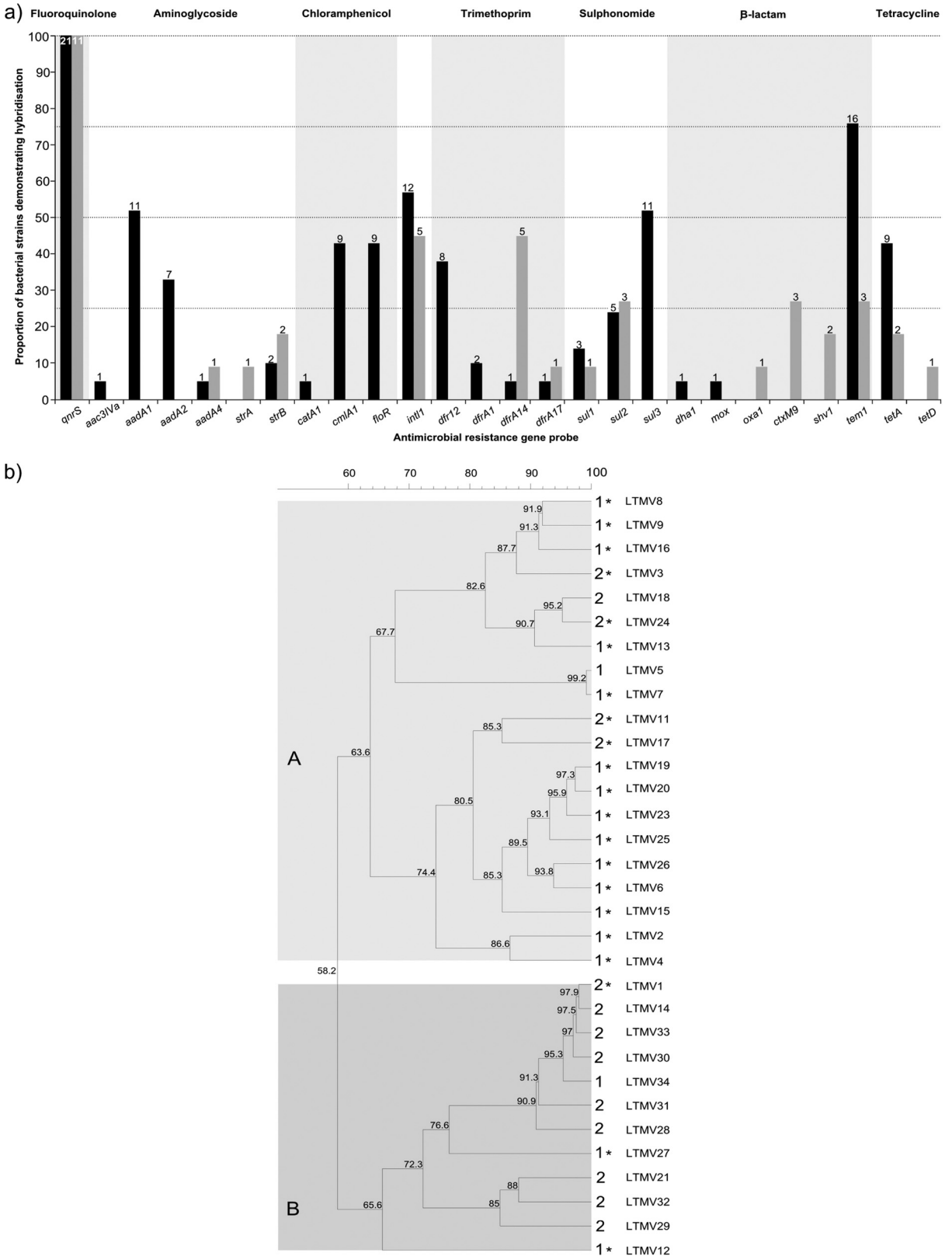

FIG. 2. The antimicrobial resistance gene content of 32 plasmids carrying *qnrS*. (a) Graph showing the proportional relationships of transformants demonstrating hybridization to 25 specific antimicrobial resistance genes and one class one integrase gene (*intI1*). The graph is subdivided into those plasmids that were isolated in the community (black bars) (n = 21) and those that were isolated in the hospital (gray bars) (n = 11). The labels at the top of the diagram highlight the group to which the indicated antimicrobial resistance gene confers resistance. (b) Neighbor-joining tree representing DNA hybridization to the antimicrobial resistance gene microarray. The 32 strains fall into main clusters A and B, which are distinguished by light and dark gray shading. Asterisks identify those strains containing community plasmids; the numbers adjacent to the strain names correspond to the EcoRI restriction digestion results (Fig. 1). The numbers on each branch correspond to percent identity between individual branches.

data demonstrate that the *qnrS1* gene is extremely widespread and capable of transfer into a number of variable replicons. Furthermore, we found different genotypic and phenotypic characteristics of plasmids carrying *qnrS1* in isolates collected in the hospital and the community, which is indicative of the selection of such replicons by the use of specific antimicrobial agents in these differing settings.

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