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Genetic characterization of the mechanisms of resistance to amoxicillin/clavulanate and third-generation cephalosporins in *Salmonella enterica* from three Spanish hospitals

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Summary. The mechanisms of antimicrobial resistance were characterized in 90 *Salmonella enterica* isolates either resistant or with intermediate resistance to amoxicillin/clavulanate ($AMC^{R/I}$) or resistant to third-generation cephalosporins ($C3G^R$). These isolates were recovered in three Spanish hospitals during 2007–2009. The $C3G^R$ phenotype was expressed by three isolates that carried the following extended-spectrum β-lactamase genes: phage-associated $bla_{CTX\ M\text{-}10}$ in *S.* Virchow, $bla_{CTX\text{-}M\text{-}14a}$ surrounded by IS*Ecp1* and IS*903* in *S.* Enteritidis, and $bla_{CTX\text{-}M\text{-}15}$ linked to IS*Ecp1* and *orf477* in *S.* Gnesta (first description in this serotype). The $AMC^{R/I}$ phenotype was found in 87 isolates (79 *S.* Typhimurim, 7 *S.* Enteritidis, and one *S.* Thompson). The $bla_{PSE\text{-}1}$ gene, followed by $bla_{OXA\text{-}1}$ was mostly found among *S.* Typhimurim, and the $bla_{TEM\text{-}1}$ gene among *S.* Enteritidis. Three different gene combinations [$bla_{PSE\text{-}1}$+*floR*+*aadA2*+*sul*+*tet*(G); $bla_{OXA\text{-}1}$+*catA*+*aadA1/strA-strB*+*sul*+*tet*(B) and $bla_{TEM\text{-}1}$+*cmlA1*+*aadA/strA-strB*+*sul*+*tet*(A)/*tet*(B) genes] were associated with the ampicillin-chloramphenicol-streptomycin-sulfonamides-tetracycline phenotype in 68 $AMC^{R/I}$ *S. enterica* isolates. Class 1 integrons were observed in 79% of the isolates and in most of them (45 isolates) two integrons including the *aadA2* and $bla_{PSE\text{-}1}$ gene cassettes, respectively, were detected. The $bla_{OXA\text{-}1}$+*aadA1* arrangement was detected in 23 isolates, and the *aac*(6′)-Ib-cr+$bla_{OXA\text{-}1}$+*catB3*+*arr3* in another one. Non-classic class 1 integrons were found in three isolates: *dfrA12*+*orfF*+*aadA2*+*cmlA1*+*aadA1* (1 isolate), *dfrA12*+*orfF*+*aadA2*+*cmlA1*+*aadA1*+*qacH*+IS*440*+*sul3* (1 isolate) and *dfrA12*+*orfF*+*aadA2*+*cmlA1*+*aadA1*+*qacH*+IS*440*+ *sul3*+*orf1*+*mef*(B)Δ-IS*26* (1 isolate). Taken together, these results underline the need for clinical concern regarding β-lactam resistance in *Salmonella* and thus for continuous monitoring. [**Int Microbiol** 2011; 14(3):173-181]

Keywords: *Salmonella enterica* · β-lactam-resistance · integrons · extended-spectrum β-lactamases (ESBL)

Introduction

Salmonella enterica is the second most frequent cause of zoonotic diseases in humans in Europe, and more than 150,000 cases of human salmonellosis were reported by The European Surveillance System during 2007 [8]. *Salmonella* Enteritidis and *Salmonella* Typhimurium are two of the ten most common serotypes confirmed in salmonellosis cases in humans, representing 81% of the isolates [8]. *S.* Typhimurium is frequently associated with multidrug resistance [3,26], in part due to the worldwide emergence of *S.* Typhimurium definitive phage type (DT) 104, which contains the chromosomal *Salmonella* genomic island type I (SGI-1). SGI-1 harbors genes that confer the ACSSuT phenotype (i.e., resistance to ampicillin, chloramphenicol, streptomycin, sul-

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fonamides, and tetracycline) [16]. Although *S.* Typhimurium DT104 is the main example of multiresistance in *S. enterica,* many antimicrobial resistance genes have been reported also in isolates of other serotypes [14].

Non-typhoidal *Salmonella* infections generally result in mild-to-moderate self-limiting gastroenteritis, and antimicrobial treatment is only required in severe cases occurring in vulnerable patient groups or to combat invasive infections. However, due to the increasing resistance of this bacterium to the conventional antimicrobial agents (ampicillin, and trimethoprim/sulfamethoxazole) used in the treatment of salmonellosis, amoxicillin/clavulanate, third-generation cephalosporins, and fluoroquinolones have become further treatment options. Resistance to β-lactams in *S. enterica* is mainly due to the production of acquired β-lactamases [14]. Among these, TEM-1, PSE-1, and OXA-1 have been described as the enzymes most frequently related to ampicillin and amoxicillin/clavulanate resistance [3,11]. The resistance of *Salmonella* to third-generation cephalosporins is primarily mediated by the production of extended-spectrum β-lactamases (ESBL) of the TEM, SHV, and CTX-M types, which are associated with different mobile genetic elements [11,14]. ESBL have been described not only in clinical *Salmonella* isolates but also in isolates from animals and food [6,21].

Mobile genetic elements such as plasmids and transposons, possibly containing integrons, are able to disseminate antimicrobial resistance by horizontal transfer in Enterobacteriaceae. Integrons are genetic elements that capture and incorporate gene cassettes by using a site-specific recombination mechanism [4]. Thus far, class 1 and, less frequently, class 2 integrons have been reported for *S. enterica* [4]. Class 1 integrons contain a 5′-conserved segment (5′-CS) that includes the integrase *intI1* gene, the *attI1* recombination site, and the Pc promoter. It is followed by a variable region where one or more gene cassettes are located. This class of integrons also contains a 3′-conserved segment (3′-CS) that includes the *sul1* and *qacE*Δ1 genes, which encode resistance to sulfonamides and ammonium quaternary compounds, respectively [4].

In recent years, resistance to amoxicillin/clavulanate among *S. enterica* isolated from different Spanish hospitals has become increasingly widespread, accompanied by the emergence of ESBL-producing isolates, detected in human samples. Consequently, there are fewer therapeutic options for the treatment of *S. enterica* infections, placing these patients at greater risk of serious morbidity and even death. The aim of the present work was to characterize the mechanisms of resistance to β-lactams and other antimicrobial agents as well as the integrons in all amoxicillin/clavulanate-resistant, intermediately resistant ($AMC^{R/I}$), and third-generation cephalosporin-resistant ($C3G^{R}$) *S. enterica* isolates recovered in three Spanish hospitals during the period 2007–2009.

Materials and methods

Isolates and antimicrobial susceptibility testing. In this study, 90 *S. enterica* isolates with the $AMC^{R/I}$ phenotype (87 isolates) or the $C3G^{R}$ phenotype (3 isolates) were recovered in three Spanish hospitals located in geographically distinct areas: Hospital General Universitario Gregorio Marañón of Madrid (HGM, 39 isolates), Hospital San Pedro of Logroño (HSP, 36 isolates), and Complejo Hospitalario of Pontevedra (CHP, 15 isolates). $AMC^{R/I}$ and $C3G^{R}$ phenotypes were detected in 12–23% and <1%, respectively, of all *S. enterica* isolated in the three hospitals. The 90 isolates were recovered from fecal (73 isolates), blood (2 isolates), urine (1 isolate) and other (14 isolates) samples from different patients during 2007 (29 isolates), 2008 (34 isolates), and 2009 (27 isolates). The serotypes of these isolates were as follows: *S.* Typhimurium (79 isolates), *S.* Enteritidis (8 isolates, one of them $C3G^{R}$), *S.* Virchow (1 isolate, $C3G^{R}$), *S.* Gnesta (1 isolate, $C3G^{R}$), and *S.* Thompson (1 isolate).

Susceptibility testing to 20 antimicrobial agents (ampicillin, AMC, cefalotin, cefazolin, ceftazidime, cefotaxime, aztreonam, cefoxitin, gentamicin, tobramycin, kanamycin, amikacin, streptomycin, nalidixic acid, ciprofloxacin, tetracycline, chloramphenicol, sulfonamides, trimethoprim, trimethoprim/sulfamethoxazole) was performed by the disc-diffusion [5] and microdilution methods (MicroScan Combo Neg panels, Siemens, Sacramento, CA, USA) according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. The AmpC phenotype was determined by comparison of the inhibition zone of cefoxitin discs (30 μg) in the presence or absence of cloxacillin (200 μg) [29]. The ESBL phenotype was determined using the double-disc synergy test with cefotaxime, ceftazidime, and aztreonam discs placed in the proximity of the AMC disc [13].

Detection of antimicrobial resistance genes. The presence of genes implicated in the resistance to β-lactams (bla_{TEM}, bla_{SHV}, bla_{CTX-M}, bla_{OXA-1} and bla_{PSE-1}), and the bla_{CTX-M} genetic environment was detected by PCR and sequencing [7,17,31]. In addition, multiplex PCR for the detection of plasmidic AmpC-type β-lactamases was carried out [20].

Tetracycline [*tet*(A)-*tet*(E),*tet*(G)], aminoglycoside [*aadA*, *strA-strB*, *aac*(3)-I, *aac*(3)-II, *aac*(3)-IV, *ant*(2″), *aph*(3′)-Ia, *aph*(3′)-IIa, *rmtB*, *armA* and *aac*(6′)-Ib], sulfonamides [*sul1, sul2* and *sul3*], trimethoprim [*dfrA*], chloramphenicol [*cmlA*, *catA* and *floR*], and quinolone [*qnrA, qnrB, qnrS* and *qepA*] resistance genes were studied by PCR and sequencing [7,24,27]. The genetic enviroments of the *sul1, sul2*, and *sul3* genes were determined as previously reported [32].

Detection and characterization of integrons. The presence of class 1, 2, and 3 integrase-encoding genes and of the 3′-CS of class 1 integrons, *qacE*Δ1+*sul1*, was analyzed by PCR. The variable regions of these integrons were PCR-amplified and subsequently sequenced to determine their gene cassette arrangements [24].

Results

Antimicrobial susceptibility in *Salmonella enterica* isolates. Table 1 shows the antimicrobial susceptibility of the 90 $AMC^{R/I}$ or $C3G^{R}$ *S. enterica* isolates included in this study. The *S.* Typhimurium isolates were highly resistant to sulfonamides (100%), tetracycline (91%), chloramphenicol (86%), and streptomycin (80%). Aminoglycosides resistance was found only among isolates of serotype *S.* Typhimurium. All isolates studied were susceptible to amikacin, cefoxitin and ciprofloxacin. A multiresistant phenotype (resistant to at least three different antimicrobial agent families) was observed among 100% of the *S.* Typhimurium and 12.5% of the *S.* Enteritidis isolates. Two *S.* Typhimurium isolates had a heptaresistant phenotype that included the ACSSuT phenotype in addition to resistance to trimethoprim and gentamicin or nalidixic acid (ACGSSuTTm and ACSSuTTmN, respectively).

The AmpC phenotype was not identified among the isolates tested. However, the ESBL phenotype was determined in three of them and corresponded to one isolate each of *S.* Enterica, *S.* Virchow, and *S.* Gnesta serotypes (Table 1). All three were resistant to cefotaxime, while *S.* Gnesta isolate was also resistant to ceftazidime and aztreonam.

Antimicrobial resistance genes. Tables 2 and 3 list the resistance genes detected in the 90 *S. enterica* isolates, according to serotype. The most frequent β-lactamase gene identified among the $AMC^{R/I}$ isolates was bla_{PSE-1}, detected in

Table 1. Number of $AMC^{R/I}$ or $C3G^{R}$ of *Salmonella enterica* isolates resistant to antimicrobial agents. The isolates were of different serotypes and obtained from three Spanish hospitals

Antimicrobial agent[a]	*S.* Typhimurium (n = 79)	*S.* Enteritidis (n = 8)	*S.* Virchow (n = 1)	*S.* Gnesta (n = 1)	*S.* Thompson (n = 1)	All *S. enterica* tested (n = 90)
AMC[b]	79	7	0	0	1	87
Cefalotin	7	1	1	1	0	10
Cefazolin	9	1	1	1	0	12
Ceftazidime	0	0	0	1	0	1
Cefotaxime	0	1	1	1	0	3
Aztreonam	0	0	0	1	0	1
Gentamicin	1	0	0	0	0	1
Tobramycin	1	0	0	0	0	1
Kanamycin	2	0	0	0	0	2
Streptomycin	63	0	0	0	0	63
Nalidixic acid	17	2	1	0	0	20
Tetracycline	72	1	1	0	1	75
Chloramphenicol	68	0	0	0	1	69
Sulfonamides	79	3	1	1	1	85
Trimethoprim	5	0	0	0	0	5
SXT[c]	5	1	0	0	0	6
ESBL phenotype	0	1	1	1	0	3

[a]All the isolates were resistant to ampicillin, but susceptible to cefoxitin, amikacin, and ciprofloxacin.
[b]AMC: Amoxicillin/clavulanate.
[c]SXT: Trimethoprim-sulfamethoxazole.

Table 2. Antimicrobial resistance genes and the resistance phenotype of *Salmonella enterica* isolates from three Spanish hospitals

Number of resistant isolates	Resistance genes	*S.* Typhimurium	*S.* Enteritidis	Other serotypes	Total (n = 90)
β-Lactams (n = 90)	bla_{PSE-1}	41	–	–	41
	bla_{OXA-1}	23	–	1	24
	bla_{TEM-1b}	9	6	–	15
	bla_{TEM-1c}	1	–	–	1
	$3bla_{PSE-1} + bla_{OXA-1}$	1	–	–	1
	$bla_{PSE-1} + bla_{TEM-1b}$	3	–	–	3
	$bla_{CTX-M-10}$	–	–	1	1
	$bla_{CTX-M-14a}$	–	1	–	1
	$bla_{CTX-M-15} + bla_{TEM-1}$ [c]	–	–	1	1
	No studied *bla* genes	1	1	–	2
Tetracycline[a] (n = 82)	*tet*(A)	5	–	–	5
	tet(B)	29	–	1	30
	tet(G)	45	–	–	45
	No studied *tet* genes	–	1	1	2
Streptomycin[b] (n = 76)	*aadA1/aadA2*	66	–	–	66
	strA-strB	5	–	–	5
	aadA1/aadA2+strA-strB	4	–	–	4
	No studied genes	1	–	–	1
Gentamicin (n = 1)	*aac*(3)-IV	1	–	–	1
Kanamycin (n = 2)	*aph*(3′)-Ia	1	–	–	1
	No studied genes	1	–	–	1
Chloramphenicol (n = 69)	*floR*	44	–	1	45
	catA	18	–	–	18
	cmlA1	3	–	–	3
	floR + *catA*	2	–	–	2
	floR + *cmlA*	1	–	–	1
Sulfonamides (n = 85)	*sul1*	54	–	–	54
	sul2	8	–	1	9
	sul1 + *sul2*	14	–	1	15
	sul2 + *sul3*	1	–	–	1
	sul1+ *sul2* + *sul3*	1	–	–	1
	No studied *sul* genes	1	3	1	5
Trimethoprim (n = 5)	*dfrA12*	3	–	–	3
	dfrA14	2	–	–	2

[a]Seven of the studied isolates with a phenotype of intermediate resistance to tetracycline harbored the *tet*(G) gene.
[b]Twelve of the studied isolates with a phenotype of intermediate resistance to streptomycin harbored the *aadA1/aadA2* gene. Three of the isolates with a susceptibility to streptomycin harbored *strA-strB* genes.
[c]bla_{TEM-1} variant showed a silent nucleotide change (T→C) at position 735 [28].

51.7% of the 87 $AMC^{R/I}$ isolates including all those belonging to *S.* Typhimurim. In addition, the gene was associated with other *bla* genes in four of these isolates (bla_{TEM-1b} or bla_{OXA-1}). The bla_{OXA-1} gene was identified in 27.6% of the $AMC^{R/I}$ isolates (23 *S.* Typhimurium and 1 *S.* Thompson), and only in one case in association with other *bla* genes. In addition, the bla_{TEM-1} gene was demonstrated in 21.8% of the $AMC^{R/I}$ isolates (13 *S.* Typhimurium and 6 *S.* Enteritidis) and associated with other *bla* genes in three of them. As shown in Table 2, bla_{TEM-1} was the most frequent *bla* gene in *S.* Enteritidis isolates.

The β-lactamase genes identified among the three $C3G^R$ isolates with an ESBL-positive phenotype were as follows: $bla_{CTX-M-14a}$ (*S.* Enteritidis), $bla_{CTX-M-15}$ (*S.* Gnesta), and $bla_{CTX-M-10}$ (*S.* Virchow). In these isolates, the IS*Ecp1*-$bla_{CTX-M-14a}$-IS*903* and IS*Ecp1*-$bla_{CTX-M-15}$-*orf477* structures were identified. The $bla_{CTX-M-15}$-positive *S.* Gnesta isolate also carried a new variant of the bla_{TEM-1} β-lactamase gene that showed a silent nucleotide change (T→C) at position 735 according to the Sutcliffe nomenclature [28]. Regarding the $bla_{CTX-M-10}$ genetic environment, the gene's upstream region included a group of ORFs (*orf2, orf3* and *orf4*) and a phage-related DNA invertase. Downstream, *orf7* was identified. All of the *S. enterica* isolates tested were negative for the plasmid-mediated quinolone resistance genes *qnrA, qnrB, qnrS*, and *qepA*.

Integron detection and characterization. Seventy-one of the 90 isolates (79%) were positive for the *intI1* gene, and six different gene cassette arrangements were determined (Table 3, Fig.1). Class 2 and 3 integrons were absent. All 45 bla_{PSE-1}-positive *S.* Typhimurium isolates showed two integrons, with variable regions of 1000 and 1200 bp, harboring the *aadA2* and bla_{PSE-1} gene cassettes, respectively. The bla_{OXA-1} + *aadA1* gene array was found in most of the bla_{OXA-1}-positive isolates (23 of 25), whereas the *S.* Thompson isolate showed the *aac*(6′)-Ib-cr+bla_{OXA-1}+*catB3* +*arr3* arrangement. Three non-classic class 1 integrons (lacking the 3′-CS) were found in three isolates (Fig. 1).

Genetic environment of *sul* genes. Of the 90 *S. enterica* isolates studied, 94.4% were resistant to sulfonamides. At least one *sul* gene was detected in 80 of them, and more than one *sul* gene in 17 of them (Table 2). The *sul1* gene was associated with class 1 integrons in all 70 *sul1*-positive isolates (Table 3).

The genetic environment of the *sul2* gene was determined in 11 of the 26 *sul2*-positive *S. enterica* isolates (42.3%). Four different structures were demonstrated (number of isolates): *repC*+*sul2*+*strA-strB*+*tnpB* (6), *repC*+*sul2*+*strA-strB*+IS*26* (2), *repC*+*sul2*+*strA-strB* (1) and *sul2* +*strA*Δ*dfrA14* -*strB* (2). In these two last isolates, the *strA* gene was truncated by the *dfrA14* gene, and a streptomycin-susceptibility phenotype was determined in both isolates. The *sul3* gene was associated with the above mentioned non-classic class 1 integrons (lacking the 3′-CS) in the two *sul3*-positive isolates.

In summary, an ACSSuT phenotype (including intermediate resistance) was confirmed in 68 *S. enterica* isolates (all of them Typhimurium), 15 of which were additionally resistant to nalidixic acid and three others to trimethoprim (Table 3). Three general gene profiles were mostly responsible for the ACSSuT multiresistant phenotype: (i) The bla_{PSE-1} and *aadA2* genes, located within two class 1 integrons (structure A, Fig. 1), were associated with the *floR, sul* and *tet*(G) genes in 45 of these isolates. In five of the 45 isolates, one non-classic class 1 integron (*dfrA12*+*orfF*+*aadA2*+*cmlA1*+*aadA1*), the bla_{TEM-1} gene, and the bla_{OXA-1} gene were additionally detected (one, three, and one isolate, respectively). (ii) The bla_{OXA-1} and *aadA1* (located within a class 1 integron of structure B, Fig. 1), *catA, sul*, and *tet*(B) gene profile occurred in 20 isolates. The *floR* gene was additionally found in two of them. (iii) An association between bla_{TEM-1b}, *cmlA1, aadA* or *strA-strB, sul*, and *tet*(A) or *tet*(B) genes was detected in three isolates. In one of them, the *aac*(3)-IV and *dfrA12* genes were additionally amplified, confirming this *S.* Typhimurium isolate's ACGSSuTTm phenotype (Table 3).

Discussion

Antimicrobial resistance in *S. enterica* is a cause of serious concern in human medicine. The drugs of choice for the treatment of complicated salmonellosis are usually ampicillin, amoxicillin/clavulanate, third-generation cephalosporins, or fluoroquinolones, but the increasing emergence of resistance to these antimicrobials limits the therapeutic choices [9,15,18]. In our study, the $AMC^{R/I}$ phenotype was detected in 12–23% of all *S. enterica* isolates recovered from human samples obtained from three Spanish hospitals. The β-lactamase-related mechanisms implicated in this $AMC^{R/I}$ phenotype were the production of the enzymes PSE-1, OXA-1 and TEM-1, as previously reported in other series [11]. The high prevalence of bla_{PSE-1} and bla_{TEM-1} observed among *S.* Typhimurium and *S.* Enteritidis isolates, respectively, was also previously reported [3,11,26]. The detection of more than one β-lactamase gene in the same isolate was infrequent in our study (4 isolates), in contrast to the data from other studies [3,11].

Table 3. Phenotypes and mechanisms of resistance detected in the 90 $AMC^{R/I}$ and $C3G^{R}$ *Salmonella enterica* isolates

	Phenotype of resistance (number of isolates)[a,b]	Genotype of resistance (number of isolates)[c]	Class 1 integron[d]
S. Typhimurium (n = 79)	SUL+TET (1)	bla_{TEM-1c} + *sul2* + *tet*(B) (1)	–
	STR+SUL+TET (7)	bla_{OXA-1} + *aadA* + *sul1* + *tet*(B) (2)	(B)
		bla_{TEM-1b} + *strA-strB* + *sul2* + *tet*(B) (3)	–
		bla_{TEM-1b} + *strA-strB* + *sul2* + *tet*(A) (1)	–
		tet(B) (1)	–
	STR+SUL+TET+NAL (1)	bla_{OXA-1} + *aadA* + *sul1*+ *tet*(B) (1)	(B)
	SUL+TET+TMP+SXT (1)	bla_{TEM-1b} + *strAΔdfrA14-strB* + *sul2*+ *tet*(A) (1)	–
	CHL+**STR**+**SUL**+**TET**+KAN (2)	bla_{PSE-1} + bla_{OXA-1} + *floR* + *aadA* + *sul1*+ *tet*(G) (1)	(A)
		bla_{PSE-1} + *floR* + *aadA* + *sul1* + *tet*(G) + *aph*(3′)-Ia (1)	(A)
	CHL+**STR**+**SUL**+**TET**+NAL (14)[f]	bla_{PSE-1} + *floR* + *aadA* + *sul1* + *tet*(G) (10)	(A)
		bla_{OXA-1} + *floR* + *catA* + *aadA* + *sul1* + *tet*(B) (1)	(B)
		bla_{OXA-1} + *catA* + *aadA* + *sul1* + *tet*(B) (3)	(B)
	SUL+TET+TMP+SXT+NAL (1)	bla_{TEM-1b} + *sul2* + *tet*(A) + *strAΔdfrA14- strB*	–
	CHL+**STR**+**SUL**+**TET**+TMP+SXT (1)	bla_{TEM-1b} + *cmlA1* + *aadA* + *strA-strB* + *sul2* + *sul3* + *tet*(A) + *dfrA12*	(E)
	CHL+**STR**+**SUL**+**TET**+TMP+SXT+NAL (1)	bla_{PSE-1} + *floR* + *cmlA1* + *aadA* + *sul1* + *tet*(G) + *dfrA12*	(A)+(D)
	CHL+**STR**+**SUL**+**TET**+GEN+TOB+TMP+SXT (1)	bla_{TEM-1b} + *cmlA1* + *aadA* + *sul1* + *sul2* + *sul3* + *tet*(A) + *aac*(3)-IV + *dfrA12*	(F)[g]
S. Enteritidis (n = 8)	None (2)	bla_{TEM-1b}	–
	NAL (1)	– (1)	–
	TET (1)	bla_{TEM-1b}	–
	SUL (2)	bla_{TEM-1b}	–
	CTX+NAL (1)	$bla_{CTX-M-14a}$	–
	SUL+SXT (1)	bla_{TEM-1b}	–
S. Gnesta (n = 1)	ATM+CAZ+CTX+SUL (1)	$bla_{CTX-M-15}$ + bla_{TEM-1}	–
S. Thompson (n = 1)	CHL+SUL+TET (1)	bla_{OXA-1} + *floR* + *catB3* + *sul1*+ *sul2* + *aac*(6′)-Ib-cr	(C)
S. Virchow (n = 1)	SUL+TET+CTX+NAL (1)	$bla_{CTX-M-10}$ + *sul2* + *tet*(B) + *strA-strB*	–

[a]Abbreviations: CAZ: ceftazidime, CTX: cefotaxime, ATM: aztreonam, GEN: gentamicin, TOB: tobramycin, KAN: kanamycin, STR: streptomycin, NAL: nalidixic acid, TET: tetracycline, CHL: chloramphenicol, SUL: sulfonamides, TMP: trimethoprim, SXT: trimethoprim/sulfamethoxazole.
[b]ACSSuT phenotype is marked in bold letters.
[c]Streptomycin resistance genes *aadA* correspond to *aadA1* or *aadA2.*
[d]Integron structures A-F correspond to those shown in Fig. 1.
[e]Six of these isolates had an intermediate phenotype with respect to tetracycline and nine isolates with respect to streptomycin.
[f]One of these isolates had an intermediate phenotype with respect to tetracycline and two with respect to streptomycin.
[g]This integron contained the putative macrolide efflux gene *mef*(B), truncated by IS*26* such that only 256 bp of *mef*(B) remained.

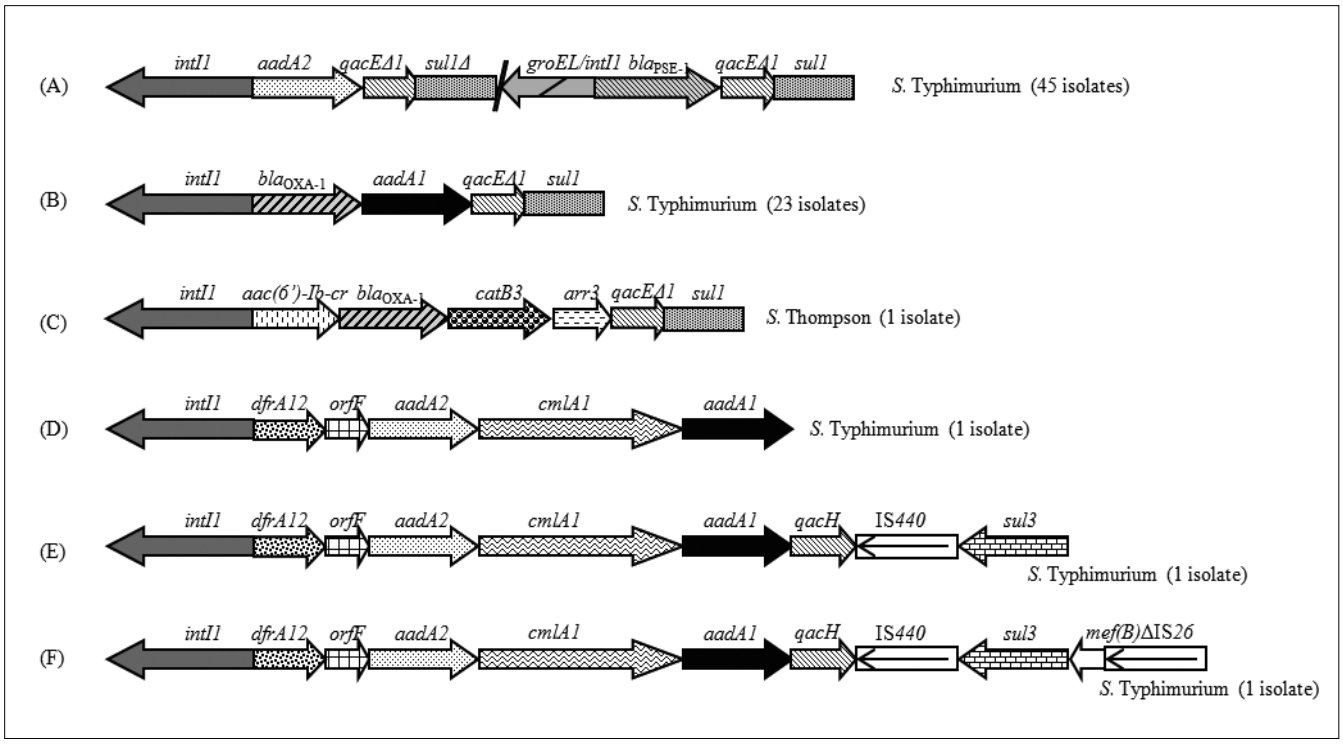

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Fig. 1. Gene cassette arrangements among class 1 integrons detected in *Salmonella enterica* isolates.

The ESBL phenotype in human clinical isolates of *S. enterica* is of particular interest but in our study it was detected in <1% of the *S. enterica* isolates obtained from the three hospitals. ESBL are spreading very rapidly among *E. coli* and *Klebsiella* spp. isolates whereas their frequency among *S. enterica* isolates is much lower [2,15,18]. The diversity of ESBL detected among our three ESBL-positive *S. enterica* isolates (CTX-M-14, CTX-M-15, and CTX-M-10) is noteworthy as is the fact that these genes were identified in unusual serotypes, i.e., *S*. Gnesta and *S*. Virchow.

The CTX-M-14 β-lactamase-encoding gene, flanked by IS*Ecp1* and IS*903* sequences, has been frequently detected in *E. coli* isolates of human and animal origin in Spain [6]. In *S. enterica,* the first description of this enzyme, in a clinical isolate of *S.* Enteritidis recovered in Spain, was that of Romero et al. [23]. However, the $bla_{\text{CTX-M-14}}$ gene has been identified in *Salmonella* of different serotypes and in several countries [2,6,9,18]. The genetic element IS*Ecp1* is a mobile and mobilizing element that may be implicated in the $bla_{\text{CTX-M-14}}$ gene mobilization [19]. Similarly, the $bla_{\text{CTX-M-15}}$ gene, flanked by IS*Ecp1* and *orf477* elements, has been shown to be disseminated throughout the world and is mostly detected among *E. coli* and *Klebsiella* isolates [6]. In our study, this enzyme was identified in a *S.* Gnesta isolate. To our knowledge, this is the first description of the presence of the CTX-M-15 β-lactamase in *S.* Gnesta, a serotype uncommonly associated with human salmonellosis.

The CTX-M-10 enzyme has been described in *E. coli, Enterobacter* spp., *Klebsiella* spp., and *S.* Virchow isolates in Spain [6,9,17,21]. In the present work, this enzyme was also found in a *S.* Virchow isolate, and the genetic environment of the $bla_{\text{CTX-M-10}}$ gene was associated with a phage-related element, similar to one previously reported [17,21].

The ACSSuT multiresistance phenotype was detected in 68 of the *S.* Typhimurium isolates. Although this phenotype is usually associated with the widely distributed chromosomal SGI-1 (contains the $bla_{\text{PSE-1}}$, *floR, aadA2*, *sul*, and *tet*(G) genes) [16,30], other gene profiles have also been described [10,12,22]. Indeed, in our study different resistant genotypes were determined; the most common one was the SGI-1 linked profile. The association of the $bla_{\text{OXA-1}}$, *catA,* [*aadA1* / *strA-strB*], *sul*, and *tet*(B) genes, with the $bla_{\text{OXA-1}}$+*aadA1* arrangement included within a 2000-bp class 1 integron, was found among 20 *S.* Typhimurium ACSSuT-resistant isolates. In addition, the gene profile $bla_{\text{TEM-1}}$, *cmlA1,* [*aadA* / *strA-strB*], *sul* and [*tet*(A) / *tet*(B)] was identified in three *S.* Typhimurium isolates. In previous studies, these latter two resistance-gene profiles were shown to be located on hybrid self-

transferable plasmids, which also contain virulence genes, such as the pUO-StVR plasmids in *S.* Typhimurium and the recently reported pUO-SeVR1 in *S.* Enteritidis [10,12,22]. Further studies of our isolates are needed to determine the plasmid localization of these ACSSuT resistance genes and/or their possible association with virulence genes.

Class 1 integrons were present in 79% of the 90 isolates tested. Note the presence of a class 1 integron with the *aac*(6′)-Ib-cr+bla_{OXA-1}+*catB3*+*arr3* structure in the *S.* Thompson isolate. While this arrangement has been previously described, it is usually associated with complex integrons containing the IS*CR1* elements, double copies of 3′-CS, and *qnr* genes, among others (e.g., GenBank accession numbers AJ971343 and AY259086). In addition, non-classical integrons (without *qacE*Δ1+*sul1* genes) were found in three isolates (4%). All three included the gene cassette organization *dfrA12*+*orfF*+*aadA2*+*cmlA1*+*aadA1*, in two of these three isolates in association with the *qacH*+IS*440*+*sul3* structure previously reported in *Salmonella* and *E. coli* [1,25].

In conclusion, bla_{PSE-1} and bla_{OXA-1} were the most frequent *bla* genes implicated in the $AMC^{R/I}$ phenotype in *S.* Typhimurium, and bla_{TEM-1} the most frequent in *S.* Enteritidis. ESBL-positive isolates, corresponding to non-*S.* Typhimurium serotypes, were identified in <1% of the *S. enterica* isolates obtained from the three hospitals. Among the three different ESBL variants detected, ours is the first description of CTX-M-15 in *S.* Gnesta. In addition, the frequent association of the β-lactamase production with nalidixic acid resistance (22%), which precludes the use of fluoroquinolones in the treatment of salmonellosis, is a cause for clinical concern and underlines the need to track the evolution of β-lactamases in *S. enterica* isolates.

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