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

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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 $bla_{\text{TEM-1}}$ 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.
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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 $bla_{\text{TEM-1}}$ 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.

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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

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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.

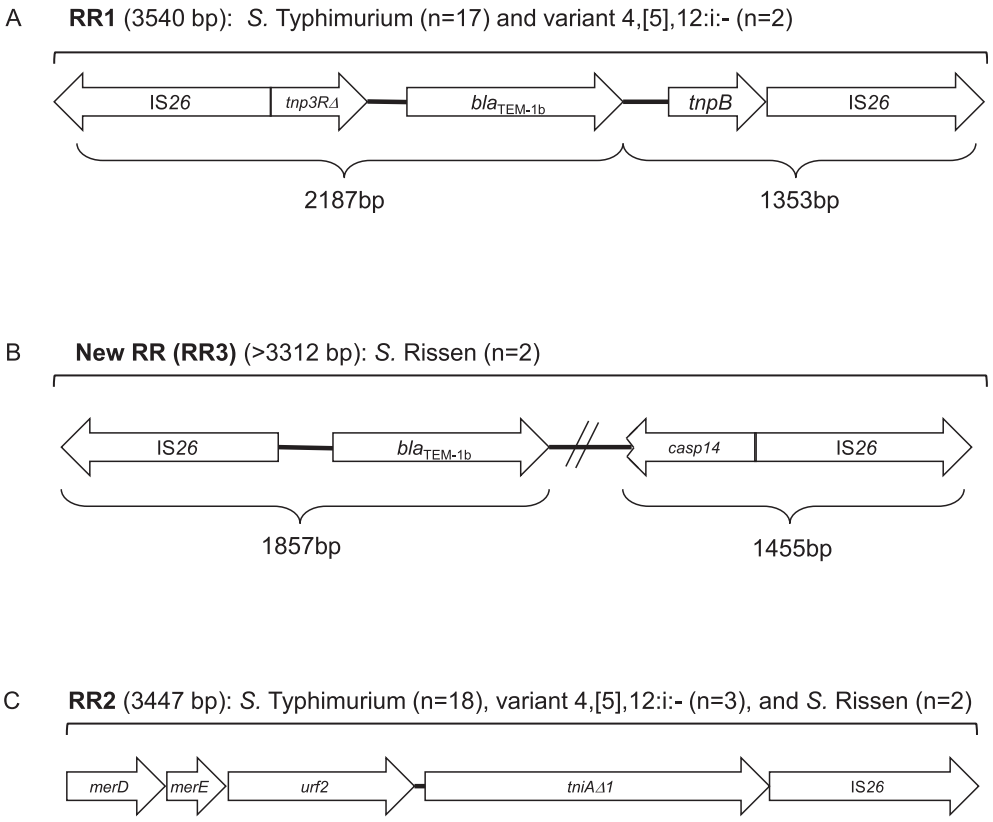

Fig. 1. Schematic representation of the genetic environment of resistance genes in R-type ASSuT *S. enterica* isolates. A: sequence of resistance region R1 (3540 bp) of 17 *S.* Typhimurium isolates and 2 monophasic variants harboring the bla_{TEM-1} gene; B: the new R3 genetic organization of bla_{TEM-1} (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.

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Conflicts of interest

There was no conflict of interest.

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