Journal of Antimicrobial Chemotherapy (2007) **60**, 1146–1150
doi:10.1093/jac/dkm352
Advance Access publication 19 September 2007

JAC

Plasmid-mediated quinolone resistance conferred by *qnrS1* in *Salmonella enterica* serovar Virchow isolated from Turkish food of avian origin

M. D. Avsaroglu[1], R. Helmuth[2], E. Junker[2], S. Hertwig[2], A. Schroeter[2], M. Akcelik[3], F. Bozoglu[1] and B. Guerra[2]*

[1]Middle East Technical University, Ankara, Turkey; [2]Federal Institute for Risk Assessment (BfR), Berlin, Germany; [3]Ankara University, Ankara, Turkey

Received 18 May 2007; returned 4 July 2007; revised 13 August 2007; accepted 14 August 2007

***Objectives*: To study the molecular characteristics of the quinolone and associated ampicillin resistance mechanisms present in *Salmonella enterica* serovar Virchow isolated from Turkish foods.**

***Methods*: Nine epidemiologically unrelated *Salmonella* Virchow strains isolated from foods (chicken and minced meat) sold in different markets in Ankara were analysed for their susceptibility to 17 antimicrobials. The strains were typed by PFGE and plasmid profiling and investigated by molecular methods (PCR/sequencing) for the presence of several resistance genes, class 1 integrons and mutations in the quinolone resistance-determining regions. Plasmids conferring quinolone resistance were analysed by restriction fragment length polymorphism (RFLP) analysis, DNA hybridization, sequencing, replicon-typing PCR and mating experiments.**

***Results*: All strains showed nalidixic acid resistance (MIC $\geq$ 128 mg/L) together with a decreased susceptibility to ciprofloxacin (three strains with an MIC of 1 mg/L and six with an MIC of 0.25 mg/L), associated with mutations within the *gyrA* gene (Asp-87→Tyr-87). In three strains, *qnrS1* genes were detected. Ampicillin resistance encoded by a $bla_{\text{CTX-M3}}$ gene and/or $bla_{\text{TEM-1-like}}$ gene was found in four strains. Three of these strains carried an ~45 kb conjugative plasmid, designated pRQ2006, harbouring *qnrS1* and a Tn*3-like* transposon. Partial sequencing and RFLP of pRQ2006 indicated its similarity to the *qnrS1* plasmid pAH03786 found in a Japanese *Shigella flexneri* 2b isolate.**

***Conclusions*: This is the first study describing the presence of *qnrS1* genes in bacterial isolates from Turkey. The pRQ2006 plasmid seems to be more related to the *S. flexneri* 2b *qnrS1* plasmid pAH0376 than to the *Salmonella qnrS1*-carrying plasmids pINF5 and TPqnrS-2.**

Keywords: *qnr*, fluoroquinolones, antimicrobial resistance, PFGE, resistance plasmids

Introduction

Fluoroquinolones and β-lactams are among the most widely used antimicrobials categorized as drugs of 'critical importance' by the World Health Organization.[1] Quinolones were introduced as antimicrobials in the 1960s and fluoroquinolones in the 1980s, and since then, bacterial resistance to these drugs has dramatically increased. Quinolone resistance is normally accompanied by a reduced susceptibility or resistance to fluoroquinolones and is mainly ascribed to mutations in the target genes *gyrA* (DNA gyrase) and *parC* (topoisomerase IV) and/or to active efflux. The combination of several mechanisms leads to high-level resistance.[1,2]

Plasmid-encoded quinolone/fluoroquinolone resistance conferred by *qnr* genes (*qnrA*, *qnrB1–8*, *qnrS1–2*) has been described as well.[2,3] In most cases, the *qnr* genes were linked to genes encoding extended-spectrum or plasmid-mediated AmpC-type β-lactamases.[2] In order to determine the prevalence of these genes, several studies on isolates of different species (i.e. *Klebsiella, Enterobacter, Citrobacter, Escherichia coli* and *Salmonella*), sources (human, animal or food) and countries (USA, India, Vietnam, France, UK, Germany, Turkey and so on) have been performed. In *Salmonella*, *qnrA*, *B* and *S* genes were detected with varying prevalence.[2,4–6] The aim of the present study was the molecular characterization of the quinolone and

*Corresponding author. Tel: +49-30-8412-2057; Fax: +49-30-8412-2000; E-mail: beatriz.guerra@bfr.bund.de

© The Author 2007. Published by Oxford University Press on behalf of the British Society for Antimicrobial Chemotherapy. All rights reserved. For Permissions, please e-mail: journals.permissions@oxfordjournals.org

Downloaded from https://academic.oup.com/jac/article/60/5/1146/2359228 by ANKARA user on 11 December 2021

associated ampicillin resistance mechanisms present in *Salmonella enterica* serotype Virchow isolated from Turkish foods.

Materials and methods

One hundred *S. enterica* isolates were obtained from foods bought in several markets in Ankara, Turkey, during 2005–06. *Salmonella* Virchow (15 isolates) predominated and 9 of these isolates (7 isolates from chicken and 2 from minced meat) were considered as epidemiologically unrelated (different isolation date or place, see Table 1) and selected for further studies. The strains were phage-typed following the scheme used at the Health Protection Agency (HPA), London, UK and tested for their susceptibility to 17 antimicrobials by broth microdilution (CLSI), as described previously.[7]

PFGE with *Xba*I was carried out following the PulseNet protocol (www.pulsenet-europe.org). Patterns were defined considering bands $\geq$50 kb. Plasmid profiling was performed by the method described by Kado and Liu.[8]

The detection of antimicrobial resistance genes, class 1 integrons and mutations in the quinolone resistance-determining regions (QRDRs) was performed by PCR amplification/sequencing, as described previously.[7]

Plasmid characterization was carried out by cloning, primer walking and/or amplification using primers deduced from published sequences. Determination of the incompatibility group, restriction fragment length polymorphism (RFLP) analysis using several endonucleases and Southern hybridization were carried out as described previously.[7,9] Conjugation experiments using an *E. coli* J53 rifampicin-resistant strain as recipient were performed at 22°C and 37°C on filters and in liquid broth. To avoid the selection of J53 *gyrA* mutants, selection was made on Eosin Methylene Blue (EMB) agar plates with ampicillin and rifampicin (200 and 100 mg/L, respectively).

For information about the primers used and their references, please see Table S1 [available as Supplementary data at *JAC* Online (http://jac.oxfordjournals.org/)].

Results and discussion

Phenotypical and molecular characterization of the strains

The nine *Salmonella* Virchow isolates were phage type PT26. All strains showed nalidixic acid resistance (MIC $\geq$ 128 mg/L) together with decreased susceptibility to ciprofloxacin (three with an MIC of 1 mg/L and six with an MIC of 0.25 mg/L). Four strains also showed ampicillin resistance. Only one of these isolates showed a multiresistant phenotype (ampicillin–ceftiofur–nalidixic acid–streptomycin–sulfamethoxazole–trimethoprim–sulfamethoxazole/trimethoprim).

PFGE with *Xba*I (Figure 1) defined three similar PFGE patterns (X1 and X2, shown by four strains, and X3 by one strain). By plasmid profiling, four patterns were defined: PP0, no plasmid (two strains); PP1, one ~45 kb plasmid (three strains); PP2, one ~45 kb plasmid and three small plasmids (<5 kb, one strain) and PP3, three small (<10 kb) cryptic plasmids (three strains) (Figure 1).

Molecular characterization of quinolone and ampicillin resistance determinants

The isolates were tested for the presence of mutations in the QRDR of both *gyrA* and *parC* genes. All strains exhibited mutations in the *gyrA* gene resulting in the amino acid substitution Asp-87→Tyr-87. They were further screened for the presence of the plasmid-encoded quinolone resistance genes *qnrA*, *qnrB* and *qnrS*. Three strains that showed resistance to ampicillin and nalidixic acid (ciprofloxacin MIC = 1 mg/L) gave PCR products of 417 bp with the *qnrS* primers.[4] Sequences of these products were identical to *qnrS1* (GenBank accession no. AB187515). Further PCR amplifications/sequencing of the flanking regions of one positive isolate (DMC9) using the Pre-qnrS primers[6] and primers designed for the present work confirmed the presence of the *qnrS1* gene (656 bp) found in different Enterobacteriaceae.[2] In the three *qnrS1* carriers, $bla_{\text{TEM-1}}$ genes coding for ampicillin resistance were detected. The fourth ampicillin-resistant isolate carried both $bla_{\text{TEM-1-like}}$ and $bla_{\text{CTX-M3}}$ extended-spectrum β-lactamase genes (Table 1).

Mapping of the qnrS1 and bla_{TEM-1} genes on pRQ2006

To examine whether the *qnrS1* gene was localized on the chromosome or on a plasmid, Southern hybridization of total (*Xba*I PFGE patterns) and plasmid (plasmid patterns) DNA was performed. Both the *qnrS1* and the $bla_{\text{TEM-1-like}}$ probes hybridized with an ~45 kb PFGE band of pattern X1 that corresponded to a plasmid, designated pRQ2006, present in the three *qnrS1*-positive isolates (two with plasmid profile PP1 and one with PP3) (Figures 1 and 2). Conjugation experiments were performed. Six selected transconjugants showed low resistance to nalidixic acid (8–16 mg/L) and a decreased susceptibility to ciprofloxacin (0.5 mg/L). They were positive for the *qnrS1* and bla_{TEM-1} genes and did not show any chromosomal mutation affecting the QRDR of the *gyrA* gene.

To determine the incompatibility group of the pRQ2006 plasmid, the 18 incompatibility group replicon-typing PCR[9] together with IncQ detection (*repB* of RS1010, accession no. M28829 and *repA* of the pGNB2-*qnrS2* positive plasmid, accession no. DQ460733) was applied. Although the scheme was useful to characterize other resistance plasmids in our laboratory (data not shown), no positive product was obtained using the DMC9 isolate or plasmid DNA as templates.

Restriction analysis of pRQ2006 was performed using several endonucleases (Figure 2). Restriction fragments generated with *Hinc*II, *Hin*dIII, *Eco*RV and *Eco*RI were chosen for DNA hybridization with $bla_{\text{TEM-1}}$ and *qnrS1* probes. Only in the case of restriction with *Eco*RI, both probes hybridized to the same fragment (~20 kb) (Figure 2).

The presence of a Tn*3*-like transposon (accession no AB187515.1), which contained the $bla_{\text{TEM-1}}$ gene, was confirmed by partial sequencing of *Hin*dIII (8 kb fragment) and *Eco*RV (2.7 kb fragment) restriction fragments that had been inserted into the vector pIV2 and introduced into the *E. coli* strain NEB5 (New England Biolabs).

Relationship of pRQ2006 to other known qnrS1-bla_{TEM-1} plasmids

Only a few *qnrS1*-carrying plasmids have been described so far. They differ in their molecular sizes, resistance genes (i.e. for β-lactamases) and bacterial host. The 47 kb plasmid pAH0376 (accession no. AB187515.1) was the first *qnrS1*-carrier plasmid described. It was found in a human clinical isolate of *Shigella*

Downloaded from https://academic.oup.com/jac/article/60/5/1146/2359228 by ANKARA user on 11 December 2021

Downloaded from https://academic.oup.com/jac/article/60/5/1146/2359228 by ANKARA user on 11 December 2021

Table 1. Strains used in the present study

Strain/ isolate ref. no.	Species/ serotype	Origin	Isolation date	Phage type[a]	PFGE pattern	Plasmid profile	Resistance phenotypes	MIC (mg/L)		Resistance genotypes	Class 1 or 2 integrons
								NAL	CIP		
DMC 5	*Salmonella* Virchow	chicken	15 November 2005	PT26	X3	PP3	NAL	>128	0.25	$gyrA^{Tyr\text{-}87}$	none
DMC 9	*Salmonella* Virchow	chicken	15 November 2005	PT26	X1	PP1	AMP–NAL	>128	1	$bla_{\text{TEM-1}}$-($gyrA^{Tyr\text{-}87}$-*qnrS1*)	none
DMC 16	*Salmonella* Virchow	chicken	4 October 2005	PT26	X1	PP2	AMP–NAL	128	1	$bla_{\text{TEM-1}}$-($gyrA^{Tyr\text{-}87}$-*qnrS1*)	none
DMC 18	*Salmonella* Virchow	chicken	15 October 2005	PT26	X1	PP0	NAL	128	0.25	$gyrA^{Tyr\text{-}87}$	none
DMC 19	*Salmonella* Virchow	chicken	7 November 2005	PT26	X2	PP1	AMP–EFT–NAL–STR–SUL–TMP–SXT	128	0.25	($bla_{\text{TEM-1}}$-$bla_{\text{CTX-M3}}$)-$gyrA^{Tyr\text{-}87}$-*strA/B-sul2-dfrA14*	none
DMC 32	*Salmonella* Virchow	chicken	4 October 2005	PT26	X2	PP3	NAL	>128	0.25	$gyrA^{Tyr\text{-}87}$	none
DMC 42	*Salmonella* Virchow	chicken	15 November 2005	PT26	X2	PP3	NAL	>128	0.25	$gyrA^{Tyr\text{-}87}$	none
DMC 68	*Salmonella* Virchow	minced meat	15 October 2005	PT26	X1	PP1	AMP–NAL	>128	1	$bla_{\text{TEM-1}}$-($gyrA^{Tyr\text{-}87}$-*qnrS1*)	none
DMC 83	*Salmonella* Virchow	minced meat	23 December 2005	PT26	X2	PP0	NAL	>128	0.25	$gyrA^{Tyr\text{-}87}$	none
J53	*E. coli*	—	—	—	—	—	susceptible	4	0.008	—	—
Tj1	*E. coli* J53	conjugation	—	—	—	PP1	AMP	16	0.5	$bla_{\text{TEM-1}}$-*qnrS1*	none
Tj2	*E. coli* J53	conjugation	—	—	—	PP1	AMP	16	0.5	$bla_{\text{TEM-1}}$-*qnrS1*	none
Tj3	*E. coli* J53	conjugation	—	—	—	PP1	AMP	8	0.5	$bla_{\text{TEM-1}}$-*qnrS1*	none

AMP, ampicillin; EFT, ceftiofur; CIP, ciprofloxacin; NAL, nalidixic acid; STR, streptomycin; SUL, sulfamethoxazole; SXT, sulfamethoxazole/trimethoprim; TMP, trimethoprim.
[a]Carried out following the scheme used at the HPA, London, UK.

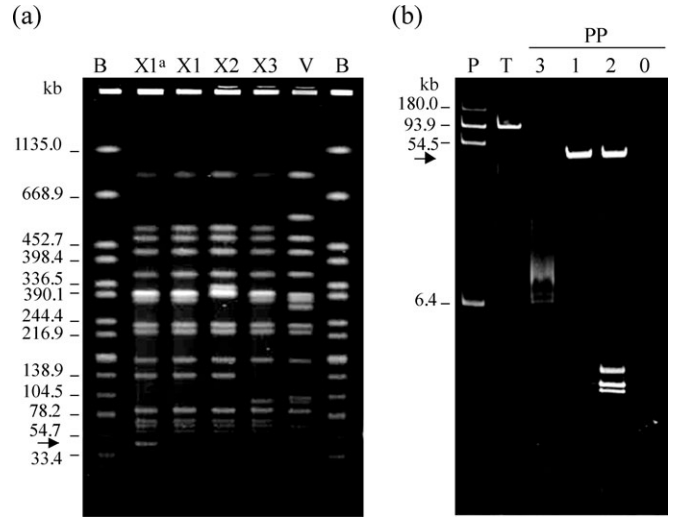


Figure 1. PFGE typing and plasmid profiling of *Salmonella* Virchow isolates. (a) PFGE patterns obtained with *Xba*I restriction. B, *Salmonella* Braenderup HB9812; V, *Salmonella* Virchow NRL-06-01122 (Germany, chicken). X1[a], shown by three *qnrS1*-positive strains, containing the plasmid pRQ2006 (→, ∼45 kb). (b) Plasmid profiles (PP). P, molecular weight markers R27, R1, RP4 and ColE1; T, plasmid pSLT90 (∼90 kb) from *Salmonella* Typhimurium LT2; PP3, PP1, PP2 and PP0 correspond to the Turkish *Salmonella* Virchow isolates DMC5, DMC9, DMC16 and DMC18, respectively.

flexneri in Japan and carries a Tn*3*-like transposon.[10] The 58 kb pINF5 plasmid (accession no. AM234722.1) also carries the Tn*3*-like transposon and was isolated from *Salmonella* Infantis recovered from poultry in Germany.[11] The pK245 plasmid (accession no. JQ449578.1) is a 98 kb plasmid, which carries a bla_{SHV-2} (ampicillin resistance) gene found in a clinical isolate of *Klebsiella pneumoniae*.[12] In *Enterobacter cloacae* isolated in France and Vietnam, several plasmids with sizes between 50 (p287) and 100 kb (i.e. pS3–5) have been described and some of them carry a new bla_{LAP-1} gene.[8,13] Recently, Hopkins *et al*.[5] identified several *qnrS1* plasmids in the UK, including the 44 kb IncN plasmid TPqnrS-2a/b, which also carries the bla_{TEM-1} gene, in *Salmonella* Virchow PT8 isolated from Thai cooked chicken.

The plasmid pRQ2006 was compared with pINF5 from *Salmonella* Infantis and pAH0376 from *S. flexneri* by primer walking and/or amplification using primers deduced from the published sequences of pINF5 (13 389 bp) and pAH0376 (11 002 bp), as well as by comparison of RFLP hybridization patterns.

First, a region of ∼3430 bp of pRQ2006 was sequenced. This region matched with nucleotides 915–3093 from pINF5 and nucleotides 2114–5545 from pAH0376 and contained the bla_{TEM-1} gene, the *tnpR* gene (encoding the resolvase) and part of the *tnpA* gene (encoding the transposase) of the Tn*3*-like transposon. The region located upstream of Tn*3* only matched with pAH0376 from *S. flexneri* (nucleotides 2114–2909). This region included a TAAAA direct repeat at the boundaries of the Tn*3* element, which differs from the TTATT repeat part of an IS*26* relic found in pINF5.[11]

Secondly, a pRQ2006 region of ∼1677 bp containing *qnrS1* was sequenced. This sequence was similar to the sequences described for pINF5 and pAH0376 (nucleotides 6871–8547 from pINF5 and 9661–10 132 from pAH0376). On the basis of the sequence of pINF5, amplification experiments targeting other genes/sequences located downstream of *qnrS1* were performed. Using primers designed for the CS12 fimbrial gene cluster of *E. coli* present in pINF5[11] (nucleotides 9661–10 143), a 100% homologous PCR product of 482 bp could be amplified. In contrast, no product targeting the *tnp* gene encoding the transposase of IS*26* of pINF5 (expected size of 429 bp corresponding to nucleotides 11 591–12 020) was obtained, showing the lack of the IS*26* relic in pRQ2006.

Kehrenberg *et al*.[11] reported that pINF5 and pAH0376 showed different *Hin*dIII restriction patterns. RFLP hybridization with *qnrS1* and bla_{TEM-1} probes had been carried out. The

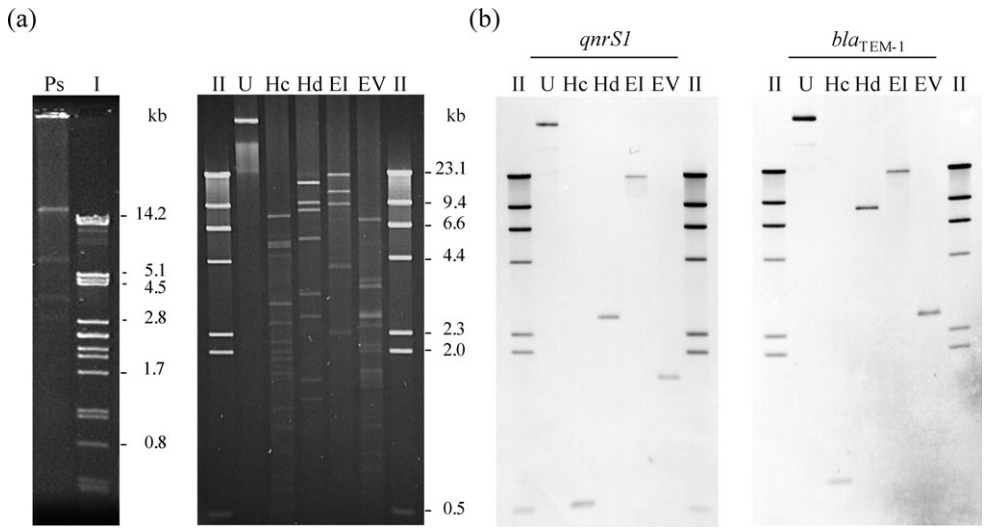


Figure 2. Restriction and hybridization analysis of the plasmid pRQ2006. (a) Plasmid restriction profiles generated by *Pst*I (Ps), *Hin*cII (Hc), *Hin*dIII (Hd), *Eco*RI (EI) and *Eco*RV (EV). (b) Hybridizations of digested DNA with *qnrS1* and bla_{TEM-1} probes. U, undigested pRQ2006. Molecular weight marker used: phage lambda DNA digested *Pst*I (I) and phage lambda DNA digested with *Hin*dIII (II; Molecular weight marker DNA II, digoxigenin labelled, Roche Applied Science).

Downloaded from https://academic.oup.com/jac/article/60/5/1146/2359228 by ANKARA user on 11 December 2021

results are similar to those obtained with pRQ2006: *qnrS1* is located on a 2.6 kb *Hin*dIII-fragment of pRQ2006 (as described for pINF5 and pAH0376) and the bla_{TEM-1} gene is located on an 8.4 kb fragment (as described for pAH0376). Furthermore, both genes are located on an ~20 kb *Eco*RI fragment (as described for pAH0376). The pRQ2006 plasmid yielded a *Pst*I-RFLP (Figure 2) similar to those shown by Hopkins *et al.*[5] for the *S. flexneri* plasmid, whereas the TPqnrS-2 plasmids found in the *Salmonella* Virchow isolates yielded very different *Pst*I-RFLPs.[5]

This is the first study describing the presence of *qnrS1* genes in bacterial strains from Turkey. The *Salmonella* Virchow strains were isolated from chicken and minced meat sold for human consumption. The pRQ2006 *qnrS1* plasmid differs from the other described plasmids found in *Salmonella* and seems to be related to the plasmid pAH0376 found in *S. flexneri* isolated from human clinical samples in Japan. The presence of these plasmids in *Salmonella* isolates from food-producing animals is another worrying step in the increasingly observed fluoroquinolone resistance.

Acknowledgements

We thank J. Hammerl for his help with the incompatibility experiments and M. Jaber and F. Scholz for their technical support. We also thank the participants in WP9 and WP21 of the Med-Ved-Net Project for their advice, especially A. Carattoli for the control DNAs used for the replicon-typing PCR. For the *qnr*-positive strains used as controls, we thank L. Martínez Martínez (*E. coli* J53 carrying pMG252 *qnrA*+) and L. Poirel (*K. pneumoniae B1 qnrB1*+ and *E. coli S7 qnrS1*+).

Funding

This work was supported by grants from the Middle East Technical University (BAP-08-11-DPT-2002K120510), Ankara University (BAP-20060705110), Med-Ved-Net (EU funded Network of Excellence for the Prevention and Control of Zoonoses) and by the Federal Institute for Risk Assessment (BfR-45-004). M. D. A. was recipient of a grant from METU no. BAP-08-11-DPT-2002K120510 project and performed a 1 year scientific visit at the BfR.

Transparency declarations

None to declare.

Supplementary data

Table S1 is available as Supplementary data at *JAC* Online (http://jac.oxfordjournals.org/).

References

1. World Health Organization. *Second Joint FAO/OIE/WHO Expert Workshop on Non-human Antimicrobial Usage and Antimicrobial Resistance: Management Options*. 15–18 March 2004, Oslo, Norway.

2. Robicsek A, Jacoby GA, Hooper DC. The worldwide emergence of plasmid-mediated quinolone resistance. *Lancet Infect Dis* 2006; **6**: 629–40.

3. Cattoir V, Poirel L, Rotimi V *et al.* Multiplex PCR for detection of plasmid-mediated quinolone resistance *qnr* genes in ESBL-producing enterobacterial isolates. *J Antimicrob Chemother* 2007; **60**: 394–7.

4. Gay K, Robicsek A, Strahilevitz J *et al.* Plasmid-mediated quinolone resistance in non-Typhi serotypes of *Salmonella enterica*. *Clin Infect Dis* 2006; **43**: 297–304.

5. Hopkins KL, Wootton L, Day MR *et al.* Plasmid-mediated quinolone resistance determinant *qnrS1* found in *Salmonella enterica* strains isolated in the UK. *J Antimicrob Chemother* 2007; **59**: 1071–5.

6. Poirel L, Leviandier C, Nordmann P. Prevalence and genetic analysis of plasmid-mediated quinolone resistance determinants QnrA and QnrS in *Enterobacteriaceae* isolates from a French university hospital. *Antimicrob Agents Chemother* 2006; **50**: 3992–7.

7. Guerra B, Junker E, Miko A *et al.* Characterization and localization of drug resistance determinants in multidrug-resistant, integron-carrying *Salmonella enterica* serotype Typhimurium strains. *Microb Drug Resist* 2004; **10**: 83–91.

8. Kado CI, Liu ST. Rapid procedure for detection and isolation of large and small plasmids. *J Bacteriol* 1981; **145**: 1365–73.

9. Carattoli A, Bertini A, Villa L *et al.* Identification of plasmids by PCR-based replicon typing. *J Microbiol Methods* 2005; **63**: 219–28.

10. Hata M, Suzuki M, Matsumoto M *et al.* Cloning of a novel gene for quinolone resistance from a transferable plasmid in *Shigella flexneri* 2b. *Antimicrob Agents Chemother* 2005; **49**: 801–3.

11. Kehrenberg C, Friederichs S, de Jong A *et al.* Identification of the plasmid-borne quinolone resistance gene *qnrS* in *Salmonella enterica* serovar Infantis. *J Antimicrob Chemother* 2006; **58**: 18–22.

12. Chen YT, Shu HY, Li LH *et al.* Complete nucleotide sequence of pK245, a 98-kilobase plasmid conferring quinolone resistance and extended-spectrum-β-lactamase activity in a clinical *Klebsiella pneumoniae* isolate. *Antimicrob Agents Chemother* 2006; **50**: 3861–6.

13. Poirel L, Cattoir V, Soares A *et al.* Novel Ambler class A β-lactamase LAP-1 and its association with the plasmid-mediated quinolone resistance determinant QnrS1. *Antimicrob Agents Chemother* 2007; **51**: 631–7.

Downloaded from https://academic.oup.com/jac/article/60/5/1146/2359228 by ANKARA user on 11 December 2021