

International collaborative study on the occurrence of plasmid-mediated quinolone resistance in *Salmonella enterica* and *Escherichia coli* isolated from animals, humans, food and the environment in 13 European countries

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Objectives: This study was initiated to collect retrospective information on the occurrence of plasmid-mediated quinolone resistance (PMQR) in *Salmonella enterica* and *Escherichia coli* isolates in Europe and to identify the responsible genes.

Methods: Databases of national reference laboratories containing MIC values for *Salmonella* and *E. coli* isolated between 1994 and 2009 in animals, humans, food and the environment from 13 European countries were screened for isolates exhibiting a defined quinolone resistance phenotype, i.e. reduced susceptibility to fluoroquinolones and nalidixic acid. PCR and sequence analysis were performed to identify the responsible PMQR genes.

Results: Screening of databases of 13 European countries resulted in a selection of 1215 *Salmonella* and 333 *E. coli* isolates. PMQR genes were identified in 59% of the *Salmonella* isolates and 15% of the *E. coli* isolates selected. In *Salmonella*, *qnrS1* ($n=125$) and variants of *qnrB* ($n=138$) were frequently identified, whereas *qnrA1* ($n=3$) and *aac(6')-1b-cr* ($n=3$) were rarely found. *qnrD* was detected in 22 *Salmonella* isolates obtained from humans and animals. In *E. coli*, *qnrS1* was identified in 19 isolates and *qnrB19* was found in one isolate. No *qnrC* or *qepA* genes were detected in either *Salmonella* or *E. coli*.

Conclusions: This study shows the occurrence and dissemination of PMQR genes in *Salmonella* and *E. coli* in Europe with a defined quinolone resistance phenotype. We also report the first detection of *qnrD* in *Salmonella* collected in Europe.

Keywords: PMQR, MICs, *qnr*, *aac(6')-1b-cr*

Introduction

Fluoroquinolones are important antibiotics for treatment of infections in both humans and animals.¹ Resistance to

fluoroquinolones can lead to treatment failure and is therefore considered a public health risk.^{2,3}

In Enterobacteriaceae, (fluoro)quinolone resistance is mainly caused by point mutations in the quinolone

resistance-determining region (QRDR) of gyrase (*gyrA* and *gyrB*) and topoisomerase (*parC* and *parE*) genes. An increase in the number of mutations leads to a stepwise increase in the level of resistance to fluoroquinolones such as ciprofloxacin. However, one point mutation in *gyrA* results in resistance to nalidixic acid.¹ In addition, efflux pumps and decreased permeability of the outer membrane can also contribute to the level of quinolone resistance.¹ In the last decade plasmid-mediated quinolone resistance (PMQR) has been increasingly reported in Enterobacteriaceae worldwide, including several European countries.^{4,5} However, studies reporting the occurrence of PMQR genes in Enterobacteriaceae on a European level are lacking.

To date, three different transferable (fluoro)quinolone resistance mechanisms have been described: (i) five different *qnr* families, each with different numbers of alleles (*qnrA1-7*, *qnrS1-4*, *qnrB1-31*, *qnrC* and *qnrD*)⁶ (<http://www.lahey.org/qnrstudies>); (ii) a modified aminoglycoside acetyl transferase gene [*aac(6′)-1b-cr*];⁷ and (iii) a specific quinolone efflux pump (*qepA*)⁸ and multidrug resistance pumps like *oqxAB*.⁹ In general, PMQR-positive isolates display a distinctive phenotypic quinolone resistance pattern, which is related to a specific affinity to fluoroquinolones, resulting in reduced susceptibility to these substances, but only a minor reduction in susceptibility to nalidixic acid.^{10,11} This phenotype is only exhibited by bacterial isolates without mutations in the QRDR, but can nevertheless be of interest in retrospective studies.

In 2008, the European Union Reference Laboratory for Antimicrobial Resistance (EURL-AR; at the National Food Institute, Lyngby, Denmark) initiated a collaborative study including all the National Reference Laboratories for Antimicrobial Resistance (NRL-AR), which routinely used MIC determinations for susceptibility testing. The aim of this study was to collect retrospective information on the emergence and occurrence of PMQR in *Salmonella* and *Escherichia coli* isolates from different sources in Europe and to identify the responsible genes.

Methods

All NRLs on antimicrobial resistance in animals and food were invited by the EURL-AR to participate in a collaborative study on the occurrence of PMQR in *Salmonella* and *E. coli*. Furthermore, the National Veterinary Institute (NVI) in Norway and the Health Protection Agency Centre for Infections (HPA) in the UK were also invited to participate in this study. A questionnaire (available as Supplementary data at JAC Online) was included to collect information concerning sample identity, maintenance of strain collections, testing methods and types of databases. The NRL-AR laboratories were asked to retrospectively screen their MIC databases for *E. coli* and *Salmonella* isolates exhibiting a defined PMQR phenotype; i.e. reduced susceptibility to ciprofloxacin and nalidixic acid. Based on epidemiological cut-off values set by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) for ciprofloxacin and enrofloxacin, inclusion criteria were set for *Salmonella* (MICs ≥ 0.125 mg/L and ≥ 0.25 , respectively) and *E. coli* (MICs ≥ 0.06 mg/L) combined with nalidixic acid MICs 4–32 mg/L. The majority of the participants tested ciprofloxacin and nalidixic acid; only Poland (until 2007) and Norway (until 2006) tested for susceptibility to enrofloxacin instead.

All databases, except for the HPA, consisted of MIC values obtained with broth microdilution according to ISO-20776-1:2006.¹² HPA used a semi-quantitative agar dilution system testing susceptibility to antibiotics at breakpoint levels. For our purpose this system provided sufficient information on the quinolone resistance phenotype as it included

testing a single concentration of nalidixic acid (16 mg/L) and two different ciprofloxacin concentrations (0.125 and 1 mg/L). Isolates exhibiting growth on 0.125 mg/L ciprofloxacin but no growth on 16 mg/L nalidixic acid were considered PMQR suspected and included in the selection.

Furthermore, participants were asked to send epidemiological data (such as source and year of isolation) concerning the selected isolates to the EURL-AR in order to build a database of the PMQR-suspected isolates. Databases containing disc diffusion results were excluded from the study, since valid information on inhibition zone diameters indicative of PMQR-positive isolates was lacking. Subsequently, all participants were asked to screen their selected isolates with PCR for all known PMQR genes. PCR protocols based on published primers for each of the genes *qnrA*,¹³ *qnrB*,¹⁴ *qnrC*,¹⁵ *qnrD*,¹⁶ *qnrS*,¹³ *qepA*¹⁷ and *aac(6′)-1b-cr*¹⁸ were distributed to all participants. For all PCR tests, positive control strains were available at the EURL-AR. Laboratories were allowed to use their in-house PCR methods. All laboratories, except for HPA, used the proposed PCR protocols. The HPA used described primers for *qnrA*, *qnrB* and *qnrS*,¹⁹ but home-designed primers for *qnrC* (5′-TGAATTATCCCATAAAACG-3′ and 5′-TGCTGGAAATAACAATCACC-3′), *qnrD* (5′-CGGGGAATAGAGTTAAAAAT-3′ and 5′-TATCGGTGAACAATAACACC-3′) and *qepA* (5′-GTAGATCGTCAGCAGCAC-3′ and 5′-TCTTGGATCCTGGACAT-3′).

Finally, participants were requested to sequence the amplicons to identify the detected resistance genes and their respective variants. For the identification of *qnrB* variants a second set of primers was used in order to analyse a larger amplicon.²⁰ To discriminate between *qnrB5* and *qnrB19* a third set of primers based on the linkage between *ISEcp1C* and *qnrB19* was used.²¹ If laboratories were not able to perform PCR and sequencing, they were invited to send their PMQR-suspected isolates either to the EURL-AR in Denmark or to the Central Veterinary Institute (CVI) in the Netherlands for further analysis.

Results

National Reference Laboratories from Belgium, the Czech Republic, Denmark, Finland, France, Germany, Ireland, Italy, Norway, Poland, Spain, the Netherlands and the UK participated in this study.

Retrospective screening of the MIC data of 661 629 *Salmonella* isolates and 31 132 *E. coli* isolates resulted in a selection of 1215 PMQR-suspected *Salmonella* and 333 PMQR-suspected *E. coli* isolates. Subsequently, 485 *Salmonella* and 133 *E. coli* were genetically characterized (Table 1). The remaining isolates were not analysed, because they were either not stored, not viable or were retested for susceptibility and based on the new results excluded from the study. The isolates tested were obtained between 1994 and 2009 from humans, turkeys (and turkey meat), fowls (broilers, laying hens and broiler meat), pigs (and pork), cattle, sheep and reptiles, but also from food (undefined meat, food products and spices), animal feed and environmental samples.

For *Salmonella*, PCR testing revealed PMQR genes in 59% (288/485) of isolates tested originating from 11 different European countries (Table 1). PMQR genes were identified in *Salmonella* isolates obtained from humans, turkeys, fowls, reptiles, pigs, sheep, food and the environment and in two isolates of unknown origin (Table 2). The earliest PMQR-positive *Salmonella* isolate detected was a *qnrS1*-positive *Salmonella* Typhimurium isolate obtained in 2002 from a human in the UK.

The *qnrS1* gene was predominant in the selected *Salmonella* isolates and was detected in 26% of the isolates (125/485). This PMQR gene variant was widespread among 25 different *Salmonella* serovars and was most frequently detected in

Table 1. Number of *S. enterica* and *E. coli* isolates included, selected and tested by country and their respective PMQR genes detected

Country	Isolates included	Isolates selected	Isolates tested	Isolates PMQR+	<i>qnrA</i>	<i>qnrB</i>	<i>qnrD</i>	<i>qnrS</i>	<i>aac(6')-1b-cr</i>
Belgium	2500 ^a /0 ^b	24/0	4/0	2/0	1/0	—	—	1/0	—
Czech Republic	536/0	1/0	1/0	1/0	—	1/0	—	—	—
Denmark	24526/11345	68/182	54/36	16/1	—	9/0	—	7/1	—
Finland	787/5238	10/54	1/11	1/2	—	1/0	—	0/2	—
France	0/3750	0/45	0/45	0/0	—	—	—	—	—
Germany	33185/0	194/0	67/0	33/0	1/0	24/0	—	8/0	1 ^c /0
Ireland	1151/0	46/0	3/0	1/0	—	1/0	—	—	—
Italy	850/0	10/0	10/0	10/0	—	—	7/0	3/0	2 ^d /0
Norway	279/3762	2/15	1/4	0/0	—	—	—	—	—
Poland	2091/898	14/16	14/16	14/16	—	0/1	—	14/15	—
Spain	4048/0	109/0	109/0	101/0	—	84/0	13/0	4/0	—
Netherlands	19886/6139	150/21	150/21	62/1	1/0	14/0	2/0	45/1	—
UK	571790/0	587/0	71/0	47/0	—	4/0	—	43/0	—
Total	661629/31132	1215/333	485/133	288/20	3/0	138/1	22/0	125/19	3/0

^aNumber of *S. enterica* isolates.

^bNumber of *E. coli* isolates.

^c*aac(6')-1b-cr* in combination with *qnrB6*.

^d*aac(6')-1b-cr* in combination with *qnrS1*.

human *Salmonella* Corvallis isolates mainly from the Netherlands and in human *Salmonella* Typhimurium isolates from the UK (Table 3). In addition, *qnrS1* was frequently identified in *Salmonella* Saintpaul isolates obtained from turkeys in Denmark, Poland and Germany. *Salmonella* isolates obtained from fowls, pigs, sheep, reptiles and food were also *qnrS1*-positive. In two *qnrS1*-positive isolates from Italy the concurrent presence of the *aac(6')-1b-cr* gene was demonstrated.

Six variants of *qnrB* (*qnrB2*, *qnrB4*, *qnrB6*, *qnrB7*, *qnrB12* and *qnrB19*) were identified in 138 *qnrB*-positive isolates. Most *qnrB*-positive isolates were obtained from turkeys, but *qnrB* gene variants were also identified in *Salmonella* isolates from other sources, such as humans, reptiles and fowls (Table 2). Within the *qnrB*-positive group, *qnrB2* was the most frequent variant found and was particularly prevalent in *Salmonella* Derby and *Salmonella* London obtained from turkeys in Spain (Table 3). Furthermore, *qnrB19* was most often detected in *Salmonella* isolated from turkeys in Germany and Denmark, including *Salmonella* Hadar and *Salmonella* Newport isolates (Tables 2 and 3). Some of these isolates were previously reported as *qnrB5* positive.²² However, in the present study we used an extra set of primers,²¹ and based on sequence analysis of the amplicons these isolates were now designated *qnrB19* positive. In addition, *qnrB19* was identified in a particular group of *Salmonella* isolated from reptiles in German zoos,²³ included in this study. In one case, *aac(6')-1b-cr* was detected together with *qnrB6* in a *Salmonella* isolate from a turtle.

The *qnrD* genes were detected in 22 isolates from eight different serovars noted in three different countries (Table 3). Most *qnrD*-positive isolates were identified in laying hens from Spain. However, *qnrD* genes were also detected in *Salmonella* isolates obtained in fowls, turkeys and food from Italy and in two human isolates from the Netherlands (Table 2).

Finally, the *qnrA1* gene was identified in two *Salmonella* Paratyphi B variant Java isolates from poultry products in Belgium and the Netherlands and in one *Salmonella* Typhimurium from turkeys in Germany (Table 3). None of the *Salmonella* isolates tested was positive for *qnrC* or *qepA* genes.

A small proportion of the PMQR-positive *Salmonella* isolates ($n=10$) demonstrated resistance to third-generation cephalosporins, indicative of the presence of extended-spectrum β -lactamases (ESBLs) or AmpC-type β -lactamases (data not shown).

In *E. coli*, 15% (20/133) of the selected isolates were PMQR-positive, originating from four different countries (Table 1), but mostly from Poland. The isolates were obtained from food, fowls, turkeys, cattle and pigs (Table 4). *qnrS1* was detected in 19 isolates, whereas one isolate from a turkey was found to be *qnrB19* positive. In this study, the earliest PMQR-positive *E. coli* detected were two isolates obtained in 2008 from pigs in Finland. None of the *E. coli* isolates tested was found to be positive for *qnrC*, *qnrD* or *qepA* genes. Furthermore, a single *qnrS1*-positive *E. coli* collected from a Dutch broiler exhibited resistance to third-generation cephalosporins.

In *Salmonella*, PMQR genes were identified in isolates with a wide range of MICs, but most PMQR genes were found in isolates with ciprofloxacin MICs of 0.25–1 mg/L combined with nalidixic acid MICs of 8–32 mg/L. The PMQR-positive *E. coli* isolates exhibited a narrow range of ciprofloxacin (0.25–0.5 mg/L) and nalidixic acid (4–16 mg/L) MICs.

Discussion

In general, the acquisition of PMQR genes leads to decreased susceptibility to fluoroquinolones, which affects the mutation

Table 2. Number of identified PMQR genes in *S. enterica* by country and source

Country	Environment	Food	Humans	Pigs	Fowls ^a	Reptiles	Sheep	Turkeys ^b	Unknown origin
Belgium	—	—	—	<i>qnrS1</i> (1)	<i>qnrA1</i> (1)	—	—	—	—
Czech Republic	—	—	—	<i>qnrB2</i> (1)	—	—	—	—	—
Denmark	—	<i>qnrS1</i> (1)	—	—	—	—	—	<i>qnrB19</i> (9) <i>qnrS1</i> (6)	—
Finland	—	—	—	—	—	—	—	<i>qnrB19</i> (1) ^c	—
Germany	—	<i>qnrB19</i> (2) <i>qnrS1</i> (2)	—	—	<i>qnrB19</i> (2)	<i>qnrB6</i> + <i>aac(6')</i> - <i>1b-cr</i> (1) <i>qnrB19</i> (9) <i>qnrS1</i> (1)	—	<i>qnrA1</i> (1) <i>qnrB2</i> (1) <i>qnrB19</i> (7) <i>qnrS1</i> (5)	<i>qnrB6</i> (2)
Ireland	<i>qnrB2</i> (1)	—	—	—	—	—	—	—	—
Italy	<i>qnrS1</i> + <i>aac(6')</i> - <i>1b-cr</i> (1)	<i>qnrD</i> (1) <i>qnrS1</i> + <i>aac(6')</i> - <i>1b-cr</i> (1)	—	—	<i>qnrD</i> (3)	—	<i>qnrS1</i> (1)	<i>qnrD</i> (3)	—
Poland	—	—	—	<i>qnrS1</i> (2)	<i>qnrS1</i> (9)	—	—	<i>qnrS1</i> (3)	—
Spain	—	—	—	—	<i>qnrB2</i> (2) <i>qnrD</i> (13) <i>qnrS1</i> (4)	—	—	<i>qnrB2</i> (82)	—
Netherlands	—	<i>qnrS1</i> (2)	<i>qnrB2</i> (4) <i>qnrB7</i> (1) <i>qnrB12</i> (1) <i>qnrB19</i> (4) <i>qnrD</i> (2) <i>qnrS1</i> (43)	—	<i>qnrA1</i> (1) <i>qnrB2</i> (1) <i>qnrB12</i> (1) <i>qnrB19</i> (2)	—	—	—	—
UK	—	—	<i>qnrB2</i> (2) <i>qnrB4</i> (1) <i>qnrB19</i> (1) <i>qnrS1</i> (43)	—	—	—	—	—	—

^aIncludes broilers, broiler meat and laying hens.^bIncludes turkeys and turkey meat.^c*Salmonella* obtained in turkey meat imported from Brazil.

Table 3. Number of identified PMQR genes in *S. enterica* by country and serovar

Serovar	Belgium	Czech Republic	Denmark	Finland	Germany	Ireland	Italy	Poland	Spain	Netherlands	UK
Agona	—	—	—	—	<i>qnrB2</i> (1)	<i>qnrB2</i> (1)	—	<i>qnrS1</i> (1)	—	—	<i>qnrS1</i> (1)
Anatum	—	—	—	—	—	—	—	—	—	<i>qnrS1</i> (1)	—
Braenderup	—	—	—	—	—	—	—	—	<i>qnrD</i> (2)	<i>qnrD</i> (1)	—
Bredeney	—	—	—	—	—	—	—	—	—	<i>qnrB2</i> (1)	—
Cerro	—	—	—	—	—	—	—	—	<i>qnrS1</i> (1)	—	—
Concord	—	<i>qnrB2</i> (1)	—	—	—	—	—	—	—	<i>qnrB2</i> (3)	—
Corvallis	—	—	—	—	—	—	—	—	—	<i>qnrS1</i> (34)	<i>qnrS1</i> (6)
Dabou	—	—	—	—	—	—	—	—	<i>qnrD</i> (1)	—	—
Derby	—	—	—	—	—	—	<i>qnrS1</i> (1)	—	<i>qnrB2</i> (56)	—	<i>qnrB19</i> (1)
Enteritidis	—	—	—	—	—	—	—	<i>qnrS1</i> (2)	<i>qnrB2</i> (1)	—	—
									<i>qnrD</i> (3)		
Give	—	—	—	<i>qnrB19</i> (1)	—	—	—	—	—	—	—
Goldcoast	<i>qnrS1</i> (1)	—	—	—	—	—	—	—	—	—	—
Hadar	—	—	<i>qnrB19</i> (6)	—	<i>qnrB19</i> (10)	—	—	—	<i>qnrB2</i> (2)	<i>qnrD</i> (1)	—
Infantis	—	—	—	—	—	—	—	<i>qnrS1</i> (1)	<i>qnrS1</i> (1)	<i>qnrS1</i> (1)	—
Kentucky	—	—	—	—	—	—	—	—	—	<i>qnrS1</i> (2)	—
Kingston	—	—	<i>qnrS1</i> (1)	—	—	—	—	—	—	—	—
Litchfield	—	—	—	—	<i>qnrB6</i> + <i>aac(6')</i> - <i>1b-cr</i> (1)	—	—	—	—	—	—
London	—	—	—	—	—	—	—	—	<i>qnrB2</i> (22)	—	—
Mbandaka	—	—	—	—	—	—	—	<i>qnrS1</i> (1)	—	<i>qnrB7</i> (1)	—
Meleagridis	—	—	—	—	—	—	—	—	—	<i>qnrB19</i> (1)	—
Montevideo	—	—	—	—	<i>qnrB6</i> (2)	—	<i>qnrD</i> (7)	—	<i>qnrB2</i> (3)	<i>qnrB12</i> (1)	<i>qnrS1</i> (1)
										<i>qnrS1</i> (3)	
Newport	—	—	<i>qnrB19</i> (3)	—	—	—	—	<i>qnrS1</i> (5)	—	—	—
Ohio	—	—	—	—	—	—	—	—	<i>qnrD</i> (5) <i>qnrS1</i> (1)	—	—
Paratyphi B var. Java	<i>qnrA1</i> (1)	—	—	—	—	—	—	—	—	<i>qnrA1</i> (1)	<i>qnrS1</i> (1)
										<i>qnrB19</i> (3)	
Pomona	—	—	—	—	—	—	—	—	—	—	—
Rissen	—	—	—	—	—	—	—	—	—	<i>qnrS1</i> (1)	—
Saintpaul	—	—	<i>qnrS1</i> (6)	—	<i>qnrS1</i> (7)	—	—	<i>qnrS1</i> (4)	—	<i>qnrS1</i> (1)	<i>qnrS1</i> (1)
Senftenberg	—	—	—	—	—	—	—	—	<i>qnrS1</i> (1)	<i>qnrB2</i> (1)	—
Stanley	—	—	—	—	—	—	—	—	—	<i>qnrS1</i> (2)	—
Subsp. <i>enterica</i> 4,[5],12:i:-	—	—	—	—	—	—	<i>qnrS1</i> + <i>aac(6')</i> - <i>1b-cr</i> (1)	—	—	—	—
Subsp. I rough	—	—	—	—	<i>qnrB19</i> (3)	—	—	—	—	—	—
Subsp. II	—	—	—	—	<i>qnrB19</i> (1)	—	—	—	—	—	—
Subsp. IV	—	—	—	—	<i>qnrB19</i> (2)	—	—	—	—	—	—
Typhimurium	—	—	—	—	<i>qnrA1</i> (1)	—	—	—	<i>qnrD</i> (2)	<i>qnrB12</i> (1)	<i>qnrB2</i> (1)
										<i>qnrB19</i> (2)	<i>qnrB4</i> (1)
											<i>qnrS1</i> (31)

Continued

Table 3. Continued

Serovar	Belgium	Czech Republic	Denmark	Finland	Germany	Ireland	Italy	Poland	Spain	Netherlands	UK
Uganda	—	—	—	—	<i>qnrB19</i> (1)	—	—	—	—	—	—
Urbana	—	—	—	—	<i>qnrB19</i> (3)	—	—	—	—	—	—
Virchow	—	—	—	—	—	—	—	—	—	—	<i>qnrB2</i> (1) <i>qnrS1</i> (1) <i>qnrS1</i> (1)
Virginia	—	—	—	—	—	—	<i>qnrS1 + aac(6')-1b-cr</i> (1)	—	—	—	—
Wandsworth	—	—	—	—	—	—	—	—	—	—	—

Table 4. Number of PMQR genes identified in *E. coli* by country and source

Country	Cattle	Food	Pigs	Fowls	Turkeys
Denmark	—	<i>qnrS1</i> (1)	—	—	—
Finland	—	—	<i>qnrS1</i> (2)	—	—
Poland	<i>qnrS1</i> (1)	—	<i>qnrS1</i> (3)	<i>qnrS1</i> (7)	<i>qnrB19</i> (1) <i>qnrS1</i> (4)
Netherlands	—	—	—	<i>qnrS1</i> (1)	—

prevention concentration (MPC) and may accelerate selection of fluoroquinolone-resistant mutants.^{24,25} Moreover, interactions between mutations in the QRDR and PMQR genes can result in higher MICs of ciprofloxacin.²⁶ Hence, data on the occurrence of PMQR genes are important for the surveillance of quinolone resistance in humans and animals. A limitation of our study was the potential exclusion of isolates in which both PMQR genes and chromosomal mutations were present. The quinolone resistance phenotype of these isolates is not indicative of the presence of PMQR genes. Isolates with mutations in the QRDR exhibit high-level resistance to nalidixic acid (MIC \geq 32 mg/L) in combination with reduced susceptibility to fluoroquinolones, independently of the presence of PMQR genes. Therefore, these phenotypes are not suitable for retrospective screening of MIC databases for PMQR. As a result, our screening method has probably underestimated the prevalence of PMQR genes in the total population, whilst coexistence of PMQR and mutations has been reported in Enterobacteriaceae from animals.^{27,28} Furthermore, the number of isolates included in this study depended on the number of isolates available in the strain collections, which varied considerably between countries (Table 1). Also, the number of isolates selected and tested, as well as the sources, varied between countries. The *Salmonella* strains in the collections originated from 11 countries and were isolated from humans, animals, food and the environment. The human clinical *Salmonella* isolates originated from the UK and the Netherlands only. The *E. coli* strains in the collections originated from six countries and were solely isolated from animals and food. Therefore, in-depth epidemiological analysis of the results by country and source was not appropriate. The analysis was limited to a description of the emergence and occurrence of PMQR and its characteristics instead of prevalences by source and country. Nevertheless, the strength of our study is the selection of isolates with a defined quinolone resistance phenotype in a large strain collection, resulting in a relatively high detection rate of PMQR genes within the selection, especially in *Salmonella*.

We detected *qnrS1* in a large number of serovars, particularly in human *Salmonella* Corvallis and *Salmonella* Typhimurium isolates. Eight *qnrS1*-positive *Salmonella* isolates from the UK originated from people with a travel history to South-East Asia (data not shown). These findings confirm earlier reports of human *qnrS1*-positive *Salmonella* isolates from Denmark and the UK associated with foreign travel or imported food from South-East Asia.^{29,30}

The occurrence of *qnrS1* has recently been described in four *Salmonella* Saintpaul isolates collected from turkey meat in

Germany and Poland.²² These four isolates were also included in our study and we identified 15 other *qnrS1*-positive *Salmonella* Saintpaul isolates collected from turkeys, fowls, humans and food. Our findings demonstrate further dissemination of this serovar to other sources. To our knowledge, we report the first *qnrS1* in serovars Agona, Cerro, Derby, Goldcoast, Kingston, Mbandaka, Newport, Senftenberg, Ohio and Wandsworth and *Salmonella enterica* serovar 4,[5],12:i-, indicating horizontal transmission of this PMQR gene within *S. enterica*.

The majority (86%) of the *qnrB*-positive *Salmonella* isolates were of animal origin. However, this number was highly influenced by the proportion of animal isolates in the total selection and, in particular, by the number of *qnrB*-positive turkey isolates from Spain. Nevertheless, we identified *qnrB* in *Salmonella* of animal origin in seven different European countries.

To date, the only case of a *qnrB2*-positive *Salmonella* Bredeney isolate of animal origin was reported in a Dutch broiler chicken.³¹ However, in the present study we identified *qnrB2* genes in serovars Agona, Derby, Enteritidis, Hadar, London and Montevideo (Table 3), most of them obtained from turkeys in Spain. The high number of *qnrB2*-positive *Salmonella* from Spain found in this study suggests a potential source for transmission of these genes to humans in this country. Nevertheless, reports on the occurrence of human *qnrB2*-positive *Salmonella* isolates in Spain are scarce.^{32,33} In our study, the number of *qnrB2*-positive human isolates was also low.

The presence of *qnrB19* has been described in different *Salmonella* serovars of human origin, including *Salmonella* Typhimurium isolates from the Netherlands and Italy.^{21,34} We describe two *qnrB19*-positive *Salmonella* Paratyphi B variant Java isolates obtained from chicken products and a human patient, all collected in the Netherlands in the same period (2007–08) suggesting transfer of this gene between animals and humans. In addition, to our knowledge, we report the first *qnrB19*-positive serovars: Derby, Meleagridis and Paratyphi B variant Java.

The first *qnrD* was described in *Salmonella* Kentucky and *Salmonella* Bovismorbificans isolates from humans in China.¹⁶ To date, *qnrD* has also been reported in *Morganella morganii* and *Proteus mirabilis* from Italy³⁵ and in *M. morganii* isolates from Algeria.³⁶ However, to our knowledge, this is the first description of *qnrD* in *Salmonella* isolates from both humans and animals in Europe. Remarkably, the first *qnrD* gene in our study was detected in a human isolate collected in 2003 from the Netherlands. Moreover, we identified *qnrD* in *Salmonella* from Spain collected in 2004–08 from laying hens. These findings reveal that this newly described PMQR gene had already been present in Europe for several years, being able to spread over different European countries, before it was identified in China. Moreover, there seems to be a cluster of *qnrD*-positive *Salmonella* isolates in Italy and Spain, which should be verified by additional studies.

Although *qnrA1* is the first PMQR gene described, this gene is rarely found in *Salmonella*.^{37–39} To our best knowledge, we describe the first *qnrA1* in *Salmonella* isolates of animal origin in two *Salmonella* Paratyphi B variant Java isolates collected from poultry products and in one *Salmonella* Typhimurium isolate collected from a turkey. Both *Salmonella* Paratyphi B variant Java isolates were multidrug resistant, including resistance to cefotaxime. Moreover, the Dutch isolate was confirmed ESBL-positive, harbouring a *bla*_{CTX-M-9} in addition to a *qnrA1* on

an IncHI2 plasmid (data not shown). Even though *qnr* genes have often been associated with plasmid-encoded ESBL genes,^{11,40} in our study low numbers of such isolates were found.

To date, European reports of PMQR genes in *E. coli* isolates of animal origin are scarce.^{41–43} In our study, 16 of the isolates originated from different healthy animals in Poland, suggesting a more frequent occurrence of PMQR-positive *E. coli* in this country. Almost all isolates were *qnrS1*-positive, but one isolate obtained from a turkey was found to be *qnrB19*-positive. PFGE revealed low genetic similarity of those isolates, indicating independent sources and routes of infection (data not shown). To our knowledge, we report the first *qnr* genes in *E. coli* isolates from animals in Finland (*qnrS1*) and Poland (*qnrS1* and *qnrB19*). These findings indicate that PMQR genes are infrequently present in the commensal *E. coli* in the gut flora of food-producing animals in Europe with reduced susceptibility to fluoroquinolones.

Within the selection the occurrence of *Salmonella* isolates with *aac(6′)-1b-cr* was low and in all cases coexistence with a *qnr* gene was found, as described in previous studies.^{27,28} In addition, the *aac(6′)-1b-cr* gene was not identified in any *E. coli* isolate. The low incidence of *aac(6′)-1b-cr* in our study might be explained by the inclusion criteria defined for this study. Isolates with merely *aac(6′)-1b-cr* exhibit a minor reduction in susceptibility to ciprofloxacin and therefore could have been missed in our selection.¹⁰

Despite all the PMQR genes identified, the resistance phenotype of a relatively large proportion of the tested isolates remains unexplained: 41% of the *Salmonella* and 85% of the *E. coli* isolates tested negative for all known PMQR genes. This could be due to the presence of new undetected PMQR genes or other quinolone resistance mechanisms present or inadequate selection criteria. Since all isolates included exhibited nalidixic MICs ≤ 32 mg/L, we did not look for mutations in the QRDR region. Therefore, additional analysis would be necessary to reveal other possible resistance mechanisms, such as the involvement of efflux pumps.

Summarizing, our study confirms the occurrence of PMQR-positive *Salmonella* isolates of human and animal origin in Europe since 2002. Moreover, the detection of PMQR genes in *Salmonella* obtained from animals in 10 different countries is indicative of their widespread occurrence in Europe. Within our selection, most PMQR-positive *Salmonella* isolates originated from Spain, the Netherlands, UK, Germany and Denmark and were predominantly obtained from humans, turkeys and fowls. Although these data are influenced by differences in the number of isolates per country and source, they suggest that poultry is the main source of PMQR determinants in Europe, with predominant serovars Derby, London, Saintpaul and Hadar. Furthermore, some of the human PMQR-positive *Salmonella* in this study were associated with foreign travel, particularly *qnrS1*-positive *Salmonella* Corvallis and *Salmonella* Typhimurium isolates. In addition, we report the first *qnrD* in *Salmonella* isolated outside of Asia. Our study also demonstrates the presence of PMQR genes in *E. coli* recently isolated from animals in four European countries. Despite the fact that fluoroquinolones have been used in food-producing animals since the end of the 1980s, PMQR genes occur relatively infrequently in animal isolates. This suggests that PMQR-positive isolates without additional mutations in the QRDR region are not selected

during treatment of animals with fluoroquinolones. Apparently the administration of fluoroquinolones in food-producing animals results in concentrations in the gastrointestinal tract that are higher than the MICs for PMQR-positive isolates. Co-selection through the use of other antibiotics may occur once PMQR genes are embedded in plasmids harbouring additional resistance genes. On the other hand, if PMQR genes do accelerate the selection of fluoroquinolone-resistant mutants during treatment of animals with fluoroquinolones, the focus of surveillance studies should also be the detection of PMQR genes in fluoroquinolone-resistant isolates. Moreover, long-term surveillance is needed for the future monitoring of trends in the occurrence of PMQR genes in Europe. Also, future studies are required focusing on plasmid analysis to understand more about the successful dissemination of PMQR genes in Enterobacteriaceae.

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Transparency declarations

None to declare.

Supplementary data

The questionnaire is available as Supplementary data at JAC Online (<http://jac.oxfordjournals.org/>).

References

- Hopkins KL, Davies RH, Threlfall EJ. Mechanisms of quinolone resistance in *Escherichia coli* and *Salmonella*: recent developments. *Int J Antimicrob Agents* 2005; **25**: 358–73.
- Threlfall EJ, de Pinna E, Day M *et al*. Alternatives to ciprofloxacin use for enteric fever, United Kingdom. *Emerg Infect Dis* 2008; **14**: 860–1.
- Gagliotti C, Buttazzi R, Sforza S *et al*. Resistance to fluoroquinolones and treatment failure/short-term relapse of community-acquired urinary tract infections caused by *Escherichia coli*. *J Infect* 2008; **57**: 179–84.
- Cattoir V, Nordmann P. Plasmid-mediated quinolone resistance in Gram-negative bacterial species: an update. *Curr Med Chem* 2009; **16**: 1028–46.
- Strahilevitz J, Jacoby GA, Hooper DC *et al*. Plasmid-mediated quinolone resistance: a multifaceted threat. *Clin Microbiol Rev* 2009; **22**: 664–89.
- Jacoby G, Cattoir V, Hooper D *et al*. *qnr* Gene nomenclature. *Antimicrob Agents Chemother* 2008; **52**: 2297–9.
- Robicsek A, Strahilevitz J, Jacoby GA *et al*. Fluoroquinolone-modifying enzyme: a new adaptation of a common aminoglycoside acetyltransferase. *Nat Med* 2006; **12**: 83–8.
- Yamane K, Wachino J, Suzuki S *et al*. New plasmid-mediated fluoroquinolone efflux pump, QepA, found in an *Escherichia coli* clinical isolate. *Antimicrob Agents Chemother* 2007; **51**: 3354–60.
- Zhao J, Chen Z, Chen S *et al*. Prevalence and dissemination of *oqxAB* in *Escherichia coli* isolates from animals, farmworkers, and the environment. *Antimicrob Agents Chemother* 2010; **54**: 4219–24.
- Cavaco LM, Aarestrup FM. Evaluation of quinolones for use in detection of determinants of acquired quinolone resistance, including the new transmissible resistance mechanisms *qnrA*, *qnrB*, *qnrS*, and *aac(6′)-Ib-cr*, in *Escherichia coli* and *Salmonella enterica* and determinations of wild-type distributions. *J Clin Microbiol* 2009; **47**: 2751–8.
- Robicsek A, Jacoby GA, Hooper DC. The worldwide emergence of plasmid-mediated quinolone resistance. *Lancet Infect Dis* 2006; **6**: 629–40.
- Anonymous. *ISO 20776-1:2006. Clinical Laboratory Testing and In Vitro Diagnostic Test Systems – Susceptibility Testing of Infectious Agents and Evaluation of Performance of Antimicrobial Susceptibility Test Devices – Part 1: Reference Method for Testing the In Vitro Activity of Antimicrobial Agents Against Rapidly Growing Aerobic Bacteria Involved in Infectious Diseases*. European Committee for Standardization, ISO Copyright Office, Geneva, Switzerland, 2006.
- Cavaco LM, Frimodt-Moller N, Hasman H *et al*. Prevalence of quinolone resistance mechanisms and associations to minimum inhibitory concentrations in quinolone-resistant *Escherichia coli* isolated from humans and swine in Denmark. *Microb Drug Resist* 2008; **14**: 163–9.
- 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.
- Wang M, Guo Q, Xu X *et al*. New plasmid-mediated quinolone resistance gene, *qnrC*, found in a clinical isolate of *Proteus mirabilis*. *Antimicrob Agents Chemother* 2009; **53**: 1892–7.
- Cavaco LM, Hasman H, Xia S *et al*. *qnrD*, a novel gene conferring transferable quinolone resistance in *Salmonella enterica* serovar Kentucky and Bovismorbificans strains of human origin. *Antimicrob Agents Chemother* 2009; **53**: 603–8.
- Richter SN, Frasson I, Bergo C *et al*. Characterisation of *qnr* plasmid-mediated quinolone resistance in Enterobacteriaceae from Italy: association of the *qnrB19* allele with the integron element ISCR1 in *Escherichia coli*. *Int J Antimicrob Agents* 2010; **35**: 578–83.
- Park CH, Robicsek A, Jacoby GA *et al*. Prevalence in the United States of *aac(6′)-Ib-cr* encoding a ciprofloxacin-modifying enzyme. *Antimicrob Agents Chemother* 2006; **50**: 3953–5.
- Robicsek A, Strahilevitz J, Sahm DF *et al*. *qnr* prevalence in ceftazidime-resistant Enterobacteriaceae isolates from the United States. *Antimicrob Agents Chemother* 2006; **50**: 2872–4.
- Jacoby GA, Walsh KE, Mills DM *et al*. *qnrB*, another plasmid-mediated gene for quinolone resistance. *Antimicrob Agents Chemother* 2006; **50**: 1178–82.

- 21 Garcia-Fernandez A, Fortini D, Veldman K et al. Characterization of plasmids harbouring *qnrS1*, *qnrB2* and *qnrB19* genes in *Salmonella*. *J Antimicrob Chemother* 2009; **63**: 274–81.
- 22 Cavaco LM, Korsgaard H, Sorensen G et al. Plasmid-mediated quinolone resistance due to *qnrB5* and *qnrS1* genes in *Salmonella enterica* serovars Newport, Hadar and Saintpaul isolated from turkey meat in Denmark. *J Antimicrob Chemother* 2008; **62**: 632–4.
- 23 Guerra B, Helmuth R, Thomas K et al. Plasmid-mediated quinolone resistance determinants in *Salmonella* spp. isolates from reptiles in Germany. *J Antimicrob Chemother* 2010; **65**: 2043–5.
- 24 Martinez-Martinez L, Pascual A, Garcia I et al. Interaction of plasmid and host quinolone resistance. *J Antimicrob Chemother* 2003; **51**: 1037–9.
- 25 Rodriguez-Martinez JM, Velasco C, Garcia I et al. Mutant prevention concentrations of fluoroquinolones for Enterobacteriaceae expressing the plasmid-carried quinolone resistance determinant *qnrA1*. *Antimicrob Agents Chemother* 2007; **51**: 2236–9.
- 26 Luo Y, Li J, Meng Y et al. Joint effects of topoisomerase alterations and plasmid-mediated quinolone-resistant determinants in *Salmonella enterica* Typhimurium. *Microb Drug Resist* 2010; doi:10.1089/mdr.2010.0074.
- 27 Gibson JS, Cobbold RN, Heisig P et al. Identification of Qnr and AAC(6′)-Ib-cr plasmid-mediated fluoroquinolone resistance determinants in multidrug-resistant *Enterobacter* spp. isolated from extraintestinal infections in companion animals. *Vet Microbiol* 2010; **143**: 329–36.
- 28 Ma J, Zeng Z, Chen Z et al. High prevalence of plasmid-mediated quinolone resistance determinants *qnr*, *aac(6′)-Ib-cr*, and *qepA* among ceftiofur-resistant *Enterobacteriaceae* isolates from companion and food-producing animals. *Antimicrob Agents Chemother* 2009; **53**: 519–24.
- 29 Cavaco LM, Hendriksen RS, Aarestrup FM. Plasmid-mediated quinolone resistance determinant *qnrS1* detected in *Salmonella enterica* serovar Corvallis strains isolated in Denmark and Thailand. *J Antimicrob Chemother* 2007; **60**: 704–6.
- 30 Hopkins KL, Day M, Threlfall EJ. Plasmid-mediated quinolone resistance in *Salmonella enterica*, United Kingdom. *Emerg Infect Dis* 2008; **14**: 340–2.
- 31 Veldman K, van Pelt W, Mevius D. First report of *qnr* genes in *Salmonella* in The Netherlands. *J Antimicrob Chemother* 2008; **61**: 452–3.
- 32 Gutierrez B, Herrera-Leon S, Escudero JA et al. Novel genetic environment of *qnrB2* associated with TEM-1 and SHV-12 on pB1004, an IncHI2 plasmid, in *Salmonella* Bredeney BB1047 from Spain. *J Antimicrob Chemother* 2009; **64**: 1334–6.
- 33 Herrera-Leon S, Gonzalez-Sanz R, Herrera-Leon L et al. Characterization of multidrug-resistant Enterobacteriaceae carrying plasmid-mediated quinolone resistance mechanisms in Spain. *J Antimicrob Chemother* 2011; **66**: 287–90.
- 34 Dionisi AM, Graziani C, Lucarelli C et al. Molecular characterization of multidrug-resistant strains of *Salmonella enterica* serotype Typhimurium and Monophasic variant (S. 4,[5],12:i:-) isolated from human infections in Italy. *Foodborne Pathog Dis* 2009; **6**: 711–7.
- 35 Koncan R, Mazzariol A, Kocsis B et al. First detection in Europe of plasmid-mediated fluoroquinolone resistance *qnrD* determinant. *Clin Microbiol Infect* 2010; **16**: S126.
- 36 Ramdani-Bouguessa N, Manageiro V, Ferreira E et al. Occurrence of PMQR determinants and ESBL in clinical Enterobacteriaceae isolates from an Algerian hospital. *Clin Microbiol Infect* 2010; **16**: S126.
- 37 Cattoir V, Weill FX, Poirel L et al. Prevalence of *qnr* genes in *Salmonella* in France. *J Antimicrob Chemother* 2007; **59**: 751–4.
- 38 Gunell M, Webber MA, Kotilainen P et al. Mechanisms of resistance in nontyphoidal *Salmonella enterica* strains exhibiting a nonclassical quinolone resistance phenotype. *Antimicrob Agents Chemother* 2009; **53**: 3832–6.
- 39 Murray A, Mather H, Coia JE et al. Plasmid-mediated quinolone resistance in nalidixic-acid-susceptible strains of *Salmonella enterica* isolated in Scotland. *J Antimicrob Chemother* 2008; **62**: 1153–5.
- 40 Nordmann P, Poirel L. Emergence of plasmid-mediated resistance to quinolones in Enterobacteriaceae. *J Antimicrob Chemother* 2005; **56**: 463–9.
- 41 Cerquetti M, Garcia-Fernandez A, Giufre M et al. First report of plasmid-mediated quinolone resistance determinant *qnrS1* in an *Escherichia coli* strain of animal origin in Italy. *Antimicrob Agents Chemother* 2009; **53**: 3112–4.
- 42 Hordijk J, Bosman AB, van Essen-Zandbergen A et al. *qnrB19* gene bracketed by IS26 on a 40-kilobase IncR plasmid from an *Escherichia coli* isolate from a veal calf. *Antimicrob Agents Chemother* 2011; **55**: 453–4.
- 43 Kirchner M, Wearing H, Teale C. Plasmid-mediated quinolone resistance gene detected in *Escherichia coli* from cattle. *Vet Microbiol* 2011; **148**: 234–5.