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Antimicrobial resistance in non-typhoidal Salmonella from food sources Colombia: evidence for an unusual plasmid located Class 1 integron in serotypes Typhimurium and Anatum

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Abstract

Seventy-two isolates representing 18 serotypes recovered from various food samples collected in Colombia were tested for antimicrobial susceptibilities. The collection was further characterised for extended-spectrum cephalosporin, aminoglycoside, and tetracycline resistance mechanisms. Multi-drug resistant (MDR) isolates were further investigated for class 1 integrons and were evaluated for the presence of conjugative plasmids along with a determination of the incompatibility group by PCR. Antiobiotic analysis showed that ceftiofur resistance was moderately high (15%). A comparable resistance pattern was also observed for neomycin and oxytetracycline (11 and 10% respectively).

There was a high prevalence of gene cassettes as part of one or more class 1 integrons (61%) many of which contained markers that contributed to the resistance profile. Class 1 integrons identified in MDR *Salmonella enterica* serotype Typhimurium and *Salmonella enterica* serotype Anatum isolates were characterised. Sequence identified several incomplete open reading frames (ORF) as part of a gene cassette (*bla-imp-13, dfr7, blr1088* and *aac8*) along with an intact gene cassette (*bla-oxa-2*) in each case. A mosaic of gene cassettes was identical in the two *Salmonella* serotypes and these organisms were cultured from food samples in different regions of Colombia. These integrons were located to a conjugative replicon. Plasmid profiling and incompatibility typing identified three plasmids belonging to Inc groups A/C, P and W. Our study highlights the role of integrons, contributing to a MDR phenotype that is capable of dissemination to other bacteria.
Introduction

*Salmonella* spp. are recognised as major food borne pathogens worldwide\(^1\). Most *Salmonella* infections in humans result from the ingestion of contaminated foods of animal origin\(^2\). Contamination can occur at multiple stages along the food chain including production, processing, distribution, handling and preparation.

The emergence and dissemination of multi-drug resistant (MDR) *Salmonella* has become a major public health issue as it threatens the efficacy of current antibiotic therapies in cases of life-threatening *Salmonellosis*\(^3, 4, 5^-8\). Of particular concern, is the continuing emergence of *Salmonella* isolates that are resistant to extended-spectrum cephalosporins\(^9, 10, 11\). Extended-spectrum cephalosporins such as ceftriaxone and ceftiofur are important therapeutic agents used to treat invasive *Salmonella* infections, particularly in children\(^4, 12\). Resistance to these agents is mediated by an AmpC-like beta-lactamase encoded by the *bla*\(_{CMY}\) gene\(^13, 14\). Epidemiological studies have shown that the extensive use of antibiotics in domestic livestock is an important risk factor for the emergence and subsequent transfer of MDR *Salmonella* to humans *via* the food chain\(^15\).

Many antimicrobial resistance genes in *Salmonella* are found on large conjugative plasmids and these can be located within transposons\(^5, 16^-18\). These genes can be also found as a component part of a gene cassette recombined
Several classes of integron have been defined and class 1 integrons are clinically important, and these are found as part of the Tn21 or Tn402 transposon family. They are widely distributed among Salmonella and are frequently located on plasmids. Plasmids of the IncF and IncL/M incompatibility group have been reported as vehicles for class 1 integrons. The association of resistance determinants with mobile genetic elements such as plasmids, transposons and integrons contributes to the widespread dissemination of resistance markers within and between bacterial species.

In this paper, we report on the resistance profiles of Salmonella isolates recovered from a variety of foods in Colombia. All were investigated for the corresponding genetic marker(s) associated with resistance. In two Salmonella serotypes, an unusual array of incomplete ORFs along with a bla-oxa-2 gene were identified and located to a 2.6-Kbp amplicon contained within the variable region of a class 1 integron. We examined the ability of these two isolates to disseminate their resistance genes via conjugative to a marked Escherichia coli strain.

Materials and Methods

Salmonella isolates

The 72 Salmonella isolates in this study, representing 18 serotypes (Table 1) were a sub-set of a collection (n = 636) of Salmonella isolates that were part of an epidemiological study carried out in the Caribbean zone of South America.
The isolates were recovered from various food samples (chicken, beef, sausage, cheese) collected from fast-food outlets in four regions of northern Colombia.

Identification and serotyping of all isolates was carried out at Instituto de Investigaciones Biologicas del Tropica, Facultad de Medicina Veterinaria y Zootecnia, Universidad de Cordoba.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was carried out by disk diffusion according to guidelines of the National Committee for Clinical Laboratory Standards. The antimicrobials and concentrations in parenthesis used included: ampicillin (10 μg), amoxicillin/clavulanic acid (30 μg) amoxicillin (25 μg), gentamicin (10 μg), cefuroxime (30 μg), ceftiofur (30 μg), framycetein (100 μg), neomycin (30 μg), oxytetracycline (30 μg), streptomycin (10 μg), and sulphamethoxazole-trimethoprim (1.25/23.75 μg) (Oxoid). The resistance breakpoints used were: ampicillin ≤ 13 mm, amoxicillin/clavulanic acid ≤ 13 mm, amoxicillin ≤ 13 mm, gentamicin ≤ 12 mm, cefuroxime ≤ 14 mm, ceftiofur ≤ 14 mm, framycetein ≤ 13 mm, neomycin ≤ 13 mm, oxytetracycline ≤ 14 mm, streptomycin ≤ 11 mm and sulphamethoxazole-trimethoprim ≤ 10 mm. *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as controls.

DNA isolation, PCR amplification and DNA sequence analysis

Total DNA was prepared using the Wizard Genomic DNA purification kit (Promega, Madison, WI). The integrity of the purified template DNA was
assessed by conventional agarose gel [1.5%, (w/v)] electrophoresis and the quantity determined using a UV spectrophotometer. The structural components of class 1 integrons were amplified by PCR (these included \textit{intI1}, \textit{qacE\Delta1}, \textit{sul1} and the variable gene cassette region). In addition the presence of a selection of antimicrobial resistance genes was determined by amplification also. All of the genes, the corresponding primers and references are shown in Table 1.

Briefly, the PCR reactions contained 100 ng of purified DNA, 50 pmol/\mu l of forward and reverse primers (MWG-Biotech AG, Ebersberg, Germany), 1 X Buffer containing 2.5 mM MgCl\textsubscript{2}, 200 \mu M dNTPs (Promega, Madison, WI) and 0.5 U \textit{Taq} DNA Polymerase (New England Biolabs, Ipswich, MA) or \textit{Pfu} Polymerase (Chimerx, Madison, WI).

PCR products of interest were gel extracted using a QIAGEN gel extraction kit (West Sussex, UK). DNA was quantified by spectrophotometry and sequenced commercially (Qiagen, Hilden, Germany). Sequences were initially compared to the current GenBank sequence databases using the BLAST suite of programs\textsuperscript{30}. CLUSTALW amino acid sequence alignments were produced for comparison\textsuperscript{31}.

\textbf{PCR-based incompatibility typing}

Inc group identification of plasmids was carried out according to the referenced method\textsuperscript{32}. Briefly, 18 pairs of primers were designed to perform 5 multiplex-and 3 simplex-PCRs, recognizing the FIA, FIB, FIC, HI1, HI2, I1-I\gamma, L/M, N, P, W, T,
A/C, K, B/O, X, Y, F and FIIA replicons representative of the major plasmid incompatibility groups circulating among the Enterobacteriaceae. Multiplex 1 recognises HI1, HI2 and I1-γ, multiplex 2 recognises X, L/M, and N, multiplex 3 recognises FIA, FIB and W, multiplex 4 recognises Y, P, and FIC and multiplex 5 recognises A/C, T and FIIAs. The three simplex PCRs recognize F, K and B/O replicons respectively. Primers and positive controls were previously described. All PCR amplifications, except F-simplex, were performed with the following amplification scheme: 1 cycle of denaturation at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 60°C for 30 s and elongation at 72°C for 1 min. A final extension of 1 cycle at 72°C for 5 min was performed. The F-simplex PCR was performed with the same amplification program but at an annealing temperature of 52°C.

Isolation of large plasmids

Plasmid DNA was isolated from strains according to the manufacturers instructions (Invitrogen, Biosciences, Dublin). The concentration and integrity was assessed as outlined above.

Conjugation experiments

Conjugation experiments were performed with E. coli JM109 (Promega, Madison, WI) as the recipient strain. This plasmid free strain was grown overnight in Luria Bertani [LB] (Oxoid, Basingstoke, Hampshire, UK) broth in the presence of nalidixic acid at a concentration of 50 μg/ml and was subsequently transferred
onto a LB plate containing 50 μg/ml of the same antibiotic. This strain then
represented the marked recipient for all conjugation experiments. The absence
of growth of the donor strains SC-28, SC-56 confirmed that both were susceptible
to nalidixic acid and these were designated Nal\textsuperscript{s}. The donor strain SC-56 [R-
type: Aml, Amp, Ot and SXT] was grown on a nutrient agar (NA) plate containing
ampicillin (10 μg/ml) whilst the recipient was grown on NA plate containing
nalidixic acid (50 μg/ml). A single colony of both the donor and recipient was
transferred to 3 ml of LB and incubated at 37°C for 24 h. The overnight culture
was pelleted and resuspended in 1 ml of LB broth without antibiotic. An aliquot of
this suspension (100 μl) was plated directly onto a dried NA plate containing
nalidixic acid (50 μg/ml) and sequentially, each of the following; ampicillin (10
μg), tetracycline (30 μg), and trimethoprim (5 μg). The second donor SC-28 was
grown and plated as described for SC-56 but in addition it was also plated onto
gentamycin (10 μg). All transconjugants were examined for the presence of
replicons using the incompatibility typing protocol described earlier. The Inc Rep
typing assay was performed prior to and after conjugation experiments.

**Nucleotide accession number**
The complete 2.6-Kbp amplicons sequenced from *S. Anatum* (SC-28) *S.
Typhimurium* (SC-56) were submitted to GenBank and assigned the Accession
numbers AM237807 and AM237806 respectively.
**Results**

**Antimicrobial susceptibility of *Salmonella* isolates**

Twenty four (33%) of the *Salmonella* strains showed resistance to at least one of the 11 antimicrobial agents tested. Table 2 summarises the resistance profiles obtained for all isolates. The highest levels of resistance were found for ceftiofur (17%) followed by neomycin (13%) and oxytetracycline (11%). Fourteen different antibiotic profiles were observed, 10 of which were multi-antimicrobial resistant (defined as isolates showing resistance to two or more classes of antimicrobials) as shown in Table 3.

**Antimicrobial resistance genes and gene cassettes**

The *bla*-*CMY-2* gene was detected in 7 of 12 isolates resistant to the extended spectrum β-lactamases. Three *S. Typhimurium* isolates were found to carry *bla-*PSE gene. β-Lactam resistant isolates did not produce any amplicons when screened for *ctx-u* or *bla*-TEM (Table 1). The *tetA* or *tetG* genes were detected in all isolates showing tetracycline resistance.

Sixty one % of the isolates contained integron associated gene cassettes with sizes ranging from 0.4- to 2.6-Kbp (data not shown). One *S. Typhimurium* isolate contained 1.0- and 1.2-Kbp amplicons corresponding to the integron profile (IP) type-1. Two *S. Typhimurium* isolates from Monteria, contained a 1.0-Kbp amplicon (Table 3). Thirty isolates from the collection contained a 1.4-Kbp amplicon alone whilst a further three isolates had the 1.4-Kbp amplicon in
addition to a 0.7-Kbp amplicon. Three isolates contained the 1.4-Kbp amplicon along with a second amplicon of 0.6-, 0.5- or 0.4-Kbp. One S. Anatum (R- Type: Amp, Cn, Eft, Fy, N, Ot and S) and two S. Typhimurium (R-Type: Aml, Amp, Ot and Sxt) contained a 2.6- and 1.2-Kbp amplicon respectively. Overall, the gene cassettes detected were not serotype specific and were found in thirteen (including an undetermined serotype recorded as Salmonella spp.) of the eighteen serotypes present in this collection. Table 3 shows a summary of the gene cassette sizes of any isolate that displayed resistance to at least one antimicrobial agent.

Molecular analysis of Integron associated gene cassettes

Sequence analysis of the 2.6-Kbp amplicons from S.Typhimurium (SC-56) and S. Anatum (SC-28) isolates were characterized further in this study. Following the complete determination of the DNA sequence in each case, initial comparisons showed that all were identical (Figure). The 2.6-Kbp sequence from each revealed four partial gene sequences (bla_imp13, dfr7, bllr1088 and aac8) and one complete ORF, (bla_oxa-2). The 5’- and 3’-conserved structures were determined by PCR (Table 1) and found to be intact, indicating the presence of a complete class 1 integron. To determine if these 2.6-Kbp amplicons were present on plasmids, large plasmids were extracted from S. Typhimurium (SC-56) and S. Anatum (SC-28). The purified plasmid DNA from both isolates was used as template DNA in a subsequent gene cassette PCR reaction. The 2.6-Kbp amplicons were successfully amplified in both cases. In addition the plasmid
profiles from *S. Typhimurium* and *S. Anatum* were examined by agarose gel electrophoresis and showed identical plasmid DNA profiles.

When the DNA sequence of the 2.6-Kbp amplicon was analysed in detail, the putative attachment site (*att1*) was located towards the proximal end (Figure). This is the recombination site recognized by the 59-base element (be) sequence found on the distal ends of gene cassettes. This sequence was located between nucleotide positions 1 through 74. Downstream of the *att1* site is the first of four incomplete ORFs that showed some identity to *bla*-*imp13*, *dfr7*, *blr1088* and *aac8* sequences, respectively (all indicated by the dashed arrowheads in the Figure).

The first incomplete gene (*bla*-*imp13*) is 53 bp in length, followed by the classic 59-be. A stem loop structure characteristic of 59-be was also identified where a region of perfect overlapping occurred (-TTTTTATAAAAACT- indicated in bold face type in the Figure). Seven base pairs downstream of the incomplete (*bla*-*imp13*) gene and within the first 59-be element –10 (-TAACAA) and –35 signals (-TGGACA) are at found (-10 and –35 signals- indicated in blue bold face type in Figure). The second incomplete gene *dfr7* was located at position 615 extending from the first codon TTG until a TAA stop codon is reached at position 1089.

BLAST searches with this incomplete ORF identified significant sequence similarity to a dihydrofolate reductase gene (*dfr7*) found in *S. Typhimurium* [AY245101], *Escherichia coli* [AJ884724] and on an IncLM plasmid from *E. coli* [X58425]. The *dfr7* partial gene coded for a truncated polypeptide of 157 aa.

Amino acid alignments of this partial DfrVII identified in the serotypes
Typhimurium and Anatum demonstrated that it differed by 32 amino acids residues when aligned with similar sequences [AF245101, X58425, Q549W7, P27422; data not shown]. These differences were found throughout the amino acid sequence and were not located towards any specific terminal end of the polypeptide.

The 59-be element associated with the incomplete dfr7 gene is located at position 1090 and extends until position 1215. Similarly both −10 (-TAACAA) and −35 (-TGGACA) signals are found at nucleotide positions 1087 to 1091 and 1221 to 1226 respectively (-indicated in blue bold face type in Figure1). Further inspection of the 59-be element DNA sequence revealed a putative ribosomal binding site (RBS). This site spanned from position 1090 and extended beyond the 59-be element until position 1224.

Situated between this feature and the third incomplete gene (blr1088) is a 143 bp DNA sequence (coloured box in Figure) that does not show homology to any sequence in the GenBank database.

BLAST searches on another DNA fragment located between positions 1359 and 1599 showed 62 and 59% sequence similarity to a hypothetical protein BLR1088 from Bradyrhizobium japonicum [BAC46353.1] and to a DNA region from Burkholderia pseudomallei, respectively. The hypothetical protein, BLR1088, is a putative GNAT family N-acetyltransferase that is important for regulation of cell growth and development. This protein also plays an important role in
transcription and DNA repair. The putative stop codon (TGA) of this 238 bp DNA partial sequence overlaps with an ATG site in the aac8 partial gene sequence (-TGATG-; as indicated by the bold face type in the Figure).

BLAST searches showed that the DNA region from position 1599 to 1749 was similar to (81% similarity) an aminoglycoside acetyltransferase gene (aac8). Directly downstream of aac8 partial gene sequence, a complete gene cassette containing a bla-oxa-2 encoding ORF was located. The translation start and stop codons for bla-oxa-2 were identified at positions 1749 and 2575 respectively. The start codon overlapped with the stop codon of the proximal aac8 partial ORF. BLAST searches on this complete ORF revealed that it was identical to an oxacillinase (class D β-lactamase) gene (bla-oxa-2). This gene was previously identified in S. Typhimurium where it was located to an IncN plasmid R46 [M95287 and X07260]. It was also detected in Pseudomonas aeruginosa [AY507153 and AJ620678] and on an integron located on a transposon in Clostridium asperum [AJ871915]. The 275 amino acid residue Bla-oxa2 polypeptide from the two study isolates (SC-28 and SC-56) was identical to a Bla-oxa2 enzyme in P. aeruginosa [AY507153, AJ620678] and in IncN plasmid R46 [M95287]. Finally, the 59-base element associated with the bla-oxa-2 gene was identified at position 2540 whilst the 3’-CS encoding the quaternary ammonium and sulfonamide resistance genes was found directly downstream of this element.
Conjugation assay and plasmid incompatibility typing.

Conjugation experiments showed that the ampicillin resistance trait from the two *Salmonella* donor strains (SC-56) and (SC-28) could be transferred to a recipient *E. coli* JM109 Nal\(^r\) strain in broth mating. Similarly, the tetracycline resistance marker of *S. Typhimurium* (SC-56) also transferred successfully to the *E. coli* recipient strain. Gentamycin and trimethoprim resistances were not transferred from either donor (SC-28 or SC-56) to the recipient strain.

A PCR based incompatibility typing assay (inc/rep) detected the presence of IncA/C, P and W replicons in the donor strains (SC-28 and SC-56) prior to conjugation assays. Following conjugation the transconjugants (Nal\(^r\) and Amp\(^r\)) obtained from *S. Anatum* (SC-28) and from *S. Typhimurium* (SC-56) were again typed using the inc/rep PCR protocol. Results showed that both sets of transconjugants were positive for IncA/C and IncP replicons. A third transconjugant denoted as Nal\(^r\), Tet\(^r\) from *S. Typhimurium* (SC-56) was also positive for IncA/C and IncP.

Discussion

In this paper, we reported on a collection of *Salmonella* spp. of various serotypes cultured from a variety of food samples; chicken, beef, sausage and cheese. These food samples were from fast-food outlets and retail markets located in four regions of Northern Colombia. Antibiogram analysis of this collection showed that
ceftiofur resistance was common, with 15 % of all isolates resistant to this
antimicrobial agent. A comparable resistance pattern was observed for
neomycin and oxytetracycline with 11 and 10% of the collection resistant to these
antibiotics. Ceftiofur resistant *Salmonella* strains have recently been isolated
from humans and food animals that are destined for the food chain and
this resistance may be a consequence of the widespread use of these
antimicrobial agents. It is interesting to speculate that a combination of the
widespread use of inexpensive antimicrobials and the high prevalence of
associated class 1 integrons containing the corresponding resistance markers
may be a contributing factor to the persistence of resistance to these agents in
*Salmonella* spp. in animals destined for the food chain.

We investigated the resistance phenotypes of the collection and described the
prevalence of class 1 integron gene sequences among them. We also examined
the collection for the following genetic markers; *bla*-TEM, *bla*-PSE, *bla*-CMY2, *ctx*-u,
tetG and tetA that in some cases may have contributed to their resistance
phenotype. Amplicons derived from the variable region of class 1 integrons from
*S. Typhimurium* (SC-56) and *S. Anatum* (SC-28) contained the complete *bla*-oxa2
gene cassette along with a number of partial ORFs and these were fully
characterized. When these sequences were compared to similar structures in
other *Salmonella* and unrelated bacteria a strong homology was evident. In
particular the *bla*-oxa2 gene was identical in other *Salmonella* and unrelated
bacteria. BLAST searches of the metallo-beta-lactamase partial gene sequence
(bla-\textit{imp}13) showed that the same gene was contained within a novel Tn5051-
transposon in a clinical \textit{P. aeruginosa} isolate. This transposon was responsible
for the dissemination of carbapenemase resistance throughout Europe.\textsuperscript{35} The
dihydrofolate reductase gene (\textit{dfr7}) and the oxacillinase gene (\textit{bla-oxa2}) have been
reported previously in \textit{S. Typhimurium} and \textit{S. Typhi} [AY348316] where they have
been located on integrons and associated with large plasmids such as IncN R46
\textsuperscript{36,37}. However, the partial ORFs identified here have not been reported in
\textit{Salmonella} spp.

The genetic organization of the 2.6-Kbp integron structures in this study showed
that the \textit{bla-oxa2} gene was located immediately downstream of the \textit{aac8} partial
gene sequence. The oxacillinase gene (\textit{bla-oxa2}) from a \textit{P. aeruginosa} [AY444814]
isolate was similarly located downstream of its \textit{aac8} gene both of which were part
of integron (In78) associated with a transposon.\textsuperscript{38} Aubert et al.,\textsuperscript{38} also found
several truncated ORFs within variable gene cassettes that they studied. These
authors reported DNA stretches within the variable cassette regions that did not
share any sequence identity with known DNA sequences available in the
GenBank database. A similar observation was made in our study wherein a 143-
bp DNA sequence was located between \textit{dfr7} and \textit{blr1088} partial gene cassettes
and which did not share any identity with known DNA sequences. We observed
the genetic organization of the gene cassettes in this study showed similarities to
those that are located on plasmids or transposons in other Gram negative
bacteria (Figure). This indicates the possible transmission or resistance markers
from food related to non-food related bacteria that can cause human infection.
The G+C content for the variable gene cassette region for S. Typhimurium (SC-56) and S. Anatum (SC-28) was 45.29 % compared with 49.17 for SGI-1 and 51-53 % for the S. Typhimurium genome. The G+C content of each cassette in this study (incomplete or complete) were as follows; dfr7 (34.11 %), bla -imp13 (35.18 %), bla-oxa2 (49.27 %), aac8 (52.63 %) and blr1088 (54.39 %).

Incompatibility typing of the donor strains (SC-28 and SC-56) showed that Inc A/C, P and W replicons were present in two serotypes Typhimurium and Anatum. Although in S. Typhimurium the integron-dependant antibiotic resistance cluster is most often associated with the chromosomally located Salmonella Genomic Island 1 (SGI1), integrons can also be found on plasmids, in particular plasmids of Inc F and Inc L/M incompatibility groups along with IncF, IncH, IncI, IncN, IncP and IncQ in decreasing order of prevalence 17. We confirmed that the 2.6 Kbp integron amplicons were located on plasmids. BLAST searches of the complete 2.6 Kbp fragment showed that the DNA fragment shared 99% and 83% DNA similarity to an integron structure located on conjugative IncN and IncP plasmids from P. aeruginosa and S. Typhimurium [AJ863750 and AY046276]. Inc/rep typing showed that Amp’, Nal’ and Tet’, Nal’ transconjugants contained plasmids belonging to groups IncA/C and IncP. These plasmids are responsible for the ampicillin and tetracycline resistance phenotypes observed.
In conclusion, our findings show that the 2.6-Kbp gene cassette from *S.* Typhimurium and *S.* Anatum food isolates have an unusual genetic organization in which they have several truncated ORFs. The gene cassettes were found to have a lower G+C content that that of *Salmonella* and SGI-1. These genetic features would suggest the possibility of one or more recombinational events occurring resulting in the evolution of this novel mosaic gene cassette structure in two *Salmonella* isolates from food sources. Comparison of our cassette structures with others indicates similar events may have occurred independently in non-food isolates.

Evidence of truncated ORFs in variable gene cassettes has been reported before where in some instances have been located to integron associated transposons. This study showed that the 2.6-Kbp gene cassette is located on a large conjugative plasmid either IncA/C or IncP, the latter being the most likely since our gene cassette showed a 83% identity to an IncP from *S.* Typhimurium. To support this, all transconjugants were positive for IncP replicon. In addition to this ampicillin resistance is found frequently on IncP plasmids.

A comparison of the 2.6-Kbp structure in this study with similar structures in clinical isolates from *Salmonella* and other bacteria highlights the horizontal transmission of antimicrobial resistance determinants via plasmids could occur from food related pathogens to those responsible for clinical infection in humans.
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References.


25. Hall R. Mobile gene cassettes and integrons: moving antibiotic resistance
genes in Gram-negative bacteria. *Ciba Found Symp* 1997; **207**: 192-202;
discussion 202-5. Review.

26. Lévesque C, Piche L, Larose C et al. PCR mapping of integrons reveals
several novel combinations of resistance genes. *Antimicrob Agents

27. Michael G, Cardoso M, Schwarz S. Class I integron-associated gene
cassettes in *Salmonella enterica subsp. Enterica serovar Agona* isolated

28. Durango J, Arrieta G, Mattar S. Presence of *Salmonella* as a risk to public
health in the Caribbean zone of Colombia. *Biomedica* 2004; **24**: 89-96.

29. National Committee for Clinical Laboratory Standards. *Performance
Standards for Antimicrobial Susceptibility Testing; Twelfth Informational
Supplement*, NCCLS document M100-S11, NCCLS, Pennsylvania, USA,
2002.

30. Altschul S, Madden T, Schaffer A et al. Gapped BLAST and PSI-BLAST:
a new generation of protein database search programs. *Nucleic Acids Res*
1997; **25**: 3389-402.

31. Thompson J, Higgins D, Gibson T. CLUSTALW: improving the sensitivity
of progressive multiple sequence alignment through sequence weighting,
positions specific gap penalties and weight matrix choice. *Nucleic Acids


Figure Legends

Figure

Schematic representation of a class 1 integron identified in S. Typhimurium (SC-56) and S. Anatum (SC-28) from Colombian food collection in this study. The figure depicts four partial gene cassettes bla-imp13, dfr7, blr1088 and aac8 along
with a complete gene cassette of \textit{bla-oxa2}. These are indicated with dotted and complete lined arrows, indicating direction of transcription. The 5' and 3' CS – conserved segments of the class 1 integron are shown as shaded boxes at either end. In the case of the 5' CS reading frame, the vertical dotted lines indicate the attachment site located between nucleotide positions 14-66 (\textit{att} site) within the 5' region. The putative 59- be elements are shown as square white boxes preceding \textit{bla-imp13, dfr7} and \textit{bla-oxa2} and are shown beneath the gene cassettes. The bold vertical lines within the first 59-be delineates the stem loop structure. The –10 and –35 signals are shown in blue bold face type. The translation start and stop codons for each gene (partial or complete) are indicated in green and red and where overlapping occurs nucleotides are shown in brown. Numbering also indicates start and stop codon positions. The 3' CS- conserved segments contained both the \textit{qacEΔ1} and \textit{sul1} determinants. The pink shaded boxes show DNA sequence that is unknown in GenBank database. Inverted repeat is depicted by black circle. Horizontal arrows above \textit{intI1} indicate transcription direction.