ELSEVIER

Institut Pasteur

Research in Microbiology xx (2015) 1–4

www.elsevier.com/locate/resmic

# First description of food-borne *Salmonella enterica* resistance regions R1 and R3 associated with IS*26* elements

Eduarda Gomes-Neves [a,b,1], Vera Manageiro [b,c,1], Eugénia Ferreira [c], José M. Correia da Costa [b,c], Manuela Caniça [c,*]

[a] *ICBAS, Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, Portugal*
[b] *CECA-ICETA, Centro de Estudos de Ciência Animal, Universidade do Porto, Porto, Portugal*
[c] *Departamento de Doenças Infeciosas, Instituto Nacional de Dr. Ricardo Jorge, Lisboa and Porto, Portugal*

Received 1 December 2014; accepted 13 March 2015
Available online ■ ■ ■

**Abstract**

In this study, we assessed the presence of IS*26* in food-borne ASSuT-type *Salmonella enterica* isolates. A new genetic region (R3) was described, that included a C14 caspase gene between IS*26* elements. R3 was present in two *Salmonella* Rissen isolates from a swine carcass and a meat handler, collected at the same abattoir. Furthermore, a new rearrangement of resistance region R1, harboring the $bla_{TEM-1}$ gene flanked by IS*26* elements, was identified in *Salmonella* Typhimurium and *Salmonella* 4,[5],12:i:-, from different samples. This study highlights the zoonotic potential of *Salmonella* spp. isolates and the possible role of IS*26* in the mobilization of resistance genes.


*Keywords:* *Salmonella* spp.; *IS*26; Swine; Portugal

## 1. Introduction

*Salmonella enterica*, like *Salmonella* Typhimurium DT104, the monophasic variant *Salmonella* 4,[5],12:i:- and *Salmonella* Rissen, has been involved in important multidrug-resistant (MDR) human infections associated with several sources [1–3]. The 4,[5],12:i:- variant has been linked to poultry, cattle, pig and pork products, with pigs being the likely reservoir of infection [1]. Indeed, as recently demonstrated, animal-husbandry-associated environments seem to contribute to enhancing *Salmonella*'s pathogenic potential [2]. In fact, in those isolates, resistance genes are often associated with integrons, insertion sequences (IS) and transposons, clustering within chromosomal antimicrobial resistance regions [4]. IS*26* has been particularly implicated in the dissemination of chromosomal regions containing resistance genes by facilitating their mobilization between distinct genetic areas [5].

Indeed, a *dfrA5*-IS*26* configuration was previously detected among *Escherichia coli* strains with different serotypes sourced from both humans and animals, acting as a conduit for the transfer of integron-related resistance genes to human pathogens [6]. In *Salmonella* strains, namely *S*. Typhimurium and the monophasic variant *S*. 4 [5],12:i:-, IS*26* elements have been linked to the presence of resistance regions conferring an R-type ASSuT resistance pattern [7,8].

In this study, we investigated the genetic environment of $bla_{TEM-1}$ genes among multidrug-resistant *S. enterica* isolates, in order to elucidate the genetic relationship of IS*26* mobile genetic elements towards these important resistance genes.

* Corresponding author.
*E-mail addresses:* egomesneves@mail.icav.up.pt (E. Gomes-Neves), vera.manageiro@insa.min-saude.pt (V. Manageiro), eugenia.ferreira@insa.min-saude.pt (E. Ferreira), jose.costa@insa.min-saude.pt (J.M. Correia da Costa), manuela.canica@insa.min-saude.pt (M. Caniça).
[1] These authors contributed equally to the work.

http://dx.doi.org/10.1016/j.resmic.2015.03.007
0923-2508/

## 2. Materials and methods

### 2.1. Sampling and phenotype characterization

A collection of 60 MDR (R-type ASSuT phenotype) serotyped *S. enterica* strains (32 *S.* Typhimurium and 3 monophasic variants *S.* 4,[5],12:i:-, 11 *S.* Derby, 4 *S.* Rissen, 3 *S.* London, 3 *S.* Mbandaka, 2 *S.* Give, 1 *S.* Enteritidis and 1 *S.* Sandiego), isolated from slaughtered swine samples, were recovered from ileocecal lymph node samples of swine, carcass swabs, meat samples and meat handlers' hands, in a previous study [9,10].

### 2.2. Analysis of sequences flanking IS26 elements

In all 60 MDR *S. enterica* isolates, we investigated the genetic organization of three resistance regions (named R1, R2 and R3), and the association of IS*26* with resistance-encoding genes, by PCR mapping and sequencing assays, using primers targeting IS*26* and $bla_{TEM}$ [11], *sul1* [12], *sul2*, *sul3*, *merD* and *urf2* (Table 1). Controls were included in all assays: *Salmonella* spp. strains [10] were used as PCR-positive controls for $bla_{TEM}$, *sul1, sul2* and *sul3*; *E. coli* INSRA1169 [13] for *merD* and *urf2*; and *E. coli* INSLA51 [12] for the IS*26* gene, respectively. Sequence alignments and generation of resistance cassette contigs were performed using *Bionumerics* software (Applied Maths). Gene identity was confirmed at the NCBI website (http://www.ncbi.nlm.nih.gov/).

### 2.3. Statistical analysis

OpenEpi software, version 3 (www.openepi.com) was used for statistical analysis. Two-sided $P$ values of $<0.05$ were considered to be statistically significant.

## 3. Results and discussion

PCR mapping identified IS*26* elements in 23/60 (38.3%) of MDR *S. enterica* isolates in three different resistance regions (R1, R2 and R3), statistically associated with antibiotic ($bla_{TEM-1}$, region R1 and R3) or mercury (*merD-merE-urf2*, region R2) resistance-encoding genes (Table 2), with $P < 0.001$. Overall, we detected 27 $bla_{TEM-1}$ genes, from which 21 were flanked up- and downstream by two copies of IS*26*, in a Tn*6029*-like structure that was described by Cain et al. [14]: the R1 region (17 *S.* Typhimurium and 2 *S.* 4,[5],12:i:-) and, here firstly described, the R3 region (2 *S.* Rissen) (Fig. 1A, Fig. 1B).

As shown in Fig. 1A, in *S.* Typhimurium strains (including monophasic strains), the genetic arrangement (3540 bp) comprised between the two IS*26* was identical to the chromosomal resistance R1 region (GenBank accession no. HQ331538) previously described in epidemiologically unrelated *Salmonella* strains recovered only from humans in Italy [7]. However, *sul*1, *sul*2 and *sul*3 resistance genes were not present in the R1 surrounding regions in this study, revealing a different genetic arrangement in our animal and meat handler isolates when compared to human *Salmonella* cases in Italy and Canada [7,15].

In two *S.* Rissen isolates (Fig. 1B) recovered in the same abattoir, from a carcass and a meat handler, the new resistance region (R3) presented an IS*26*-$bla_{TEM-1}$ genetic platform (with 1857 bp); this structure was followed by a peptidase C14 caspase catalytic subunit P20-encoding gene, plus another copy of IS*26* found downstream of the $bla_{TEM-1}$ gene (with 1455 bp). Interestingly, the C14 caspase gene was also found in an *Actinomycete* integrating conjugative element, which catalyzed the mobilization of other genetic elements such as genomic islands and virulence plasmids [16]. The truncated Tn*3* transposon of the R1 region was not present in this R3 genetic region (Fig. 1B).

The subsequent use of primers specific to known resistance-encoding genes generated a PCR amplicon (R2 region, 3447 bp) both in the 21 *S.* Typhimurium (including the three monophasic variants) and the 2 *S.* Rissen isolates (Table 2 and Fig. 1C). Indeed, this region was the only one identical to that described by Lucarelli et al. (GenBank accession no. HQ331538), presenting part of a mercury resistance operon and flanked downstream by an IS*26* element [7]. This operon (which has been reported to be a conserved region among *Salmonella* strains) is of great concern, since its co-existence with antibiotic resistance regions suggests indirect selection of antibiotic resistance in *S. enterica* strains [7,17].

Insertion sequence IS*26* plays a key role in dissemination of antibiotic resistance genes, namely in *Salmonella* spp. [5,6,18], both in plasmids and in chromosomal genomic islands [19,20]. In this study, we described three different chromosomal regions containing antibiotic or mercury resistance genes that are flanked by, and eventually interspersed with, copies of IS*26*, including the new R3. The presence of multiple copies of IS*26* enhances the mobilization of large MDR regions, which might include resistance and pathogenicity-encoding genes and then build new MDR regions. Indeed, a recent study indicated that transposition of IS*26*, presumably donated by plasmids originally acquired by biphasic *S.* Typhimurium, was involved in the deletion of the *fljAB* operon and surrounding genes and hence was responsible for the monophasic phenotype of *S.* 4,5,12:i:– isolates [21].

In conclusion, the presence of RR1 plus RR2 in both *S.* Typhimurium and in its monophasic variant (previously

Table 1
Primers designed in this study and used for PCR mapping analysis.

| Gene | Primer name | Primer sequence (5′ → 3′) |
|---|---|---|
| *sul*2 | Sul2-F | ATGAATAAATCGCTCATCATTTTC |
| | Sul2-R | TTAACGAATTCTTGCGGTTTC |
| *sul*3 | Sul3-F | ATGAGCAAGATTTTTGGAATC |
| | Sul3-R | CTAACCTAGGGCTTTGGA |
| *mer*D | MerD-F | CCTTCGAGGCGGGTATC |
| *urf*2 | Urf2-R | TGTTGCAGGCAGGAATAGC |

Please cite this article in press as: Gomes-Neves E, et al., First description of food-borne *Salmonella enterica* resistance regions R1 and R3 associated with IS*26* elements, Research in Microbiology (2015), http://dx.doi.org/10.1016/j.resmic.2015.03.007

Table 2
Characteristics of 28 *Salmonella* isolates containing resistance regions (R1, R2, R3) and/or producing TEM-1 β-lactamase.

| Serotype (no. of isolates) | Resistance phenotype[a] | Sampled material (number of isolates) | $bla_{TEM-1}$ (n = 27) | Resistance region[b,c] | | |
|---|---|---|---|---|---|---|
| | | | | R1 *(IS26-tnp3RΔ -$bla_{TEM-1b}$-tnpB-IS26)* (n = 19) | R2 *(merD-merE-urf2-tniA Δ1-IS26)* (n = 23) | R3 *(IS26-$bla_{TEM-1b}$-casp 14-IS26)* (n = 2) |
| 4,[5],12:i:- (3) | ASSuT | Meat (1); lymph node (1) | + | + | + | − |
| | ST | Lymph node (1) | − | − | + | − |
| Typhimurium (20) | ASSuT | Lymph node (5); carcass (5); Meat (5) | + | + | + | − |
| | AST | Meat handler (1) | + | + | + | − |
| | AT | Lymph node (1) | + | + | + | − |
| | | Carcass (1) | + | − | + | − |
| | ASuTW | Lymph node (1) | + | − | − | − |
| | CASSuT | Meat (1) | + | − | − | − |
| Rissen (3) | CASSuTW | Carcass (1) | + | − | + | + |
| | ASSuTW | Meat handler (1) | + | − | + | + |
| | ASSuT | Lymph node (1) | + | − | − | − |
| London (2) | ANSSuT | Carcass (2) | + | − | − | − |

[a] A, ampicillin; C, chloramphenicol; S, streptomycin; Su, sulfamethoxazole; T, tetracycline; W, trimethoprim.
[b] +, positive result; −, negative result.
[c] Regions presented in Fig. 1.

reported only in human isolates), as well as RR2 plus RR3 in *S.* Rissen from diverse food-chain-related reservoirs, highlights the zoonotic potential of such isolates and the possible role of IS*26* in the mobilization of resistance genes within, to and from animal settings. Thus, it is imperative and mandatory to view food-producing animals as reservoirs of non-typhoidal *Salmonella* in order to monitor this situation in humans, animals and food.

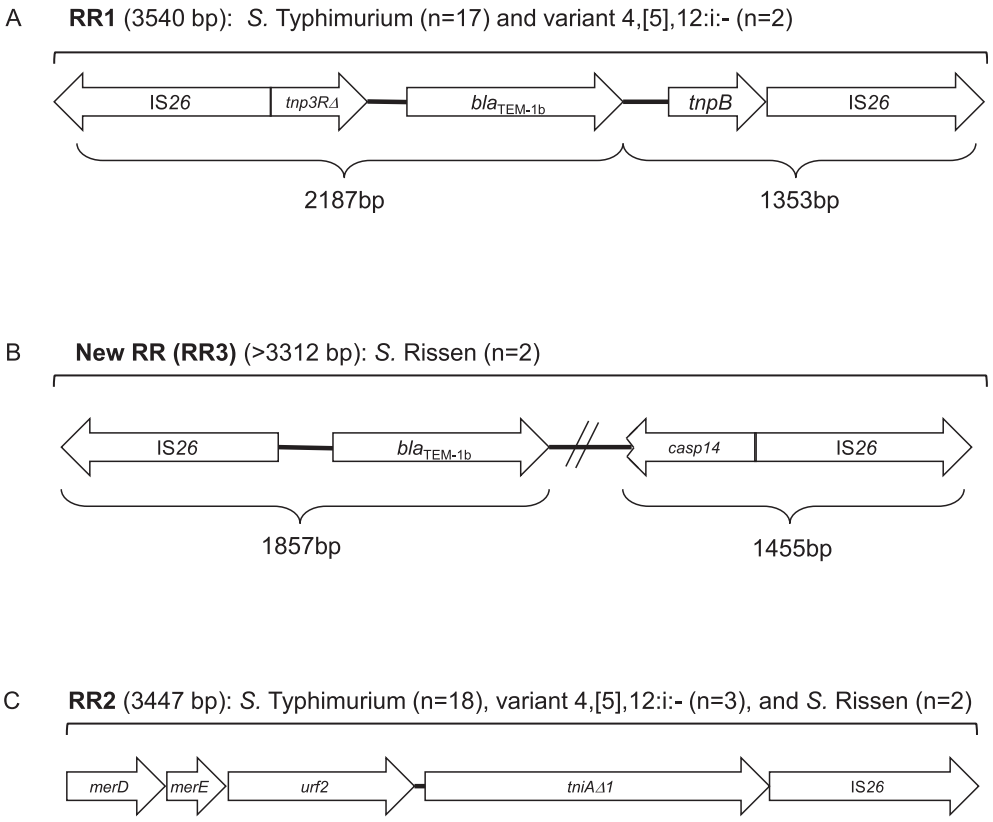


Fig. 1. Schematic representation of the genetic environment of resistance genes in R-type ASSuT *S. enterica* isolates. A: sequence of resistance region R1 (3540 bp) of 17 *S.* Typhimurium isolates and 2 monophasic variants harboring the $bla_{TEM-1}$ gene; B: the new R3 genetic organization of $bla_{TEM-1}$ (R3, >3312 bp) found in 2 *S.* Rissen isolates; C: genetic organization of mercury resistance operon (R2, 3447 bp), in both 18 *S.* Typhimurium and 3 variants 4,[5],12:i:-, as well as 2 *S.* Rissen isolates. The directions of transcription of the corresponding genes are depicted by arrows.

Please cite this article in press as: Gomes-Neves E, et al., First description of food-borne *Salmonella enterica* resistance regions R1 and R3 associated with IS*26* elements, Research in Microbiology (2015), http://dx.doi.org/10.1016/j.resmic.2015.03.007

## Conflicts of interest

There was no conflict of interest.

## Acknowledgments

V. Manageiro was supported by grant SFRH/BPD/77486/2011 from the FCT, Lisbon, Portugal. This study was in part supported by NIH internal funding. The authors also thank the Fundação para a Ciência e a Tecnologia (FCT) for project grant PEst-OE/AGR/UI0211/2011-2014, Strategic Project UI211-2011-2014.

## References

[1] Hopkins KL, Kirchner M, Guerra B, Granier SA, Lucarelli C, Porrero MC, et al. Multiresistant *Salmonella enterica* serovar 4,[5],12:i:- in Europe: a new pandemic strain? Euro Surveill 2010;15:19580.

[2] Oliveira M, Wijnands L, Abadias M, Aarts H, Franz E. Pathogenic potential of *Salmonella* Typhimurium DT104 following sequential passage through soil, packaged fresh-cut lettuce and a model gastrointestinal tract. Int J Food Microbiol 2011;148:149–55.

[3] EFSA/ECDC. The European Union summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2012. EFSA J 2014;2014(12):3590.

[4] Miriagou V, Carattoli A, Fanning S. Antimicrobial resistance islands: resistance gene clusters in *Salmonella* chromosome and plasmids. Microbes Infect 2006;8:1923–30.

[5] Harmer CJ, Moran RA, Hall RM. Movement of IS26-associated antibiotic resistance genes occurs via a translocatable unit that includes a single IS*26* and preferentially inserts adjacent to another IS*26*. mBio 2014:5.

[6] Dawes FE, Kuzevski A, Bettelheim KA, Hornitzky MA, Djordjevic SP, Walker MJ. Distribution of class 1 integrons with IS*26*-mediated deletions in their 3'-conserved segments in *Escherichia coli* of human and animal origin. PLoS One 2010;5:e12754.

[7] Lucarelli C, Dionisi AM, Filetici E, Owczarek S, Luzzi I, Villa L. Nucleotide sequence of the chromosomal region conferring multidrug resistance (R-type ASSuT) in *Salmonella* Typhimurium and monophasic *Salmonella* Typhimurium strains. J Antimicrob Chemother 2012;67:111–4.

[8] Laorden L, Herrera-León S, Martínez I, Sanchez A, Kromidas L, Bikandi J, et al. Genetic evolution of the Spanish multidrug-resistant *Salmonella enterica* 4,5,12:i:- monophasic variant. J Clin Microbiol 2010;48:4563–6.

[9] Gomes-Neves E, Antunes P, Tavares A, Themudo P, Cardoso MF, Gärtner F, et al. *Salmonella* cross-contamination in swine abattoirs in Portugal: carcasses, meat and meat handlers. Int J Food Microbiol 2012;157:82–7.

[10] Gomes-Neves E, Antunes P, Manageiro V, Gärtner F, Caniça M, da Costa JM, et al. Clinically relevant multidrug resistant *Salmonella enterica* in swine and meat handlers at the abattoir. Vet Microbiol 2014;168:229–33.

[11] Mendonça N, Leitão J, Manageiro V, Ferreira E, Caniça M. Spread of extended-spectrum β-lactamase CTX-M-producing *Escherichia coli* clinical isolates in community and nosocomial environments in Portugal. Antimicrob Agents Chemother 2007;51:1946–55.

[12] Jones-Dias D, Manageiro V, Francisco AP, Martins AP, Domingues G, Louro D, et al. Assessing the molecular basis of transferable quinolone resistance in *Escherichia coli* and *Salmonella* spp. from food-producing animals and food products. Vet Microbiol 2013;167:523–31.

[13] Manageiro V, Ferreira E, Pinto M, Fonseca F, Ferreira M, Bonnet R, et al. Two novel CMY-2-type β-lactamases encountered in clinical *Escherichia coli* isolates. Ann Clin Microbiol Antimicrob 2015;14:12.

[14] Cain AK, Liu X, Djordjevic SP, Hall RM. Transposons related to Tn*1696* in IncHI2 plasmids in multiply antibiotic resistant *Salmonella enterica* serovar Typhimurium from Australian animals. Microb Drug Resist 2010;16:197–202.

[15] Mulvey MR, Finley R, Allen V, Ang L, Bekal S, El Bailey S, et al. Emergence of multidrug-resistant *Salmonella* enterica serotype 4,[5],12:i:- involving human cases in Canada: results from the Canadian integrated Program on antimicrobial resistance surveillance (CIPARS), 2003-10. J Antimicrob Chemother 2013;68:1982–6.

[16] Ghinet MG, Bordeleau E, Beaudin J, Brzezinski R, Roy S, Burrus V. Uncovering the prevalence and diversity of integrating conjugative elements in actinobacteria. PLoS One 2011;6:e27846.

[17] Baker-Austin C, Wright MS, Stepanauskas R, McArthur JV. Co-selection of antibiotic and metal resistance. Trends Microbiol 2006;14:176–82.

[18] Djordjevic SP, Stokes HW, Chowdhury PR. Mobile elements, zoonotic pathogens and commensal bacteria: conduits for the delivery of resistance genes into humans, production animals and soil microbiota. Front Microbiol 2013;4:86.

[19] Doublet B, Praud K, Weill F-X, Cloeckaert A. Association of IS*26*-composite transposons and complex In4-type integrons generates novel multidrug resistance loci in *Salmonella* genomic island 1. J Antimicrob Chemother 2009;63:282–9.

[20] Holt KE, Thomson NR, Wain J, Phan MD, Nair S, Hasan R, et al. Multidrug-resistant *Salmonella enterica* serovar Paratyphi A harbors *IncHI1* plasmids similar to those found in serovar Typhi. J Bacteriol 2007;189:4257–64.

[21] García P, Malorny B, Hauser E, Mendoza MC, Rodicio MR. Genetic types, gene repertoire, and evolution of isolates of the *Salmonella enterica* serovar 4,5,12:i:– spanish clone assigned to different phage types. J Clin Microbiol 2013;51:973–8.

Please cite this article in press as: Gomes-Neves E, et al., First description of food-borne *Salmonella enterica* resistance regions R1 and R3 associated with IS*26* elements, Research in Microbiology (2015), http://dx.doi.org/10.1016/j.resmic.2015.03.007