Eur J Clin Microbiol Infect Dis (2009) 28:1087–1093
DOI 10.1007/s10096-009-0753-1

ARTICLE

Detection of *Salmonella enterica* serovar Typhimurium with pUO-StVR2-like virulence-resistance hybrid plasmids in the United Kingdom

A. Herrero · M. C. Mendoza · E. J. Threlfall · M. R. Rodicio

Received: 21 December 2008 / Accepted: 22 April 2009 / Published online: 12 May 2009

Abstract The aim of this study was to investigate the presence in the United Kingdom (UK) of *Salmonella enterica* serovar Typhimurium isolates carrying pUO-StVR2-like virulence-resistance hybrid plasmids that originated from pSLT. One hundred and fifty ampicillin-resistant isolates of *S.* Typhimurium, collected in different regions of the UK during 2006, were screened for the presence of $bla_{\text{OXA-1}}$ carried by an InH-like integron (2000 bp/$bla_{\text{OXA-1}}$-*aadA1*) characteristic of pUO-StVR2. Positive isolates were tested for the presence of a large plasmid that hybridised with probes specific for the $bla_{\text{OXA-1}}$ and *spvC* genes, used as resistance and virulence markers of the hybrid plasmid, respectively. Eleven out of the 150 isolates fulfilled both criteria and were assigned to the *S.* Typhimurium pUO-StVR2 group. Nine were resistant to ampicillin, chloramphenicol, streptomycin/spectinomycin, sulfonamides and tetracycline, encoded by $bla_{\text{OXA-1}}$, *catA1*, *aadA1*-like, *sul1* and *tet*(B), respectively, and carried a pUO-StVR2-like plasmid of ca. 130 kb. Two contained hybrid plasmids of smaller size and lacked resistance(s) to chloramphenicol or chloramphenicol and tetracycline. The eleven isolates, which showed five and six closely related *Xba*I and *Bln*I profiles, respectively, were resistant to nitrofurantoin. In conclusion, multidrug-resistant *S.* Typhimurium isolates of the pUO-StVR2 group, which are endemic in Spain, were also detected in the UK, albeit with a low frequency (7.3%).

A. Herrero · M. C. Mendoza · M. R. Rodicio (✉)
Departamento de Biología Funcional, Área de Microbiología,
Universidad de Oviedo,
Julián Clavería 6,
33006 Oviedo, Spain
e-mail: rrodicio@fq.uniovi.es

E. J. Threlfall
Salmonella Reference Unit, Laboratory of Enteric Pathogens,
Health Protection Agency Centre for Infections,
61 Colindale Avenue,
London NW 5EQ, UK

Introduction

Isolates of *Salmonella enterica* serovar Typhimurium (*S.* Typhimurium) carrying a virulence-resistance hybrid plasmid, termed pUO-StVR2, were first detected in the Principality of Asturias (PA), a region in the north coast of Spain, over the 1993–2000 period [1]. By 2001–2002, these isolates had became the second most frequent multidrug-resistant (MDR) group of *S.* Typhimurium in the PA [2], only preceded by the pandemic definitive phage type (DT) 104 [3]. By 2002–2004, they were widely spread in Spain, where they can be now regarded as endemic [4]. Most isolates (>89.0%) shared the following features: (i) resistance to ampicillin, chloramphenicol, streptomycin/spectinomycin, sulfonamides and tetracycline (AMP-CHL-STR-SUL-TET phenotype) encoded by the $bla_{\text{OXA-1}}$, *catA1*, *aadA1*, *sul1* and *tet*(B) genes, respectively; (ii) a class 1 integron (InH) with the $bla_{\text{OXA-1}}$-*aadA1* gene cassettes in its variable region of ca. 2000 bp; (iii) the *spvC*, *rck*, *samA*, *oriT*, *traT*, *traX*, *repA* (RepFIIA) and *parA/B* genes (but not *repA2* (IncFIB), *rsk* and *pefABCD*) of pSLT, the virulence plasmid specific of serovar *S.* Typhimurium; and (iv) a hybrid plasmid termed pUO-StVR2 (of ca. 130 kb) where the resistance and virulence genes are located [4]. In addition, more than 50.0% of the analysed isolates shared an *Xba*I-*Bln*I pulsed field electrophoresis (PFGE) profile (termed X1-B1), a fact consistent with clonal spread of the isolates of the group. Intra-group

diversity was also observed and a total of four resistance phenotypes, five resistance genotypes, two integron profiles, four plasmid variants (termed pUO-StVR4 to pUO-StVR7, which differed from pUO-StVR2 in mass and restriction and/or resistance patterns), 15 *Xba*I-*Bln*I combined PFGE profiles and five phage types were identified [2, 4]. These results are in agreement with the evolution of pUO-StVR2, intra-group diversification and/or horizontal spread of the hybrid plasmid.

Information on the international distribution of the *S.* Typhimurium pUO-StVR2 group is not available. Isolates containing a 2000 bp/bla_{OXA-1}-*aadA1* integron and showing resistance phenotypes and macrorestriction profiles similar to those collected in Spain have been recovered in Portugal from clinical samples and food products [5]. Moreover, the 2000 bp/bla_{OXA-1}-*aadA1* integron has been detected in five AMP-CHL-STR-SUL-TET isolates recovered from patients hospitalised in Norway [6]. Although one of the isolates was reported as domestically acquired, the remaining four had been acquired in Spain, hinting a probable spread of *S.* Typhimurium pUO-StVR2 as far as Northern Europe. This possibility is a cause of concern, since MDR salmonellae, apart from the difficulties in therapeutic treatment, have been associated with increased risk of hospitalisation, invasive illness and death in different countries [7].

Within this scenario, the present study aimed to evaluate the epidemiological impact of *S.* Typhimurium pUO-StVR2-like isolates in the United Kingdom (UK). For this, a two-step procedure, which includes: (i) a presumptive test, in which randomly selected ampicillin-resistant isolates of *S.* Typhimurium are screened for the bla_{OXA-1} gene and the InH-like integron, followed by (ii) a confirmative test, based on the detection of a virulence-resistance hybrid plasmid [2, 4], was applied. Positive isolates were further characterised for intra-group and plasmid diversity, to extend the information on the routes of dissemination of the isolates and on the evolution of pUO-StVR2.

Materials and methods

S. Typhimurium isolates

A total of 150 isolates were randomly selected among ampicillin-resistant clinical isolates of *S.* Typhimurium recorded at the Health Protection Agency Centre for Infections (HPA, London) and collected during 2006 in different regions of the UK. *S.* Typhimurium LT2 (pSLT); LSP 146/02 (pUO-StVR2, bla_{OXA-1}, InH: 2000 bp/bla_{OXA-1}-*aadA1*); LSP 14/92 (bla_{PSE-1}, InC: 1200 bp/bla_{PSE-1} and InD: 1000 bp/*aadA2*); and LSP 389/97 (bla_{TEM-1}, InI: 1900 bp/*dfrA12-aadA2* and In0: 150 bp) were used as controls strains [1, 2, 8, 9].

Antimicrobial susceptibility

Antimicrobial susceptibility was determined by a breakpoint method in Sensitest agar (Oxoid). The antimicrobials tested were ampicillin, chloramphenicol, ciprofloxacin, gentamicin, nalidixic acid, nitrofurantoin, spectinomycin, streptomycin, sulfonamide, tetracyclines and trimethoprim. The results were interpreted according to the criteria of the CLSI [10].

PCR procedures and DNA sequencing

Single and multiple polymerase chain reactions (PCRs), using previously described primers and conditions [1, 2, 4], were performed for the detection of resistance genes (bla_{OXA-1}, bla_{PSE-1}, bla_{TEM-1}-like, *catA1*, *strA/B*, *aadA1*-like, *sul1*, *sul2*, *tet*(B), *merA* and *qacEΔ1*), class 1 integrons and pSLT genes (*spvC*, *rck*, *rsk*, *pefA*, *pefB*, *pefC*, *pefD*, *parA* and *parB*). The insertion of the bla_{OXA-1} and *aadA1*-like gene cassettes into variable regions of ca. 2000 bp, generated with the 5′CS/3′CS primers, was investigated by nested PCR amplification, using the 2000-bp fragment as the template and primers specific for the indicated genes. Amplicons obtained from the *gyrA*, *gyrB*, *parC* and *parE* genes of isolates resistant to nalidixic acid were sequenced at the Federal Institute for Risk Assessment (Bfr), Berlin, Germany. For sequence analysis, the BLAST and CLUSTAL W programs were used [11, 12].

Plasmid analysis and Southern hybridisation

Plasmid DNA was purified by the Kado and Liu method [13]. Plasmids ranging in size from 7 to 150 kb, extracted from *Escherichia coli* 39R861 (NCTC 50192), were used as size standards. Selected plasmid profiles were sequentially hybridised with probes specific for the *spvC* and bla_{OXA-1} genes. The probes were obtained from LT2 (*spvC*) and LSP 146/02 (bla_{OXA-1}) by PCR amplification using the PCR DIG labelling mix (Roche Applied Science), followed by gel extraction with the GFX™ DNA and Gel Band Purification Kit (Amersham Biosciences).

Genomic macrorestriction pulsed-field gel electrophoresis (PFGE) analysis and phage typing

Genomic DNA from pUO-StVR2-like positive isolates and from the control strains was independently digested with *Xba*I (Roche Diagnostics, 30 U; 4 h at 37°C) and *Bln*I (Fermentas, 20 U; 4 h at 37°C). The generated fragments were separated by PFGE, performed in the CHEF-DR III

System (Bio-Rad Laboratories) under the standardised conditions recommended by the Salm-gene project [14]. DNA from *S.* Typhimurium LT2 digested with either *Xba*I or *Bln*I was used as the size standard [15]. Phage typing of the isolates was performed at the HPA using standard techniques.

Results

Incidence of the bla_{OXA-1}-InH-like positive isolates in the UK

One hundred and fifty ampicillin-resistant isolates of *S.* Typhimurium, randomly selected for the present study, were initially tested for the type of *bla* gene and the integron profile. Total DNA from 67 (44.7%), 65 (43.3%) and 12 (8.0%) isolates generated the amplicons expected for bla_{PSE-1} (419 bp), bla_{TEM-1}-like (503 bp) and bla_{OXA-1} (708 bp), respectively, and two (1.3%) carried both bla_{PSE-1} and bla_{TEM-1}-like amplicons. In the remaining four, none of the three genes could be detected.

Ninety isolates (60.0%) carried conventional class 1 integron(s), arranged in different profiles. InC: 1200 bp/*aadA2* and InD: 1000 bp/bla_{PSE-1} were both present in all bla_{PSE-1}-positive isolates (69 isolates, including the two that also carried bla_{TEM-1}-like); InH-like: 2000 bp/bla_{OXA-1}-*aadA1*-like was found in all bla_{OXA-1}-positive isolates, either alone (11 isolates) or together with another integron with a second copy of the bla_{OXA-1} gene cassette in its ca. 1200 bp variable region (one isolate). In contrast, the majority of the bla_{TEM-1}-like positive isolates (57 out of 65; 87.7%) were negative for conventional class 1 integron(s), though six and one carried variable regions of ca. 1600 and 1400 bp, respectively. Finally, variable regions of ca. 1000 and 700 bp were both detected in a single isolate whose ampicillin resistance determinant was not identified. Accordingly, only 12 out of the 150 isolates fulfilled the criteria for presumptive assignation to the pUO-StVR2 group of *S.* Typhimurium, and these were selected for further characterisation.

Detection of pUO-StVR2-like hybrid plasmids in bla_{OXA-1}-InH-like positive isolates and assessment of diversity

The 12 bla_{OXA-1}-InH-like positive isolates contained a large plasmid with a size approximately coinciding with that expected for pUO-StVR2 (ca. 130 kb), in addition to one or more plasmids of smaller size (Fig. 1). All of the large plasmids hybridised with the bla_{OXA-1} probe and all except that carried by HPA 91/06 also hybridised with *spvC*. By this means, 11 out of the 12 isolates were shown to contain

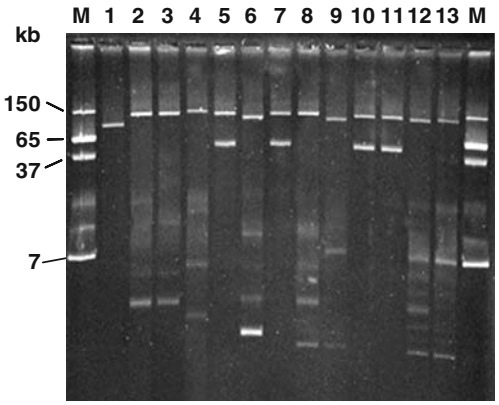

Fig. 1 Plasmid detection in *Salmonella enterica* serovar Typhimurium isolates from the UK positive for the 2000 bp/bla_{OXA-1}-*aadA1*-like integron. Lanes 1 to 13: *S.* Typhimurium LT2 (pSLT), HPA 5/06, HPA 52/06, HPA 91/06, HPA 104/06, HPA 107/06, HPA 112/06, HPA 113/06, HPA 127/06, HPA 154/06, HPA 155/06, HPA 156/06 and HPA 157/06, respectively. M: molecular size standard. The largest fragments in all lanes, except lanes 1 and 4, were identified as pUO-StVR2-like hybrid plasmids by Southern hybridisation (not shown)

a hybrid plasmid and were assigned to the *S.* Typhimurium pUO-StVR2 group, whose incidence in the UK corresponds to 7.3% of the analysed isolates. As expected, the *spvC* probe, but not bla_{OXA-1}, hybridised with the 94 kb plasmid detected in *S.* Typhimurium LT2, which was included as the control.

When the 11 isolates were tested for antimicrobial susceptibility, three distinct resistance phenotypes were identified, associated with four resistance genotypes (Table 1). Nine isolates showed the AMP-CHL-STR-SUL-TET phenotype and carried the bla_{OXA-1}, *catA1*, *aadA1*-like, *sul1* and *tet*(B) genes characteristic of pUO-StVR2. One of them was also positive for the *strA/B* and *sul2* genes, which, respectively, confer resistance to streptomycins, like *aadA1*-like (that also gives resistance to spectinomycin), and sulfonamides, like *sul1*. Two other isolates were AMP-STR-SUL-TET (HPA 107/06) or AMP-STR-SUL (HPA 127/06), and lacked *catA1* or *catA1* and *tet*(B), respectively. HPA 127/06 was also resistant to nalidixic acid, due to a GAC into AAC mutation in the chromosomal *gyrA* gene, which changed Asp87 into Asn. In contrast, mutations in *gyrB*, *parC* and *parD* were not identified. HPA 127/06 showed reduced susceptibility to ciprofloxacin (CIP_L; MIC 0.25–1.0 mg/L). The resistance profile of the outgroup HPA 91/06 isolate differed from that of HPA 127/06 by additional resistances to tetracycline (*tet*(B)) and gentamicin (although the responsible gene was not identified). It is also of note that all isolates belonging to the *S.* Typhimurium pUO-StVR2 group, but not HPA 91/06, were resistant to nitrofurantoin. With regard to pSLT genes, *spvC*, *parA*, *parB*, *rck*, *rsk* and *pefABCD* did not amplify when DNA from HPA 91/06

was used as the template. In contrast, the 11 isolates of the *S.* Typhimurium pUO-StVR2 group were positive for *spvC*, *parA*, *parB* and *rck*, but lacked *pefABCD* and *rsk*, as expected.

The *S.* Typhimurium pUO-StVR2-like isolates were also subjected to macrorestriction analysis with *Xba*I and *Bln*I, followed by PFGE (Fig. 2; Table 1). Using this method, they could be distributed into five closely related *Xba*I profiles: X1, X2, X12 (already described) [1, 2, 4], X16 and X17 (of new description), and six closely related *Bln*I profiles: B1, B2, B8, B17, B18 and B19 (with the last three also being of new description). The outgroup strains *S.* Typhimurium LT2 and HPA 91/06 showed clearly distinct profiles (X0-B0 and X18-B20, respectively). A relatively high diversity was revealed by phage typing of the *S.* Typhimurium pUO-StVR2-like isolates, with four, three, two, one and one assigned to DT120, U311, DT93, U288 and U302, respectively.

Discussion

A two-step procedure, consisting of the screening of ampicillin-resistant isolates of *S.* Typhimurium for the $bla_{\text{OXA-1}}$ gene carried by an InH-like integron, followed by the detection of a virulence-resistance hybrid plasmid among the positive isolates, proved to be valuable for the epidemiological surveillance of *S.* Typhimurium pUO-StVR2-like isolates in the UK. Using this method, 7.3% of the isolates tested (11 out of 150) obtained in geographically dispersed regions could be assigned to the group. In Spain, its incidence was remarkably higher, accounting for 26.9 and 42.5% of the ampicillin-resistant *S.* Typhimurium isolates recovered in the PA during 2001–2002 and in the whole country during 2002–2004, respectively [2, 4]. The latter figures reflect an efficient spread of the isolates carrying pUO-StVR2-like plasmids, causing concern about their detection in the UK.

Table 1 Differential features of *Salmonella enterica* serovar Typhimurium isolates from the UK carrying an integron with the 2000 bp/$bla_{\text{OXA-1}}$-*aadA1*-like variable region

Resistance phenotype (*N*)	Plasmid type; size	PFGE profiles	Phage type (*N*)
Resistance genotype (*N*)		*Xba*I-*Bln*I (*N*)	Strain(s)[a]
AMP-CHL-STR-SUL-TET-FUR (9)	VR; ca. 130 kb	X1B1 (2)	U288
$bla_{\text{OXA-1}}$-*catA1*-*aadA1*-like-*sul1*-*tet*(B)-nd (8)			HPA 113/06
			U311
			HPA 157/06
		X1–B8 (2)	DT120 (2)
			HPA 154/06
			HPA 155/06
		X16–B19 (2)	DT120 (2)
			HPA 104/06
			HPA 112/06
		X17B17	U311
			HPA 156/06
		X2–B2	DT93
			HPA 5/06
$bla_{\text{OXA-1}}$-*catA1*-[*aadA1*-like-*strA/B*]-[*sull*-*sul2*]-*tet*(B)-nd		X2–B2	DT93
			HPA 52/06
AMP-STR-SUL-TET-FUR	VR; <130 kb	X12–B18	U302
$bla_{\text{OXA-1}}$-*aadA1*-like-*sul1*-*tet*(B)-nd			HPA 107/06
AMP-STR-SUL-FUR-NAL-CIP$_{\text{L}}$/	VR; <130 kb	X17–B18	U311
$bla_{\text{OXA-1}}$-*aadA1*-like-*sul1*-nd-*gyrA* (Asp87 to Asn)			HPA 127/06
AMP-GEN-STR-SUL-TET-NAL-CIP$_{\text{L}}$	R; >130 kb	X18–B20	NT
$bla_{\text{OXA-1}}$-nd-*aadA1*-like-*sul1*-*tet*(B)-*gyrA*$^{\text{Asp87 to Asn}}$			HPA 91/06[b]

N = number of isolates with the corresponding feature when there was more than one; AMP = ampicillin; CHL = chloramphenicol; CIP = ciprofloxacin; FUR = nitrofurantoin; GEN = gentamicin; NAL = nalidixic acid; STR = streptomycin; SUL = sulfonamide; TET = tetracycline; L = low; nd = not determined. Genes between square brackets confer resistance to the same antimicrobial (STR or SUL)

[a] All strains carried an InH-like integron (2000 bp/$bla_{\text{OXA-1}}$-*aadA1*) and HPA 157/06 contained a second integron (1200 bp/$bla_{\text{OXA-1}}$). NT = non-typeable

[b] Outgroup strain

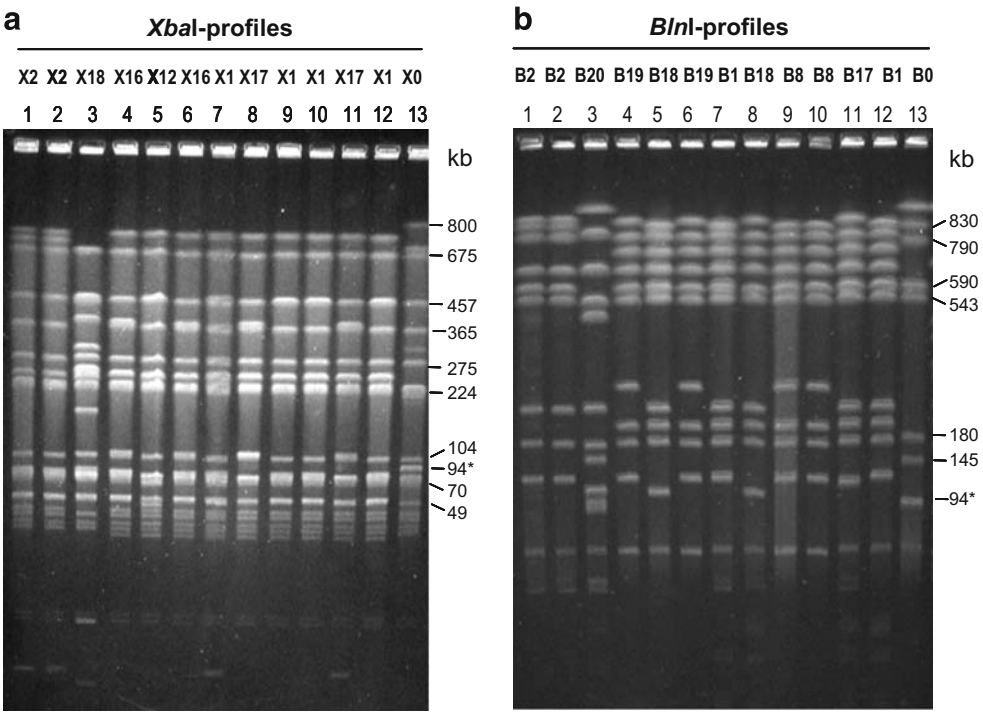

Fig. 2 *Xba*I (A) and *Bln*I (B) macrorestriction-PFGE analysis of the *Salmonella enterica* serovar Typhimurium isolates from the UK, positive for the 2000 bp/bla_{OXA-1}-*aadA1*-like integron. Lanes 1 to 13: HPA 5/06, HPA 52/06, HPA 91/06 (outgroup strain), HPA 104/06, HPA 107/06, HPA 112/06, HPA 113/06, HPA 127/06, HPA 154/06, HPA 155/06, HPA 156/06, HPA 157/06 and *S.* Typhimurium LT2, respectively. The *Xba*I and *Bln*I profiles of the latter strain (X0 and B0) were used as molecular size standards. The 94 kb fragment, which corresponds to pSLT, is marked with an *asterisk* in the two profiles

Like in Spain, the resistance pattern (AMP-CHL-STR-SUL-TET/bla_{OXA-1}-*catA1*-*aadA1*-like-*sul1*-*tet*(B)) characteristic of pUO-StVR2 (ca. 130 kb) was clearly predominant in the UK (eight of the 11 isolates of the *S.* Typhimurium pUO-StVR2 group). However, AMP-STR-SUL-TET/bla_{OXA-1}-*aadA1*-like-*sul1*-*tet*(B) and AMP-STR-SUL-NAL-CIP_L/bla_{OXA-1}-*aadA1*-like-*sul1*-*gyrA*$^{Asp87\ to\ Asn}$ variants were also detected. The corresponding isolates carried smaller hybrid plasmids (of ca. 125 kb), which have probably originated from deletions affecting either the *catA1* or the *catA1* and *tet*(B) gene(s) of pUO-StVR2. Two variants lacking *catA1* and *tet*(B) and carrying deleted versions of pUO-StVR2, termed pUO-StVR6 and pUO-StVR7, were also recorded in Spain [4]. Since the procedure applied to identify members of the pUO-StVR2 group preselects for ampicillin resistance, possible derivatives lacking bla_{OXA-1} will escape detection.

Macrorestriction-PFGE strongly supports a clonal relationship between the UK isolates, since the profiles revealed by digestions with *Xba*I or *Bln*I were identical or nearly identical. Moreover, they were also identical or very similar to the predominant profiles that are circulating in Spain (X1 and X2; B1 and B2) [2, 4]. In contrast, a relatively high diversity was revealed by phage typing. The 11 isolates were assigned to five phage types, with four, three and two being DT120, U311 and DT93, respectively. In Spain, most of the analysed isolates were NT or DT104b, although DT120 was also found. If the UK isolates are clonally related, changes in phage susceptibility, such as those reported by Lawson et al. [16], might be relatively frequent.

Of particular interest is the finding that all isolates of the pUO-StVR2 group detected in the UK were resistant to nitrofurantoin. Nitrofurans have been applied for nearly five decades in the treatment of certain bacterial and protozoal infections in humans and animals, and have also been used extensively as growth promoters for food-producing animals. The latter application has been banned in the European Community since the mid-1990s [17, 18], because of the potential carcinogenic properties of these drugs and the risk of residues in food products. In the UK, Rampling et al. [19] suggested that the widespread use of nitrofurans in the poultry industry before the 1990s may have contributed to the prevalence of *S.* Enteritidis PT4 in chickens and, consequently, to the overall increase of human salmonellosis around that time. In Portugal, a remarkable incidence of *Salmonella* isolates from several sources (predominantly humans and poultry) with decreased susceptibility to nitrofurantoin (MIC ≥64 mg/L) has been reported [20]. Most of them belonged to *S.* Enteritidis, but decreased susceptibility to nitrofurantoin was also observed among MDR isolates of *S.* Typhimurium. It has been suggested that the illegal use of nitrofurans after being banned might have contributed not only to the selection and prevalence of *S.* Enteritidis in food animals and humans, but also to the emergence of two MDR clones of *S.* Typhimurium which are widespread in Portugal. The first is the pandemic DT104, whereas the second carries an integron with the same variable region as InH and shows resistance phenotypes and macrorestriction profiles similar to those identified in Spain [7]. Of the 150

S. Typhimurium isolates from the UK that were analysed in the present work, only those assigned to the pUO-StVR2 group and a single isolate carrying bla_{TEM-1}-like amplicons were resistant to nitrofurantoin. These data prompted a retrospective study in which 44 Spanish isolates of the pandemic DT104 group and 64 Spanish isolates of the pUO-StVR2 group, including the oldest, were tested for susceptibility to this antimicrobial. Using a disk diffusion method (300 μg) and the interpretative criteria of the CLSI [10], all isolates of the latter group proved to be fully resistant to nitrofurantoin. In contrast, 42 DT104 isolates showed intermediate resistance and only two were resistant (A. Herrero, unpublished results). Accordingly, resistance to nitrofurantoin could assist the epidemiological surveillance of the *S.* Typhimurium pUO-StVR2-like isolates, at least in the UK and Spain.

Taken together, the results of the present and previous investigations support that pUO-StVR2 might have originated from pSLT within a *S.* Typhimurium nitrofurantoin-resistant isolate with the X1 macrorestriction profile. In fact, both features were shared by the earliest isolates of the group, detected in the PA over 1993–1999. Further dissemination of *S.* Typhimurium isolates carrying pUO-StVR2-like plasmids to other countries, including the UK, might have occurred through human travel and/or by the import of contaminated food products, as previously proposed for resistant isolates of *S.* Enteritidis PT1, *S.* Typhimurium DT104 and *S.* Virchow [21–23]. It will be interesting to see whether the measures adopted by the European Union in recent years to prevent food-borne zoonoses and to combat the threat of antimicrobial resistance [24–26] will be sufficient to stop further dissemination of the *S.* Typhimurium pUO-StVR2 group.

Acknowledgements This work was mostly performed during a short-term visit of A. Herrero at the Health Protection Agency (HPA). We are most grateful to the staff of the Health Protection Agency Centre for Infections, especially to Dr. K. Hopkins, for their valuable help. We also thank Dr. B. Guerra (Federal Institute for Risk Assessment, Berlin, Germany) for the sequencing of the *gyr* and *par* genes, helping with the hybridisation experiments and the useful comments. A. Herrero was the recipient of a grant from the Fundación para el Fomento en Asturias de la Investigación Científica Aplicada y la Tecnología (FICYT, ref. BP03-014). The work in Spain was supported by project FIS PI080656 from the Spanish Ministry of Science and Innovation.

References

1. Guerra B, Soto S, Helmuth R et al (2002) Characterization of a self-transferable plasmid from *Salmonella enterica* serotype Typhimurium clinical isolates carrying two integron-borne gene cassettes together with virulence and drug resistance genes. Antimicrob Agents Chemother 46:2977–2981. doi:10.1128/AAC.46.9.2977-2981.2002
2. Herrero A, Rodicio MR, González-Hevia MA et al (2006) Molecular epidemiology of emergent multidrug-resistant *Salmonella enterica* serotype Typhimurium strains carrying the virulence resistance plasmid pUO-StVR2. J Antimicrob Chemother 57:39–45. doi:10.1093/jac/dki400
3. Threlfall EJ (2000) Epidemic *Salmonella typhimurium* DT 104—a truly international multiresistant clone. J Antimicrob Chemother 46:7–10. doi:10.1093/jac/46.1.7
4. Herrero A, Rodicio MR, Echeita MA et al (2008) *Salmonella enterica* serotype Typhimurium carrying hybrid virulence-resistance plasmids (pUO-StVR): a new multidrug-resistant group endemic in Spain. Int J Med Microbiol 298:253–261. doi:10.1016/j.ijmm.2007.04.008
5. Antunes P, Machado J, Sousa JC et al (2004) Dissemination amongst humans and food products of animal origin of a *Salmonella typhimurium* clone expressing an integron-borne OXA-30 β-lactamase. J Antimicrob Chemother 54:429–434. doi:10.1093/jac/dkh333
6. Lindstedt B-A, Heir E, Nygård I et al (2003) Characterization of class I integrons in clinical strains of *Salmonella enterica* subsp. *enterica* serovars Typhimurium and Enteritidis from Norwegian hospitals. J Med Microbiol 52:141–149. doi:10.1099/jmm.0.04958-0
7. Helms M, Ethelberg S, Mølbak K; DT104 Study Group (2005) International Salmonella Typhimurium DT104 infections, 1992–2001. Emerg Infect Dis 11:859–867
8. McClelland M, Sanderson KE, Spieth J et al (2001) Complete genome sequence of *Salmonella enterica* serovar Typhimurium LT2. Nature 413:852–856. doi:10.1038/35101614
9. Guerra B, Soto SM, Argüelles JM et al (2001) Multidrug resistance is mediated by large plasmids carrying a class 1 integron in the emergent *Salmonella enterica* serotype [4,5,12:i:-]. Antimicrob Agents Chemother 45:1305–1308. doi:10.1128/AAC.45.4.1305-1308.2001
10. Clinical and Laboratory Standards Institute (CLSI) (2005) Performance standards for antimicrobial susceptibility testing—15th informational supplement. Approved standard M100–S15. CLSI, Wayne, PA
11. Altschul SF, Madden TL, Schäffer AA et al (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 25:3389–3402. doi:10.1093/nar/25.17.3389
12. Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res 22:4673–4680. doi:10.1093/nar/22.22.4673
13. Kado CI, Liu ST (1981) Rapid procedure for detection and isolation of large and small plasmids. J Bacteriol 145:1365–1373
14. Peters TM, Maguire C, Threlfall EJ et al (2003) The Salm-gene project: a European collaboration for DNA fingerprinting for food-related salmonellosis. Euro Surveill 8:46–50
15. Liu S-L, Hessel A, Sanderson KE (1993) The *Xba*I-*Bln*I-*Ceu*I genomic cleavage map of *Salmonella typhimurium* LT2 determined by double digestion, end labelling, and pulsed-field gel electrophoresis. J Bacteriol 175:4104–4120
16. Lawson AJ, Dassama MU, Ward LR et al (2002) Multiply resistant (MR) *Salmonella enterica* serotype Typhimurium DT 12 and DT 120: a case of MR DT 104 in disguise? Emerg Infect Dis 8:434–436
17. Council Regulation (EEC) No. 2901/93 of 18 October 1993 amending Annexes I, II, III and IV to Regulation (EEC) No. 2377/90 laying down a Community procedure for the establishment of maximum residue limits of veterinary medicinal products in foodstuffs of animal origin. Official Journal L 264:1–4

18. Council Regulation (EEC) No. 1442/95 of 26 June 1995 on amending Annexes I, II, III and IV of Council Regulation (EEC) No. 2377/90 laying down a Community procedure for the establishment of maximum residue limits of veterinary medicinal products in foodstuffs of animal origin. Official Journal L 143:26–30
19. Rampling A, Upson R, Brown DFJ (1990) Nitrofurantoin resistance in isolates of *Salmonella enteritidis* phage type 4 from poultry and humans. J Antimicrob Chemother 25:285–290. doi:10.1093/jac/25.2.285
20. Antunes P, Machado J, Peixe L (2006) Illegal use of nitrofurans in food animals: contribution to human salmonellosis? Clin Microbiol Infect 12:1047–1049
21. Davis MA, Hancock DD, Besser TE (2002) Multiresistant clones of *Salmonella enterica*: the importance of dissemination. J Lab Clin Med 140:135–141. doi:10.1067/mlc.2002.126411
22. Threlfall EJ, Day M, de Pinna E et al (2006) Assessment of factors contributing to changes in the incidence of antimicrobial drug resistance in *Salmonella enterica* serotypes Enteritidis and Typhimurium from humans in England and Wales in 2000, 2002 and 2004. Int J Antimicrob Agents 28:389–395. doi:10.1016/j.ijantimicag.2006.07.009
23. Meakins S, Fisher IST, Berghold C et al (2008) Antimicrobial drug resistance in human nontyphoidal *Salmonella* isolates in Europe 2000–2004: a report from the Enter-net International Surveillance Network. Microb Drug Resist 14:31–35. doi:10.1089/mdr.2008.0777
24. European Union Council Directive 2003/99/EC of the European Parliament and of the Council of 17th of November 2003 on the monitoring of zoonoses and zoonotic agents amending Council Decision 90/424/EEC and repealing Council Directive 92/117/EEC. Official Journal L 325:31–40
25. European Union Regulation No. 2160/2003 on the control of salmonella and other specified food-borne zoonotic agents. Official Journal L 325:1–15
26. Bronzwaer S, Lönnroth A, Haigh R (2004) The European Community strategy against antimicrobial resistance. Euro Surveill 9:30–34