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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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2 **Submitted to *Microbial Drug Resistance***

3

4 **Antimicrobial resistance in non-typhoidal *Salmonella* from food sources**
5 **Colombia: evidence for an unusual plasmid located Class 1 integron in serotypes**
6 **Typhimurium and Anatum**
7

8

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31 **Keywords:** *Salmonella*, class 1 integron, *bla*-$_{oxa-2}$, IncAC, P and IncW.

32 **Abstract**

33 Seventy-two isolates representing 18 serotypes recovered from various food
34 samples collected in Colombia were tested for antimicrobial susceptibilities. The
35 collection was further characterised for extended-spectrum cephalosporin,
36 aminoglycoside, and tetracycline resistance mechanisms. Multi-drug resistant
37 (MDR) isolates were further investigated for class 1 integrons and were
38 evaluated for the presence of conjugative plasmids along with a determination of
39 the incompatibility group by PCR. Antibiogram analysis showed that ceftiofur
40 resistance was moderately high (15%). A comparable resistance pattern was
41 also observed for neomycin and oxytetracycline (11 and 10% respectively).
42 There was a high prevalence of gene cassettes as part of one or more class 1
43 integrons (61%) many of which contained markers that contributed to the
44 resistance profile. Class 1 integrons identified in MDR *Salmonella enterica*
45 serotype Typhimurium and *Salmonella enterica* serotype Anatum isolates were
46 characterised. Sequence identified several incomplete open reading frames
47 (ORF) as part of a gene cassette (*bla-imp-13*, *dfr7*, *blr1088* and *aac8*) along with
48 an intact gene cassette (*bla*-$_{oxa\text{-}2}$) in each case. A mosaic of gene cassettes was
49 identical in the two *Salmonella* serotypes and these organisms were cultured
50 from food samples in different regions of Colombia. These integrons were
51 located to a conjugative replicon. Plasmid profiling and incompatibility typing
52 identified three plasmids belonging to Inc groups A/C, P and W. Our study
53 highlights the role of integrons, contributing to a MDR phenotype that is capable
54 of dissemination to other bacteria.

55

56 **Introduction**

57 *Salmonella* spp. are recognised as major food borne pathogens worldwide[1].

58 Most *Salmonella* infections in humans result from the ingestion of contaminated

59 foods of animal origin [2]. Contamination can occur at multiple stages along the

60 food chain including production, processing, distribution, handling and

61 preparation.

62

63 The emergence and dissemination of multi-drug resistant (MDR) *Salmonella* has

64 become a major public health issue as it threatens the efficacy of current

65 antibiotic therapies in cases of life-threatening Salmonellosis [3, 4, 5-8]. Of particular

66 concern, is the continuing emergence of *Salmonella* isolates that are resistant to

67 extended-spectrum cephalosporins [9, 10,11]. Extended-spectrum cephalosporins

68 such as ceftriaxone and ceftiofur are important therapeutic agents used to treat

69 invasive *Salmonella* infections, particularly in children [4, 12]. Resistance to these

70 agents is mediated by an AmpC-like beta-lactamase encoded by the bla-$_{CMY}$

71 gene [13, 14]. Epidemiological studies have shown that the extensive use of

72 antibiotics in domestic livestock is an important risk factor for the emergence and

73 subsequent transfer of MDR *Salmonella* to humans *via* the food chain [15].

74

75 Many antimicrobial resistance genes in *Salmonella* are found on large

76 conjugative plasmids and these can be located within transposons [5, 16-18]. These

77 genes can be also found as a component part of a gene cassette recombined

78 within an integron(s) [19]. Several classes of integron have been defined and class
79 1 integrons are clinically important ,and these are found as part of the Tn*21* or
80 Tn*402* transposon family. They are widely distributed among *Salmonella* and are
81 frequently located on plasmids [20, 21]. Plasmids of the IncF and IncL/M
82 incompatibility group have been reported as vehicles for class 1 integrons [16, 22-24].
83 The association of resistance determinants with mobile genetic elements such as
84 plasmids, transposons and integrons contributes to the widespread dissemination
85 of resistance markers within and between bacterial species [25, 26, 27]
86
87 In this paper, we report on the resistance profiles of *Salmonella* isolates
88 recovered from a variety of foods in Colombia. All were investigated for the
89 corresponding genetic marker(s) associated with resistance. In two *Salmonella*
90 serotypes, an unusual array of incomplete ORFs along with a *bla*-$_{\text{oxa-2}}$ gene were
91 identified and located to a 2.6-Kbp amplicon contained within the variable region
92 of a class 1 integron. We examined the ability of these two isolates to
93 disseminate their resistance genes *via* conjugative to a marked *Escherichia coli*
94 strain.
95
96 **Materials and Methods**
97 <u>Salmonella isolates</u>
98 The 72 *Salmonella* isolates in this study, representing 18 serotypes (Table 1)
99 were a sub-set of a collection (n = 636) of *Salmonella* isolates that were part of
100 an epidemiological study carried out in the Caribbean zone of South America [28].

101 The isolates were recovered from various food samples (chicken, beef, sausage,
102 cheese) collected from fast-food outlets in four regions of northern Colombia.
103 Identification and serotyping of all isolates was carried out at Instituto de
104 Investigaciones Biologicas del Tropica, Facultad de Medicina Veterinaria y
105 Zootechnia, Universidad de Cordoba.

106

107 Antimicrobial susceptibility testing

108 Antimicrobial susceptibility testing was carried out by disk diffusion according to
109 guidelines of the National Committee for Clinical Laboratory Standards [29]. The
110 antimicrobials and concentrations in parenthesis used included: ampicillin (10
111 μg), amoxicillin/clavulanic acid (30 μg) amoxicillin (25 μg), gentamicin (10 μg),
112 cefuroxime (30 μg), ceftiofur (30 μg), framycetein (100 μg), neomycin (30 μg),
113 oxytetracycline (30 μg), streptomycin (10 μg), and sulphamethoxazole-
114 trimethoprim (1.25/23.75 μg) (Oxoid). The resistance breakpoints used were:
115 ampicillin ≤ 13 mm, amoxicillin/clavulanic acid ≤ 13 mm, amoxicillin ≤ 13 mm,
116 gentamicin ≤ 12 mm, cefuroxime ≤ 14 mm, ceftiofur ≤ 14 mm, framycetein ≤ 13
117 mm, neomycin ≤ 13 mm, oxytetracycline ≤ 14 mm, streptomycin ≤ 11 mm and
118 sulphamethoxazole-trimethoprim ≤ 10 mm. *Escherichia coli* ATCC 25922 and
119 *Pseudomonas aeruginosa* ATCC 27853 were used as controls.

120

121 DNA isolation, PCR amplification and DNA sequence analysis

122 Total DNA was prepared using the Wizard Genomic DNA purification kit
123 (Promega, Madison, WI). The integrity of the purified template DNA was

124 assessed by conventional agarose gel [1.5%, (w/v)] electrophoresis and the
125 quantity determined using a UV spectrophotometer. The structural components
126 of class 1 integrons were amplified by PCR (these included *intI1*, *qacEΔ1*, *sul1*
127 and the variable gene cassette region). In addition the presence of a selection of
128 antimicrobial resistance genes was determined by amplification also. All of the
129 genes, the corresponding primers and references are shown in Table 1.

130

131 Briefly, the PCR reactions contained 100 ng of purified DNA, 50 pmol/μl of
132 forward and reverse primers (MWG-Biotech AG, Ebersberg, Germany), 1 X
133 Buffer containing 2.5 mM $MgCl_2$, 200 μM dNTPs (Promega, Madison, WI) and
134 0.5 U *Taq* DNA Polymerase (New England Biolabs, Ipswich, MA) or *Pfu*
135 Polymerase (Chimerx, Madison, WI).

136

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

142

143 PCR-based incompatibility typing

144 Inc group identification of plasmids was carried out according to the referenced
145 method [32]. Briefly, 18 pairs of primers were designed to perform 5 multiplex-and
146 3 simplex-PCRs, recognizing the FIA, FIB, FIC, HI1, HI2, I1-Iγ, L/M, N, P, W, T,

147 A/C, K, B/O, X, Y, F and FIIA replicons representative of the major plasmid
148 incompatibility groups circulating among the *Enterobacteriaceae*. Multiplex 1
149 recognises HI1, HI2 and I1-Iγ, multiplex 2 recognises X, L/M, and N, multiplex 3
150 recognises FIA, FIB and W, multiplex 4 recognises Y, P, and FIC and multiplex 5
151 recognises A/C, T and FIIAs. The three simplex PCRs recognize F, K and B/O
152 replicons respectively. Primers and positive controls were previously described
153 [32]. All PCR amplifications, except F-simplex, were performed with the following
154 amplification scheme: 1 cycle of denaturation at 94°C for 5 min, followed by 30
155 cycles of denaturation at 94°C for 1 min, annealing at 60°C for 30 s and
156 elongation at 72°C for 1 min. A final extension of 1 cycle at 72°C for 5 min was
157 performed. The F- simplex PCR was performed with the same amplification
158 program but at an annealing temperature of 52°C.
159

160 Isolation of large plasmids

161 Plasmid DNA was isolated from strains according to the manufacturers
162 instructions (Invitrogen, Biosciences, Dublin). The concentration and integrity
163 was assessed as outlined above.
164

165 Conjugation experiments

166 Conjugation experiments were performed with *E. coli* JM109 (Promega, Madison,
167 WI) as the recipient strain. This plasmid free strain was grown overnight in Luria
168 Bertani [LB] (Oxoid, Basingstoke, Hampshire, UK) broth in the presence of
169 nalidixic acid at a concentration of 50 μg/ml and was subsequently transferred

170 onto a LB plate containing 50 μg/ml of the same antibiotic. This strain then
171 represented the marked recipient for all conjugation experiments. The absence
172 of growth of the donor strains SC-28, SC-56 confirmed that both were susceptible
173 to nalidixic acid and these were designated Nal^s. The donor strain SC-56 [R-
174 type: Aml, Amp, Ot and SXT] was grown on a nutrient agar (NA) plate containing
175 ampicillin (10 μg/ml) whilst the recipient was grown on NA plate containing
176 nalidixic acid (50 μg/ml). A single colony of both the donor and recipient was
177 transferred to 3 ml of LB and incubated at 37°C for 24 h. The overnight culture
178 was pelleted and resuspended in 1 ml of LB broth without antibiotic. An aliquot of
179 this suspension (100 μl) was plated directly onto a dried NA plate containing
180 nalidixic acid (50 μg/ml) and sequentially, each of the following; ampicillin (10
181 μg), tetracycline (30 μg), and trimethoprim (5 μg). The second donor SC-28 was
182 grown and plated as described for SC-56 but in addition it was also plated onto
183 gentamycin (10 μg). All transconjuants were examined for the presence of
184 replicons using the incompatibility typing protocol described earlier. The Inc Rep
185 typing assay was performed prior to and after conjugation experiments.
186
187 Nucleotide accession number
188 The complete 2.6-Kbp amplicons sequenced from *S*. Anatum (SC-28) *S.*
189 Typhimurium (SC-56) were submitted to GenBank and assigned the Accession
190 numbers AM237807 and AM237806 respectively.
191
192

193
194 **Results**

195 Antimicrobial susceptibility of *Salmonella* isolates

196 Twenty four (33%) of the *Salmonella* strains showed resistance to a least one of
197 the 11 antimicrobial agents tested. Table 2 summarises the resistance profiles
198 obtained for all isolates. The highest levels of resistance were found for ceftiofur
199 (17%) followed by neomycin (13%) and oxytetracycline (11%). Fourteen different
200 antibiotic profiles were observed, 10 of which were multi-antimicrobial resistant
201 (defined as isolates showing resistance to two or more classes of antimicrobials)
202 as shown in Table 3.
203

204 Antimicrobial resistance genes and gene cassettes

205 The *bla*-$_{CMY-2}$ gene was detected in 7 of 12 isolates resistant to the extended
206 spectrum β-lactamases. Three *S*. Typhimurium isolates were found to carry *bla*-
207 $_{PSE}$ gene. β-Lactam resistant isolates did not produce any amplicons when
208 screened for *ctx-u* or *bla*-$_{TEM}$ (Table 1). The *tetA* or *tetG* genes were detected in
209 all isolates showing tetracycline resistance.
210

211 Sixty one % of the isolates contained integron associated gene cassettes with
212 sizes ranging from 0.4- to 2.6-Kbp (data not shown). One *S*. Typhimurium isolate
213 contained 1.0- and 1.2-Kbp amplicons corresponding to the integron profile (IP)
214 type-1 [33, 34]. Two *S*. Typhimurium isolates from Monteria, contained a 1.0-Kbp
215 amplicon (Table 3). Thirty isolates from the collection contained a 1.4- Kbp
216 amplicon alone whilst a further three isolates had the 1.4-Kbp amplicon in

217 addition to a 0.7-Kbp amplicon. Three isolates contained the 1.4-Kbp amplicon
218 along with a second amplicon of 0.6-, 0.5- or 0.4-Kbp. One *S.* Anatum (R- Type:
219 Amp, Cn, Eft, Fy, N, Ot and S) and two *S.* Typhimurium (R-Type: Aml, Amp, Ot
220 and Sxt) contained a 2.6- and 1.2-Kbp amplicon respectively. Overall, the gene
221 cassettes detected were not serotype specific and were found in thirteen
222 (including an undetermined serotype recorded as *Salmonella* spp.) of the
223 eighteen serotypes present in this collection. Table 3 shows a summary of the
224 gene cassette sizes of any isolate that displayed resistance to at least one
225 antimicrobial agent.
226
227 <u>Molecular analysis of Integron associated gene cassettes</u>
228 Sequence analysis of the 2.6-Kbp amplicons from *S.*Typhimurium (SC-56) and *S.*
229 Anatum (SC-28) isolates were characterized further in this study. Following the
230 complete determination of the DNA sequence in each case, initial comparisons
231 showed that all were identical (Figure). The 2.6-Kbp sequence from each
232 revealed four partial gene sequences (bla_{-imp13}, *dfr7*, *blr1088* and *aac8*) and one
233 complete ORF, (bla_{-oxa-2}). The 5'- and 3'-conserved structures were determined
234 by PCR (Table 1) and found to be intact, indicating the presence of a complete
235 class 1 integron. To determine if these 2.6-Kbp amplicons were present on
236 plasmids, large plasmids were extracted from *S.* Typhimurium (SC-56) and *S.*
237 Anatum (SC-28). The purified plasmid DNA from both isolates was used as
238 template DNA in a subsequent gene cassette PCR reaction. The 2.6-Kbp
239 amplicons were successfully amplified in both cases. In addition the plasmid

240 profiles from *S*. Typhimurium and *S*. Anatum were examined by agarose gel
241 electrophoresis and showed identical plasmid DNA profiles.
242

243 When the DNA sequence of the 2.6-Kbp amplicon was analysed in detail, the
244 putative attachment site (*att1*) was located towards the proximal end (Figure).
245 This is the recombination site recognized by the 59-base element (be) sequence
246 found on the distal ends of gene cassettes. This sequence was located between
247 nucleotide positions 1 through 74. Downstream of the *att1* site is the first of four
248 incomplete ORFs that showed some idenity to *bla*-$_{imp13}$, *dfr7*, *blr1088* and *aac8*
249 sequences, respectively (all indicated by the dashed arrowheads in the Figure).
250 The first incomplete gene (*bla*$_{-imp13}$) is 53 bp in length, followed by the classic 59-
251 be. A stem loop structure characteristic of 59-be was also identified where a
252 region of perfect overlapping occurred (-G<u>TTTTT</u>AT<u>AAAAA</u>CT- indicated in bold
253 face type in the Figure). Seven base pairs downstream of the incomplete (*bla*$_{-}$
254 $_{imp13}$) gene and within the first 59-be element –10 (-TAACAA) and –35 signals (-
255 TGGACA) are at found (-10 and –35 signals- indicated in blue bold face type in
256 Figure). The second incomplete gene *dfr7* was located at position 615 extending
257 from the first codon TTG until a TAA stop codon is reached at position 1089.
258 BLAST searches with this incomplete ORF identified significant sequence
259 similarity to a dihydrofolate reductase gene (*dfr7*) found in *S*. Typhimurium
260 [AY245101], *Escherichia coli* [AJ884724] and on an IncLM plasmid from *E. coli*
261 [X58425]. The *dfr7* partial gene coded for a truncated polypeptide of 157 aa.
262 Amino acid alignments of this partial DfrVII identified in the serotypes

263 Typhimurium and Anatum demonstrated that it differed by 32 amino acids
264 residues when aligned with similar sequences [AF245101, X58425, Q549W7,
265 P27422; data not shown]. These differences were found throughout the amino
266 acid sequence and were not located towards any specific terminal end of the
267 polypeptide.
268
269 The 59-be element associated with the incomplete *dfr7* gene is located at
270 position 1090 and extends until position 1215. Similarly both –10 (-TAACAA) and
271 –35 (-TGGACA) signals are found at nucleotide positions 1087 to 1091 and 122l
272 to 1226 respectively (-indicated in blue bold face type in Figure1). Further
273 inspection of the 59-be element DNA sequence revealed a putative ribosomal
274 binding site (RBS). This site spanned from position 1090 and extended beyond
275 the 59-be element until position 1224.
276 Situated between this feature and the third incomplete gene (*blr1088*) is a 143 bp
277 DNA sequence (coloured box in Figure) that does not show homology to any
278 sequence in the GenBank database.
279
280 BLAST searches on another DNA fragment located between positions 1359 and
281 1599 showed 62 and 59% sequence similarity to a hypothetical protein BLR1088
282 from *Bradyrhizobium japonicum* [BAC46353.1] and to a DNA region from
283 *Burkholderia pseudomallei*, respectively. The hypothetical protein, BLR1088, is a
284 putative GNAT family N-acetyltransferase that is important for regulation of cell
285 growth and development. This protein also plays an important role in

286 transcription and DNA repair. The putative stop codon (TGA) of this 238 bp DNA
287 partial sequence overlaps with an ATG site in the *aac8* partial gene sequence (-
288 TGATG-; as indicated by the bold face type in the Figure).
289
290 BLAST searches showed that the DNA region from position 1599 to 1749 was
291 similar to (81% similarity) an aminoglycoside acetyltransferase gene (*aac8*).
292 Directly downstream of *aac8* partial gene sequence, a complete gene cassette
293 containing a $bla_{\text{-oxa-2}}$-encoding ORF was located. The translation start and stop
294 codons for $bla_{\text{-oxa-2}}$ were identified at positions 1749 and 2575 respectively. The
295 start codon overlapped with the stop codon of the proximal *aac8* partial ORF.
296 BLAST searches on this complete ORF revealed that it was identical to an
297 oxacillinase (class D β lactamase) gene ($bla_{\text{-oxa-2}}$). This gene was previously
298 identified in *S*. Typhimurium where it was located to an IncN plasmid R46
299 [M95287 and X07260]. It was also detected in *Pseudomonas aeruginosa*
300 [AY507153 and AJ620678] and on an integron located on a transposon in
301 *Clostridium asperum* [AJ871915]. The 275 amino acid residue Bla-oxa2
302 polypeptide from the two study isolates (SC-28 and SC-56) was identical to a
303 Bla-oxa2 enzyme in *P*. aeruginosa [AY507153, AJ620678] and in IncN plasmid
304 R46 [M95287]. Finally, the 59-base element associated with the $bla_{\text{-oxa-2}}$ gene
305 was identified at position 2540 whilst the 3'-CS encoding the quaternary
306 ammonium and sulfonamide resistance genes was found directly downstream of
307 this element.
308

309 Conjugation assay and plasmid incompatibility typing.

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

316

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

325

326

327 **Discussion**

328 In this paper, we reported on a collection of *Salmonella* spp. of various serotypes
329 cultured from a variety of food samples; chicken, beef, sausage and cheese.
330 These food samples were from fast-food outlets and retail markets located in four
331 regions of Northern Colombia. Antibiogram analysis of this collection showed that

332 ceftiofur resistance was common, with 15 % of all isolates resistant to this
333 antimicrobial agent. A comparable resistance pattern was observed for
334 neomycin and oxytetracycline with 11 and 10% of the collection resistant to these
335 antibiotics. Ceftiofur resistant *Salmonella* strains have recently been isolated
336 from humans [39] and food animals that are destined for the food chain [40, 41] and
337 this resistance may be a consequence of the widespread use of these
338 antimicrobial agents. It is interesting to speculate that a combination of the
339 widespread use of inexpensive antimicrobials and the high prevalence of
340 associated class 1 integrons containing the corresponding resistance markers
341 may be a contributing factor to the persistence of resistance to these agents in
342 *Salmonella* spp. in animals destined for the food chain.
343
344 We investigated the resistance phenotypes of the collection and described the
345 prevalence of class 1 integron gene sequences among them. We also examined
346 the collection for the following genetic markers; $bla\text{-}_{TEM}$, $bla\text{-}_{PSE}$, $bla\text{-}_{CMY2}$, *ctx-u*,
347 *tetG* and *tetA* that in some cases may have contributed to their resistance
348 phenotype. Amplicons derived from the variable region of class 1 integrons from
349 *S*. Typhimurium (SC-56) and *S*. Anatum (SC-28) contained the complete $bla\text{-}_{oxa2}$
350 gene cassette along with a number of partial ORFs and these were fully
351 characterized. When these sequences were compared to similar structures in
352 other *Salmonella* and unrelated bacteria a strong homology was evident. In
353 particular the $bla\text{-}_{oxa2}$ gene was identical in other *Salmonella* and unrelated
354 bacteria. BLAST searches of the metallo-beta-lactamase partial gene sequence

355 (*bla*-$_{imp13}$) showed that the same gene was contained within a novel Tn*5051*-
356 transposon in a clinical *P*. aeruginosa isolate. This transposon was responsible
357 for the dissemination of carbapenemase resistance throughout Europe [35]. The
358 dihyrofolate reductase gene (*dfr7*) and the oxacillanase gene (*bla*-$_{oxa2}$) have been
359 reported previously in *S*. Typhimurium and *S*. Typhi [AY348316] where they have
360 been located on integrons and associated with large plasmids such as IncN R46
361 [36, 37]. However, the partial ORFs identified here have not been reported in
362 *Salmonella* spp.
363

364 The genetic organization of the 2.6-Kbp integron structures in this study showed
365 that the *bla*-$_{oxa2}$ gene was located immediately downstream of the *aac8* partial
366 gene sequence. The oxcillinase gene (*bla*-$_{oxa2}$) from a *P*. aeruginosa [AY444814]
367 isolate was similarly located downstream of its *aac8* gene both of which were part
368 of integron (In*78*) associated with a transposon [38]. Aubert et al., [38] also found
369 several truncated ORFs within variable gene cassettes that they studied. These
370 authors reported DNA stretches within the variable cassette regions that did not
371 share any sequence identity with known DNA sequences available in the
372 GenBank database. A similar observation was made in our study wherein a 143-
373 bp DNA sequence was located between *dfr7* and *blr1088* partial gene cassettes
374 and which did not share any identity with known DNA sequences. We observed
375 the genetic organization of the gene cassettes in this study showed similarities to
376 those that are located on plasmids or transposons in other Gram negative
377 bacteria (Figure). This indicates the possible transmission or resistance markers

378 from food related to non-food related bacteria that can cause human infection.
379 The G+C content for the variable gene cassette region for *S*. Typhimurium (SC-
380 56) and *S*. Anatum (SC-28) was 45.29 % compared with 49.17 for SGI-1 and 51-
381 53 % for the *S*. Typhimurium genome. The G+C content of each cassette in this
382 study (incomplete or complete) were as follows; *dfr7* (34.11 %), *bla -imp13* (35.18
383 %), *bla-*$_{oxa2}$ (49.27 %), *aac8* (52.63 %) and *blr1088* (54.39 %).
384

385 Incompatibility typing of the donor strains (SC-28 and SC-56) showed that Inc
386 A/C, P and W replicons were present in two serotypes Typhimurium and Anatum.
387 Although in *S*. Typhimurium the integron-dependant antibiotic resistance cluster
388 is most often associated with the chromosomally located *Salmonella* Genomic
389 Island 1 (*S*GI1), integrons can also be found on plasmids, in particular plasmids
390 of Inc F and Inc L/M incompatibility groups along with IncF, IncH, IncI, IncN, IncP
391 and IncQ in decreasing order of prevalence [17]. We confirmed that the 2.6 Kbp
392 integron amplicons were located on plasmids. BLAST searches of the complete
393 2.6 Kbp fragment showed that the DNA fragment shared 99% and 83% DNA
394 similarity to an integron structure located on conjugative IncN and IncP plasmids
395 from *P. aeruginosa* and *S*. Typhimurium [AJ863750 and AY046276]. Inc/rep
396 typing showed that Amp^r, Nal^r and Tet^r, Nal^r transconjugants contained plasmids
397 belonging to groups IncA/C and IncP. These plasmids are responsible for the
398 ampicillin and tetracycline resistance phenotypes observed.
399

400 **Conclusion**

401 In conclusion, our findings show that the 2.6-Kbp gene cassette from *S*.
402 Typhimurium and *S*. Anatum food isolates have an unusual genetic organization
403 in which they have several truncated ORFs. The gene cassettes were found to
404 have a lower G+C content that that of *Salmonella* and SGI-1. These genetic
405 features would suggest the possibility of one or more recombinational events
406 occurring resulting in the evolution of this novel mosaic gene cassette structure in
407 two *Salmonella* isolates from food sources. Comparison of our cassette
408 structures with others indicates similar events may have occurred independently
409 in non-food isolates.
410
411 Evidence of truncated ORFs in variable gene cassettes has been reported before
412 where in some instances have been located to integron associated transposons.
413 This study showed that the 2.6-Kbp gene cassette is located on a large
414 conjugative plasmid either IncA/C or IncP, the latter being the most likely since
415 our gene cassette showed a 83% identity to an IncP from *S*. Typhimurium. To
416 support this, all transconjugants were positive for IncP replicon. In addition to this
417 ampicillin resistance is found frequently on IncP plasmids.
418
419 A comparison of the 2.6-Kbp structure in this study with similar structures in
420 clinical isolates from *Salmonella* and other bacteria highlights the horizontal
421 transmission of antimicrobial resistance determinants via plasmids could occur
422 from food related pathogens to those responsible for clinical infection in humans.

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624 **Figure Legends**

625 **Figure**

626 Schematic representation of a class 1 integron identified in *S*. Typhimurium (SC-
627 56) and *S*. Anatum (SC-28) from Colombian food collection in this study. The
628 figure depicts four partial gene cassettes *bla-imp13*, *dfr7*, *blr1088* and *aac8* along

629 with a complete gene cassette of *bla-*$_{oxa2}$. These are indicated with dotted and
630 complete lined arrows, indicating direction of transcription. The 5' and 3' CS –
631 conserved segments of the class 1 integron are shown as shaded boxes at either
632 end. In the case of the 5' CS reading frame, the vertical dotted lines indicate the
633 attachment site located between nucleotide positions 14-66 (*att* site) within the 5'
634 region. The putative 59- be elements are shown as square white boxes
635 preceding *bla-imp13*, *dfr7* and *bla-*$_{oxa2.}$ and are shown beneath the gene
636 cassettes. The bold vertical lines within the first 59-be delineates the stem loop
637 structure. The –10 and –35 signals are shown in blue bold face type. The
638 translation start and stop codons for each gene (partial or complete) are indicated
639 in green and red and where overlapping occurs nucleotides are shown in brown.
640 Numbering also indicates start and stop codon positions. The 3' CS- conserved
641 segments contained both the *qacEΔ1* and *sul1* determinants. The pink shaded
642 boxes show DNA sequence that is unknown in GenBank database. Inverted
643 repeat is depicted by black circle. Horizontal arrows above *intI1* indicate
644 transcription direction.
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