Molecular epidemiology of emergent multidrug-resistant
Salmonella enterica serotype Typhimurium strains carrying the
virulence resistance plasmid pUO-StVR2

A. Herrero1, M. R. Rodicio1, M. A. González-Hevia2 and M. C. Mendoza1*

1Departamento de Biología Funcional, Área de Microbiología, Universidad de Oviedo, C/Julián Clavería 6, 33006 Oviedo, Spain; 2Laboratorio de Salud Pública, Oviedo, Spain

Received 21 July 2005; accepted 5 October 2005

Objectives: To evaluate the incidence of a distinct multidrug-resistant (MDR) grouping of Salmonella serotype Typhimurium strains carrying the hybrid virulence resistance plasmid pUO-StVR2, and its possible evolution in the region where it was first detected [Principality of Asturias (PA), Spain].

Methods: pUO-StVR2-containing isolates were tentatively identified by two genetic markers: the blaOXA-39 gene and the class 1 integron InH:2000 bp/blaOXA-39-8aadA1a. Positive isolates were examined for resistance profile (RP), plasmid content, virulence profile (VP) and genomic polymorphisms using macrorestriction–PFGE.

Results: A total of 182 out of 248 Typhimurium clinical isolates recorded in the PA over 2001–02 were ampicillin-resistant and could be distributed into several MDR groupings. A MDR grouping carrying pUO-StVR2, with a defined RP (AMP/blaOXA-39, CHL/catA1, [STR-SPT]/[strA/B,aadA1a], SUL/[sul1,sul2], TET/tet(B), qacEDA1, merA, TMP/dfrA12, and containing InH), was represented by 49 isolates. The VPs of these isolates (24 genes screened) differed from that of the type strain LT2 by the absence of the sopE1 and pef genes. Macrorestriction analysis established six combined XbaI/BlnI PFGE profiles, and supported a clonal relationship among most of the isolates.

Conclusions: During 2001–02, the isolates carrying pUO-StVR2 constituted the second most frequent S. Typhimurium MDR grouping recorded in the PA, preceded only by the pandemic pentaresistant DT104. Polymorphisms on the genomic DNA, different phage types, different plasmid profiles and the detection of trimethoprim resistance in one isolate encoded by an additional plasmid, were consistent with both intra-cluster evolution and horizontal transfer of the hybrid plasmid.

Keywords: multidrug resistance, hybrid plasmid, PFGE, virulence genotype

Introduction

Salmonella enterica serotype Typhimurium (S. Typhimurium) is one of the most common bacteria causing food-borne disease in developed countries.1 As with many other non-typhoid serotypes, infection usually results in self-limited gastroenteritis, which does not require antimicrobial therapy. However, bacteria can occasionally be invasive, and even fatal, particularly for patients with underlying risk factors, such as immunosuppression. In these cases, effective antimicrobial therapy is necessary. A wide diversity of virulence (V) factors have been described for Salmonella, and they can be located on the bacterial chromosome, frequently as part of pathogenicity islands, on plasmids and prophages.2–4 The V plasmid of S. Typhimurium (pSLT) has a size of 94 kb, belongs to the incompatibility group IncFI and contains the spvABCDR locus (responsible for an increase in the bacterial growth rate in mice during the systemic phase of disease), the pefBACDI operon (for biosynthesis of fimbriae involved in adherence to the intestinal epithelium) and the rck and rsk genes (resistance to complement killing), in addition to genes encoding for essential plasmid functions (such as plasmid replication and maintenance).4,5 S. Typhimurium LT2 (the type strain of Typhimurium) is susceptible to antimicrobial drugs, as were the majority of Salmonella strains circulating until the 1980s. Since then, multidrug-resistant (MDR) groupings have emerged and, nowadays, isolates pertaining to them are far more frequent than drug-susceptible isolates.1,6 A MDR clone, defined as S. Typhimurium

*Corresponding author. Fax: +34-985103148; E-mail: cmendoza@uniovi.es

© The Author 2005. 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
DT104, with resistance (R) to ampicillin, chloramphenicol–
florfenicol, streptomycin–spectinomycin, sulfonamides and
and tetracyclines (AMP-CHL-STR-SUL-TEM phenotype), was
initially detected in animals and later in humans. Today it is widely
spread and could be considered as pandemic. In this MDR
close, the genes encoding the five antimicrobial-R [blaOXA-1,
blaTEM, catA1, strA/B, adaA1a, sul1, sul2, tetB], dfrA1 and
dfrA12] and class 1 integrons. Primers for other plasmid and
chromosomal V genes were designed for the present work: pElB
(GGAAGAACGATCCTATGTCGTGUCC), pFd (CTTATAGGCTACCGGCAAGCCGACGATTTCT),
parX (GAATGCACCTGACACCAACAGGAGCATCTACAT
GCTTACGAG), sodCI (CCAGTGGAGACGTGTTAATC
GGTGCGCTATCGTGTTC), orgA (GATAAGACGTCTAA
TTGATAAGCCGAAGCAAGCATTG), tnrC (GTTCGCCGAC
AACATATTCTTTC/TACGAGATATATATTACGAGC
GGCATGATGGTG), msdG (AGGTGTACAACTGCTTGC
CTTCGAAGATGCGCATGCTCGTGC), spI4R (GATATTAGT
TCATGCCTACAGACCAATCTCAGAGGATATGTCG), spI4D
(GAATAGAAGACAAAAAGCGCACTACGTGGTTAAT
AGCGCTTCGCAAGCCAAC
ATCAAAACTACACTCA) and pipA (CCTCTTGGATGATTTCC
TTTCTTTA/TTTATCTCAGCGCGGCGGTTG).

For a correct description of the type of blaOXA gene carried by pUO
SV2R2/NiH, the 708 bp amplicons obtained with the blaOXA primers
from LSP 319/3 and LSP 153/02 were sequenced. In both cases the
gene was identified as blaOXA_30. Confirmation of the insertion of
blaOXA_30 and adaA1a into the 2000 bp amplicons generated with
the 5′/3′/5′ primers was achieved by nested-PCR, using the
2000 bp amplicon as the template DNA, and primers specific for
the blaOXA_30 and adaA1a genes.

Plasmid analysis and Southern hybridization
Plasmid DNA was routinely purified by the method of Kado and Liu.
However, for a better resolution of large plasmids with a similar size,
plasmid extraction from selected isolates was also performed by
S1-PFGE. Plasmids ranging in size from 7 to 150 kb extracted from
Escherichia coli 39R601 (NCTC 50192) and λ, Ladder PFG
Marker (New England BioLabs) were used as molecular size standards
for undigested and S1-digested DNA, respectively. Selected plasmid
profiles were sequentially hybridized with probes specific for
spvC, blaOXA_30 and dfrA12. The probes were obtained from LT2 (spvC),
LSP 319/3 (blaOXA_30) and LSP 174/01 (dfrA12) by PCR amplification
using DIG-labelled dNTPs (PCR DIG labelling mix; Roche Applied
Science), followed by gel extraction and purification with the GFX
DNA and Gel Band Purification Kit (Amersham Biosciences).
Molecular epidemiology of multidrug-resistant S. enterica

Table 1. Features of ampicillin-resistant Salmonella serotype Typhimurium isolates and control strains

<table>
<thead>
<tr>
<th>Grouping (no.)</th>
<th>Antigenic formula</th>
<th>bla gene</th>
<th>In profilea</th>
<th>Phage type (no.)</th>
<th>Originb</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1 (91)</td>
<td>[4,(5),12;i:1,2]</td>
<td>blaPSE</td>
<td>In-P1:1200 + 1000c</td>
<td>DT104 (41)</td>
<td>B: 1  F: 40  AF: -</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>U302 (36)</td>
<td>B: 1  F: 35  AF: -</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>NT (7)</td>
<td>B: -   F: 7    AF: -</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>RDNC (4)</td>
<td>B: -   F: 4    AF: -</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>others (3)d</td>
<td>B: -   F: 3    AF: -</td>
</tr>
<tr>
<td>G2 (10)</td>
<td>[4,(5),12;i:-]</td>
<td>blaTEM</td>
<td>In-P2:1900 ± 150e</td>
<td>DT 193 (5)</td>
<td>B: -   F: 5    AF: -</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>DT U302 (4)</td>
<td>B: -   F: 4    AF: -</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>NT (1)</td>
<td>B: -   F: 1    AF: -</td>
</tr>
<tr>
<td>G3 (49)</td>
<td>[4,(5),12;i:1,2]</td>
<td>blaOXA</td>
<td>In-P3:2000f</td>
<td>RDNC (26)</td>
<td>B: 1  F: 24  AF: 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>NT (9)</td>
<td>B: -   F: 9    AF: -</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>DT104 (4)</td>
<td>B: 1  F: 3    AF: -</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>others (10)e</td>
<td>B: 2   F: 8    AF: -</td>
</tr>
<tr>
<td>Others (32)</td>
<td>[4,(5),12;i:1,2]</td>
<td>blaTEM</td>
<td>In-P4:1600</td>
<td>U302 (1)</td>
<td>B: -   F: 1    AF: -</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>DT 193 (13)</td>
<td>B: -   F: 13   AF: -</td>
</tr>
<tr>
<td></td>
<td>[4,(5),12;i:1,2]</td>
<td>blaTEM</td>
<td>-</td>
<td>NT (5)</td>
<td>B: -   F: 5    AF: -</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>others (7)b</td>
<td>B: -   F: 7    AF: -</td>
</tr>
<tr>
<td></td>
<td>[4,(5),12;i:1,2]</td>
<td>NI</td>
<td>-</td>
<td>RDNC (4)</td>
<td>B: -   F: 4    AF: -</td>
</tr>
<tr>
<td></td>
<td>[4,(5),12;i:-]</td>
<td>blaTEM</td>
<td>-</td>
<td>DT208 (1)</td>
<td>B: -   F: 1    AF: -</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>PT195 (1)</td>
<td>B: -   F: 1    AF: -</td>
</tr>
<tr>
<td>Controls</td>
<td></td>
<td></td>
<td></td>
<td>RDNC</td>
<td>B: -   F: -    AF: -</td>
</tr>
<tr>
<td>LT2</td>
<td>[4,(5),12;i:1,2]</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>B: -   F: -    AF: -</td>
</tr>
<tr>
<td>LSP 14/92 (G1)</td>
<td>[4,(5),12;i:1,2]</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>B: -   F: -    AF: -</td>
</tr>
<tr>
<td>LSP 389/97 (G2)</td>
<td>[4,(5),12;i:-]</td>
<td>blaPSE</td>
<td>In-P1:1000 + 1200c</td>
<td>DT104</td>
<td>B: -   F: -    AF: -</td>
</tr>
<tr>
<td>LSP 31/93 (G3)</td>
<td>[4,(5)12;i:1,2]</td>
<td>blaTEM</td>
<td>In-P2:1900 + 150e</td>
<td>DTU302</td>
<td>B: -   F: -    AF: -</td>
</tr>
<tr>
<td></td>
<td>[4,(5)12;i:1,2]</td>
<td>blaOXA</td>
<td>In-P3:2000f</td>
<td>DT104b</td>
<td>B: -   F: -    AF: -</td>
</tr>
<tr>
<td></td>
<td>[4,(5),12;i:-]</td>
<td>blaTEM</td>
<td>In-P4:1600</td>
<td>-</td>
<td>B: -   F: -    AF: -</td>
</tr>
<tr>
<td></td>
<td>[4,(5),12;i:-]</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>B: -   F: -    AF: -</td>
</tr>
<tr>
<td></td>
<td>[4,(5),12;i:-]</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>B: -   F: -    AF: -</td>
</tr>
</tbody>
</table>

No., number of isolates; NI, not identified; NT, non-typeable; RDNC, reaction does not conform.
aSize in bp of the amplicon generated by 5’CS/3’CS primers.
bNumber of isolates from blood (B), faeces (F) and ascitic fluid (AF).
cAssociated with InC and InD.10
dNI (1), DT312 (1) and DT193 (1).
 eAssociated with InI and In0.10
fAssociated with InH.10
gDT104b (2), DT193 (2), DT208 (2), DT27 (1), DT160 (1), DT 41 (1) and DTU302 (1).
hDT208 (2), U302 (2), DT104 (2) and NI (1).

Results

S. Typhimurium ampicillin resistance in the Principality of Asturias

A total of 1427 S. enterica isolates causing human disease were recorded at the LSP over the period 2001–02 (702 in 2001 and 725 in 2002), corresponding to rates of 66.2 and 68.4 per 105 inhabitants/year, during 2001 and 2002, respectively. Of them, 248 belonged to S. Typhimurium [rates of 10.2 and 13.2 per 105 inhabitants/year, during 2001 (108 isolates) and 2002 (140 isolates), respectively]. Regarding the antigenic formula the S. Typhimurium isolates could be subdivided into typical or biphasic [4,(5),12;i:1,2] and atypical or monophasic [4,(5),12;i:-] variants, with 231 and 17 isolates, respectively.

Regarding ampicillin, 182 (73.4%) out of the 248 S. Typhimurium isolates were resistant. All of them were tested for the presence of three bla genes and for In profile by PCR procedures. Results showed that 91, 37 and 49 isolates generated the expected amplicons for blaPSE (419 bp), blaTEM (503 bp) and blaOXA (708 bp), respectively, while in the remaining five, the ampicillin-R determinants were not identified. In addition, 151 isolates were In-positive, and could be differentiated into four In profiles, In-P1 (1000 + 1200 bp); In-P2 (1900 – 150 bp); In-P3 (2000 bp); and In-P4 (1600 bp), with 91, 10, 49 and 1 isolate(s), respectively.

Both traits, bla gene and In profile, together with the antigenic formula, were used to discriminate the isolates into three major groupings: G1 (blaPSE, In-P1), G2 (blaTEM, In-P2) and G3 (blaOXA, In-P3), apart from other, as yet undefined, groupings (Table 1). The presence of blaOXA and the aadA1a gene within the 2000 bp amplicon characteristic of In-P3 (InH) was confirmed by nested-PCR amplification using specific primers for each gene (not shown).

Characterization of G3 isolates

The 49 isolates belonging to G3 and the control strains LT2 and LSP 31/93 were tested for the presence of pSLT determinants,
plasmid profile, resistance profile (RP) and virulence profile (VP). All these, as well as the control LSP 31/93 strain, were spvC-, rck- and parA/B-positive and pefABCD-negative, and carried a plasmid of the size expected for pUO-StVR2 (~140 kb; readily visualized after extraction by the Kado and Liu method). As expected, G3 isolates showed the RP conferred by pUO-StVR2, but one of them (LSP 174/01) was also trimethoprim-R. PCR amplification revealed that dfrA12 was the gene responsible for such resistance (not shown). Plasmid extraction from this and several other isolates (LSP 31/93, LSP 238/02 and LT2) by S1-PFGE, followed by Southern hybridizations, demonstrated that, together with pUO-StVR2, a plasmid of ~110 kb (labelled pUO-StR12) was present in LSP 174/01, and the dfrA12 mapped on it (Figure 1a and d). In contrast, the blaOXA and spvC probes mapped on the 140 kb plasmid from the G3 isolates (LSP 31/93 and LSP 238/02) and the second also on the 94 kb plasmid from LT2 (Figure 1b and c).

To further characterize the isolates belonging to G3, they were analysed for VP. Results revealed that all of them, as well as the control strains, were positive for the genes used as indicators of five Salmonella pathogenicity islands (inv/E/A and org/A, SPI1; ttrC and ssaQ, SPI2; mgtC and misL, SPI3; spi4R and spi4D, SPI4; and sopB and pipA, SPI5). In addition, they were also positive for seven out of nine other V genes tested (phoP/Q, agfA, slyA, strA/B, invE2A, org/A, ttrC, ssaQ, mgtC, misL, spi4R, spi4D, sopB, pipA and parA/B). VP1, all V-pSLT and V chromosome genes tested except sefD; VP2, all V genes tested except pefABCD, sefD and sopE1. Only 16 out of the 49 G3 isolates, representing the five XbaI profiles, were tested by BlnI macrorestriction.

with the second endonuclease matched those obtained with XbaI. In fact, all except one of the isolates tested with BlnI could be assigned to the B0–B5 profiles, which corresponded to the X0–X5 profiles. The exception was LSP 174/01 (dfrA12-positive) that generated the X2 profile but a distinct BlnI profile, termed B6 (Table 2 and Figure 2b), which differed from B2 by the presence of an additional ~110 kb fragment (probably corresponding to pUO-StR12). The XbaI and BlnI profiles were combined, and a dendrogram of similarity was constructed (Figure 2c). At a cut-off point of S = 0.73 all profiles from pUO-StVR2 isolates fall into a single cluster, which was related to LT2 at S = 0.54. It is interesting to note that the newly described profiles differed from the earliest X1–B1 by 2–6 bands. Moreover, the two combined profiles that included most of the analysed isolates (X1–B1 and X2–B2) differed only by three bands. Accordingly, the G3 grouping appears to be highly clonal.22

Discussion

Using two genetic markers, type of bla gene and In profile, three well-defined MDR groupings (G1–G3), together with other not well-defined groupings of S. Typhimurium could be distinguished as a cause of human salmonellosis in the PA over the period 2001–02. As in other countries,23 the pentaresistant DT104 clone (here defined as G1: blaOXA-cataI1, strE/B, adaA1a, sulI/I2 and rmtB), S. susceptible to antimicrobials; RP1, all pUO-StVR2 resistance determinants; RP2, RP1 and dfrA12 positive.

Table 2. Differential traits of G3 isolates

<table>
<thead>
<tr>
<th>Representative strain—phage type</th>
<th>R profile</th>
<th>VP profile</th>
<th>XbaI (no.)</th>
<th>BlnI (no.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LT2 — RDNC</td>
<td>S</td>
<td>VP1</td>
<td>X1 (5)</td>
<td>B0 (7)</td>
</tr>
<tr>
<td>LSP 31/93—DT104b</td>
<td>RP1</td>
<td>VP2</td>
<td>X2 (28)</td>
<td>B2 (5)</td>
</tr>
<tr>
<td>LSP 137/01—RDNC</td>
<td>RP1</td>
<td>VP2</td>
<td>X3 (1)</td>
<td>B3 (1)</td>
</tr>
<tr>
<td>LSP 355/01—DT41</td>
<td>RP1</td>
<td>VP2</td>
<td>X4 (2)</td>
<td>B4 (1)</td>
</tr>
<tr>
<td>LSP 509/01—DT1104b</td>
<td>RP1</td>
<td>VP2</td>
<td>X5 (3)</td>
<td>B5 (2)</td>
</tr>
<tr>
<td>LSP 200/02—DT208</td>
<td>RP2</td>
<td>VP2</td>
<td>X2 (1)</td>
<td>B6 (1)</td>
</tr>
</tbody>
</table>

No., number of isolates; RDNC, reaction does not conform.

The 49 clinical isolates and the control LSP 31/93 were positive for the R genes blaOXA-cataI1, strE/B, adaA1a, sulI/I2 and rmtB, S. susceptible to antimicrobials; RP1, all pUO-StVR2 resistance determinants; RP2, RP1 and dfrA12 positive.

All were positive for the V genes spvC, rck, iroB, phoP/Q, agfA, slyA, sodCI, invE2A, org/A, ttrC, ssaQ, mgtC, misL, spi4R, spi4D, sopB, pipA and parA/B. VP1, all V-pSLT and V chromosome genes tested except sefD; VP2, all V genes tested except pefABCD, sefD and sopE1.

Only 16 out of the 49 G3 isolates, representing the five XbaI profiles, were tested by BlnI macrorestriction.

Dispersion of pUO-StVR2 between genomic types of S. Typhimurium

By XbaI-macrorestriction PFGE analysis the clinical isolates could be discriminated into five XbaI profiles (X1–X5), with most isolates (85.7%) belonging to X1 and X2. LSP 31/93, the G3 strain from the previous period, generated the X1 profile while LT2 gave a distinct profile, here termed X0 (Table 2 and Figure 2a). Total DNA from representative isolates of the different XbaI profiles (6, 6, 1, 1 and 2 belonging to X1–X5, respectively; Table 2), and the controls LSP 31/93 and LT2, were also analysed by BlnI macrorestriction (Figure 2b). In general, results obtained...
Molecular epidemiology of multidrug-resistant S. enterica

profiles). Apart from the outbreak, 38 sporadic episodes were of gastroenteritis (two isolates were collected at different days from the same patient, and showed different phage type: DT104 and DT193). The remaining four corresponded to bacteraemia, and isolates with identical traits were recovered from both blood and ascitic fluid in one patient, and from blood and faeces in the other three. The 49 G3-isolates represented 3.4 and 19.7% of S. enterica and S. Typhimurium, respectively, recorded at the LSP during 1993–2002. A third MDR grouping, G2, corresponding to the [4,(5),12:i:-] variant (here identified as positive for bla

profiles, supported that most G3 isolates are

profiles in Italy.28 This plasmid, apparently non-conjugative, was of ~110 kb in size, conferred the AMP-GEN-KAN-STR-SUL-TET phenotype, and belonged to the incompatibility group IncFII. The Portuguese isolates29 recovered from humans and pork, carried InH-type integrons on self-transferable plasmids (reported as >70 kb and showing different restriction profiles), which encoded the AMP-STR-SUL-TET ± CHL R phenotype. These isolates generated XbaI profiles similar to X1 and X2 found in the PA isolates. Finally, blaOXA-aadA1 integrons were also detected in four AMP-STR-SUL-TET strains from patients hospitalized in Norway.30 Interestingly, although one of the strains was reported as domestically acquired, the remaining three were acquired in Spain.

With respect to virulence, V determinants common to LT2 and G3 included 10 chromosomal genes representing the 5 SPIs as well as 6 non-SPI located genes (phoP/Q, afgA, styK, str and iroB)31–35 and the bacteriophage-associated sodC1 gene.36 However, the sopE1 gene was present in LT2 but not in pUO-StVR2-positive isolates. This gene, located on a temperate bacteriophage, encodes an effector protein of the SPI1 type III secretion system, which contributes to host cell invasion and intestinal inflammation in animal models.37 As expected, LT2 and G3 isolates were negative for sfd2, a gene of the sfd operon, which encodes the SFD18 fimbriae in Salmonella Enteritidis.38,39 With respect to plasmid genes, all G3 isolates contained spvC, rck, parA and parB but lacked the pef operon, while LT2 was positive for all these genes. According to this, deletions in the pef operon could have occurred when the R region was inserted into pSLT to originate the hybrid plasmid. Such a possibility is currently being investigated.

It is interesting to note that 12 G3 isolates, collected during 1993–2000, and previously subjected to PFGE analysis, showed a single XbaI profile (X1).40 In contrast, the 49 isolates recovered over 2001–02 generated five XbaI profiles (X1–X5), although >85% belonged to X1 and X2. Comparisons of these, and the corresponding BlnI profiles, supported that most G3 isolates are

Figure 2. Macrorestriction–PFGE analysis of representative S. Typhimurium pUO-StVR2 isolates. (a) XbaI profiles: lanes 1–6, profiles shown by LSP strains 31/93, 60/01, 174/01, 353/01, 509/01 and 238/02, respectively. L, X0-profile generated by LT2. The arrow indicates the fragment corresponding to pSLT. (b) BlnI profiles: lanes 1–7, profiles shown by LSP strains 31/93, 60/01, 137/01, 353/01, 509/01, 238/02 and 174/01, respectively. L, B0-profile generated by LT2. The arrow indicates the fragment corresponding to pSLT. (c) Dendogram of similarity of XbaI–BlnI profiles corresponding to G3 isolates. At S = 0.73, the six profiles generated by G3 isolates were clustered, while the LT2 profile remains out of the group (S = 0.54). Asterisk indicates the branch in which the G3 prototype strain (LSP 31/93) was also included.

Downloaded from https://academic.oup.com/jac/article-abstract/57/1/39/915018 by guest on 23 February 2019
clonally related. Accordingly, vertical transmission could be the main way for the spread of pUO-StRV2, although horizontal transfer of the plasmid cannot be ruled out. In fact, the observed polymorphisms on the genomic DNA, together with the different plasmid profiles and the acquisition of a new trimethoprim-R determinant (dfrA12), are compatible not only with the evolution of the progeny of an original G3 strain, but also with the transfer of the plasmid to relatively related S. Typhimurium strains. Further epidemiological surveillance will be required to determine the potential of a wider distribution of the emergent MDR cluster carrying pUO-StRV2.

Acknowledgements

We thank the personnel of the Microbiology Laboratories of the ‘Hospital Central de Asturias’ (Oviedo), ‘Hospital San Agustín’ (Avilés), ‘Hospital de Jirró’, ‘Hospital de Cabueñas’ (Gijón), and ‘Hospital Carmen y Severo Ochoa’ (Cangas de Narcea) for their invaluable collaboration with the LSP in registering clinical isolates of Salmonella. We also thank Dr B. Guerra for his helpful comments. This work has been supported by a grant from the ‘Fondo de Investigación Sanitaria’ (ref. 02/0172). A. H. is the recipient of a grant from the ‘Fundación para el Fomento en Asturias de la Investigación Científica Aplicada y la Tecnología’ (FICYT, ref. BP04-086).

References


Molecular epidemiology of multidrug-resistant *S. enterica*


