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Antibiotic resistance genes, integrons and multiple antibiotic resistance in thirty-five serotypes of *Salmonella enterica* isolated from humans and animals in the UK

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***Objectives*: To examine 397 strains of *Salmonella enterica* of human and animal origin comprising 35 serotypes for the presence of *aadB, aphAI-IAB, aadA1, aadA2, bla(Carb$_2$)* or *pse1, bla(Tem), cat1, cat2, dhfr1, floR, strA, sul1, sul2, tetA(A), tetA(B)* and *tetA(G)* genes, the presence of class 1 integrons and the relationship of resistance genes to integrons and antibiotic resistance.**

***Results*: Some strains were resistant to ampicillin (91), chloramphenicol (85), gentamicin (2), kanamycin (14), spectinomycin (81), streptomycin (119), sulfadiazine (127), tetracycline (108) and trimethoprim (45); 219 strains were susceptible to all antibiotics. *bla(Carb$_2$), floR* and *tetA(G)* genes were found in *S.* Typhimurium isolates and one strain of *S.* Emek only. Class 1 integrons were found in *S.* Emek, Haifa, Heidelberg, Mbandaka, Newport, Ohio, Stanley, Virchow and in Typhimurium, mainly phage types DT104 and U302. These strains were generally multi-resistant to up to seven antibiotics. Resistance to between three and six antibiotics was also associated with class 1 integron-negative strains of *S.* Binza, Dublin, Enteritidis, Hadar, Manhattan, Mbandaka, Montevideo, Newport, Typhimurium DT193 and Virchow.**

***Conclusion*: The results illustrate specificity of some resistance genes to *S.* Typhimurium or non-*S.* Typhimurium serotypes and the involvement of both class 1 integron and non-class 1 integron associated multi-resistance in several serotypes. These data also indicate that the *bla(Carb$_2$), floR* and *tetA(G)* genes reported in the SG1 region of *S.* Typhimurium DT104, U302 and some other serotypes are still predominantly limited to *S.* Typhimurium strains.**

Keywords: Typhimurium, plasmids, ampicillin, streptomycin, tetracycline

Introduction

Multidrug-resistant (MDR) *Salmonella enterica* serotype Typhimurium phage type DT104 is currently the second most prevalent serotype isolated in England and Wales and is becoming increasingly common in other countries.[1,2] Resistance to ampicillin, chloramphenicol, streptomycin, sulphonamides and tetracycline (abbreviated ACSSuT) is common, although resistance to other antibiotics and other resistance patterns occur.[1,3] High level multidrug resistance is normally associated with mobile genetic elements (e.g. plasmids, transposons, integrons, phages, etc.) that encode specific resistance genes.[1,4,5] Many gene cassettes within integrons such as the *blaP1* cassette which encodes the Pse-1 or Carb-2 protein (different names for the same protein) have been described previously.[5]

The genetic make-up of many isolates of *S.* Typhimurium DT104 with the ACSSuT resistance phenotype is similar, comprising the *floR* and *tetA(G)* genes bracketed by two class 1 integrons carrying the *aadA2, bla(Carb$_2$)* or *pse1* [*bla(Carb$_2$)* and *pse1* are different names for the same gene] cassettes clustered on a 14 kb region of the genome.[2,3,6–9] This region has recently been described as *S. enterica* genomic Island 1 (SGI1)[10] and in *S.* Typhimurium DT104 these antibiotic resistance genes are an integral part of the chromosome.[3,11] Experiments have shown that these resistance genes can be efficiently transduced by P22-like phage ES18 and by phage PDT17 that is released by all DT104 isolates so far examined.[12] With this in mind, it is interesting to note that *S.* Typhimurium U302 and DT120 strains and *S.* Agona possess the same antibiotic resistance genes as MDR *S.* Typhimurium DT104.[1,9] More recently, the DT104 MDR profile has been detected within the complete *S. enterica* genomic Island 1 in *Salmonella* serotype Paratyphi B from tropical fish in Singapore.[13] These data indicate the potential for emergence of multidrug resist-

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ance in other *S. enterica* serotypes, possibly encoded by identical or similar gene clusters. In order to test this hypothesis, a panel of 397 strains of *S. enterica* that comprised 35 serotypes was examined. Specifically, each strain was tested for the presence of *aadA1, aadA2, aadB, aphAI-IAB, bla(Carb$_2$), bla(Tem), cat1, cat2, dhfr1, floR, strA, sul1, sul2, tetA(A), tetA(B)* and *tetA(G)* genes, class 1 integrons, antimicrobial resistance and for the relationship between antibiotic resistance genes, antibiotic resistance and class 1 integrons.

Materials and methods

Bacterial strains

The panel comprised 397 strains; the veterinary isolates were obtained from the Veterinary Laboratories Agency (VLA), Weybridge, UK and the human isolates from the Public Health Laboratory, Colindale, UK. Strains were selected with a bias for major serotypes and were isolated from poultry ($n = 170$), humans ($n = 55$), cattle ($n = 43$), the environment ($n = 42$, mainly farm environment such as animal housing, litter, etc.), pigs ($n = 38$), sheep ($n = 26$), domestic animals ($n = 8$), unknown sources ($n = 10$) and feed ($n = 5$). Sixty-five percent of the strains were isolated in 1999 with 88% of strains isolated in 1998–2000. The remaining strains were isolated between 1994 and 1997.

Strains P3749380 and P4320350[3] were used as positive controls for class 1 integron PCR. The following strains were used as positive controls for PCR and as DNA templates to generate antibiotic resistance gene probes: *S.* Enteritidis 7564/96 (*aadA1*), *S.* Typhimurium DT104 4665/99 [*aadA2, bla(Carb$_2$), floR, sul1, tetA(G)*], *S.* Seftenberg 7509/99 (*aadB*), *S.* Newport 6306/99 (*aphAI-IAB, cat1, dhfr1*), *S.* Liverpool 9510/97 [*bla(Tem), strA, sul2*], *S.* Typhimurium DT104 P453991 (*cat2*), *S.* Typhimurium DT193 1725/99 [*tetA(A)*], *S.* Typhimurium DT208 1859/99 [*tetA(B)*].

Antibiotics and chemicals

Antibiotics and chemicals used were obtained from Sigma–Aldrich (Poole, Dorset, UK) except ciprofloxacin which was kindly donated by Bayer (Newbury, Berkshire, UK).

MICs

MICs were determined by an agar doubling dilution method similar to that of the NCCLS,[14] with the main exception that Iso-Sensitest agar (Oxoid, Hants, UK) rather than Mueller–Hinton agar (Oxoid) was used. Bacteria were grown overnight at 37°C in Luria–Bertani (LB) broth, diluted 1/10 in normal saline and inoculated using a multi-point inoculator onto the agar with suitable dilutions of the antibiotics ampicillin, chloramphenicol, gentamicin, kanamycin, spectinomycin, streptomycin, sulfadiazine, trimethoprim and tetracycline. Strains with MICs >16 mg/L of ampicillin, chloramphenicol, gentamicin, kanamycin, streptomycin, tetracycline and trimethoprim, >64 mg/L of spectinomycin and >1024 mg/L of sulfadiazine were taken to be resistant. These values were selected as the most suitable to separate strains with resistance genes from strains without resistance genes on the basis of their MICs.

Preparation of probe and colony dot blots

Nylon membranes (Hybond N$^+$, Amersham Pharmacia Biotech, Bucks, UK) for colony dot blots were prepared as previously described.[15] PCR amplicons (see below) comprising antibiotic resistance genes were extracted from gels using a Qiagen gel extraction kit (Qiagen, Sussex, UK). The resulting DNA was labelled using an Alkphos labelling kit (Amersham Pharmacia Biotech) according to the manufacturer's instructions and used separately to hybridize to colony dot blots.

PCR amplification

The oligonucleotide primers for antibiotic resistance genes and for class 1 integrons are shown in Table 1 with respective annealing temperatures. Primers were synthesized by Eurogentec Laboratories, Southampton, UK.

All PCR amplifications contained 1× $MgCl_2$ free buffer (Promega, Madison, USA), 100 ng of each primer, 0.5 μL of *Taq* DNA polymerase (Promega), 1 μL of dNTPs mix (Promega), 2 mM $MgCl_2$ (Promega) and HPLC H_2O made up to 50 μL. The DNA template (1 μL) for PCR was a washed overnight culture grown at 37°C in LB broth. The PCR conditions were similar to those previously described.[9]

Detection of aadA1 and aadA2 genes

The *aadA1* probe was found to hybridize to *aadA2* positive strains. However, by PCR *aadA1* and *aadA2* primers were specific for their respective genes. As such, the panel of strains was probed for the presence of *aadA2* and then the streptomycin-resistant strains were checked by PCR for the presence of the *aadA1* gene. Strains positive for both *aadA1* and *aadA2* were then tested for by PCR using *aadA2* primers to confirm or otherwise the presence of the *aadA2* gene.

Presence of resistance genes within integrons

The presence of resistance genes within class 1 integrons was determined as follows. Integron primer set A (Table 1) was used to amplify the variable region of InC which in *S.* Typhimurium DT104 contains the *aadA2* gene and the variable region of InD which in *S.* Typhimurium DT104 contains the *bla(Carb$_2$)* (also known as *pse1*) gene (Figure 1). To test if *aadA2* and *bla(Carb$_2$)* genes were contained within the amplified segment, the amplified segment was blotted onto nylon membranes (Hybond N$^+$) as previously described.[15] Membranes were then hybridized with either the *aadA2* or *bla(Carb$_2$)* gene using an Alkphos labelling kit (Amersham Pharmacia Biotech) according to the manufacturer's instructions. Integron primer set B (Table 1) amplified the 3′ conserved region of both InC and InD; the *sul1* and *qacE* genes are contained in this region (Figure 1).[2,16] Therefore a positive PCR result was taken as evidence that the *sul1* gene was within the integron.

Association of resistance genes with integrons

The *floR* and *tetA(G)* genes have been reported to be between the two class 1 integrons InC and InD (Figure 1).[16] Having tested for the presence of the *aadA2, bla(Carb$_2$)* and *sul1* genes within integrons, the association of *dhfr1, floR, strA* and *sul2* genes with the integrons was determined as follows. Chromosomal DNA was digested with *Xba*I (Promega), electrophoresed for 16 h in 0.7% agarose with a 1 kb plus ladder (Invitrogen) as a marker, then blotted onto membranes as described previously.[15] Membranes were then hybridized with probes comprising separately *dhfr1* or *floR* or *strA* or *sul1* or *sul2* genes and the 1 kb plus ladder. Location of genes within the same sized fragment as that obtained when blots were hybridized with the *sul1* probe was taken as an indication of co-location of antibiotic resistance genes with the integrons (Figure 1).[16]

Table 1. Primer sequences for PCR

Gene of interest	F or R[a]	5′ to 3′ DNA sequence of primers	T_m (°C)	EMBL accession no., nucleotide position	Reference
aadA1	F	TATCAGAGGTAGTTGGCGTCAT	54	AY125351, 112–133	this study
aadA1	R	GTTCCATAGCGTTAAGGTTTCATT		AY125351, 573–596	
aadA2	F	TGTTGGTTACTGTGGCCGTA	62	AF071555, 2019–2128	9
aadA2	R	GATCTCGCCTTTCACAAAGC		AF071555, 2712–2731	
aadB	F	GAGCGAAATCTGCCGCTCTGG	61	AF078527, 3821–3841	21
aadB	R	CTGTTACAACGGACTGGCCGC		AF078527, 4120–4140	
aphAI-IAB	F	AAACGTCTTGCTCGAGGC	55	AF024666, 8881–8898	21
aphAI-IAB	R	CAAACCGTTATTCATTCGTGA		AF024666, 9321–9341	
bla(Carb$_2$)	F	GCTTCGCAACTATGACTAC	52	AF071555, 11644–11662	9
bla(Carb$_2$)	R	GTTCACCATCCAAGACTC		AF071555, 11898–11915	
bla(Tem)	F	CATTTCCGTGTCGCCCTTAT	55	J01749, 221–240	9
bla(Tem)	R	TCCATAGTTGCCTGACTCCC		J01749, 995–1014	
cat1[b]	F	CCTATAACCAGACCGTTCAG	56	V00622, 338–357	this study
cat1[b]	R	TCACAGACGGCATGATGAAC		V00622, 814–833	
cat2[b]	F	CCGGATTGACCTGAATACCT	56	X53796, 198–217	this study
cat2[b]	R	TCACATACTGCATGATGAAC		X53796,745–770	
dhfr1	F	GTGAAACTATCACTAATGGTAGCT	54	AF203818, 84–107	22
dhfr1	R	ACCCTTTTGCCAGATTTGGTAACT		AF203818, 531–554	
floR	F	AACCCGCCCTCTGGATCAAGTCAA	60	AF118107, 1413–1436	this study
floR	R	CAAATCACGGGCCACGCTGTATC		AF118107, 1939–1961	
strA	F	AGCAGAGCGCGCCTTCGCTC	59	NC_001740, 761–780	16
strA	R	CCAAAGCCCACTTCACCGAC		NC_001740, 1464–1445	
sul1	F	TCACCGAGGACTCCTTCTTC	60	AF071555, 12744–12763	9
sul1	R	AATATCGGGATAGAGCGCAG		AF071555, 13041–13060	
sul2	F	CGGTCCGGCATCCAGCAATCC	64	M36657, 455–475	this study
sul2	R	CGAGAGCCACGACCGCGCC		M36657, 878–896	
tetA(A)[b]	F	GGTTCACTCGAACGACGTCA	56	X00006, 1432–1451	this study
tetA(A)[b]	R	CTGTCCGACAAGTTGCATGA		X00006, 1989–2008	
tetA(B)[b]	F	CCTCAGCTTCTCAACGCGTG	56	J01830, 1972–1991	this study
tetA(B)[b]	R	GCACCTTGCTCATGACTCTT		J01830, 2586–2605	
tetA(G)	F	CCGGTCTTATGGGTGCTCTA	56	AF071555, 6869–6888	9
tetA(G)	R	CCAGAAGAACGAAGCCAGTC		AF071555, 7453–7472	
Integron A[c]	F	GGCATCCAAGCAGCAAGC	50	AF071555, 11126–11142	2
Integron A	R	AAGCAGACTTGACCTGAT		AF071555, 12305–12322	
Integron B[d]	F	ATCGCAATAGTTGGCGAGT	53	X15370, 211–230	2
Integron B	R	GCAAGGCGGAAACCCGCGCC		X12869, 1217–1236	

[a]F, forward; R, reverse.
[b]Kindly donated by James Cariss, University of Birmingham.
[c]Amplifies *aadA2* and *bla(Carb$_2$)* regions of InC and InD, respectively.[17]
[d]Amplifies *sul1* region in both InC and InD.[17]

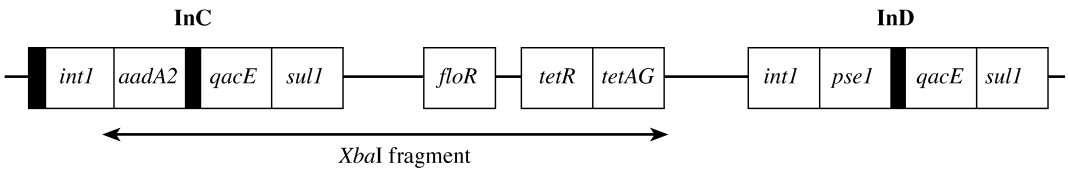

Figure 1. Schematic representation of genes within and between InC and InD integrons of *Salmonella* Typhimurium DT104. Adapted (not drawn to scale) from Figure 1 of Carattoli *et al.*[16]

Results

Antibiotic resistance

Salmonella ($n = 397$) were screened for resistance to nine antibiotics and the following numbers of strains were resistant to ampicillin (91), chloramphenicol (85), gentamicin (2), kanamycin (14), spectinomycin (81), streptomycin (119), sulfadiazine (127), tetracycline (108) and trimethoprim (45). However, 219 strains were susceptible to all antibiotics. With the exception of the five *S.* Fischerkietz strains tested, which were susceptible to all antibiotics tested (Table 2), all major serotypes of *Salmonella* had examples of strains that showed resistance to some of the antibiotics tested.

Table 2. Number of strains within each serotype resistant to antibiotics[a]

Serotype	No. tested (human)	Number of strains resistant to specific antibiotics									Number of strains fully susceptible
		AMP	CHL	GEN	KAN	SPT	STR	SDZ	TET	TMP	
Binza	15 (0)	0	0	0	0	1	3	2	2	1	12
Dublin	30 (0)	0	5	0	0	2	4	2	0	0	22
Enteritidis	63 (15)	4	0	0	2	1	2	6	1	2	53
Fischerkietz	5 (0)	0	0	0	0	0	0	0	0	0	5
Hadar	19 (5)	4	0	0	1	0	10	0	10	0	9
Heidelberg	10 (0)	2	0	0	0	2	6	2	0	0	3
Indiana	5 (0)	0	0	0	0	0	1	1	1	0	4
Livingstone	11 (0)	0	5	0	0	0	0	0	1	0	5
Mbandaka	11 (0)	1	0	0	2	2	4	4	4	3	6
Montevideo	32 (0)	2	1	0	1	1	1	2	2	2	26
Newport	23 (0)	4	2	0	1	0	6	8	1	3	12
Senftenberg	24 (0)	0	3	1	1	1	1	0	0	0	20
Typhimurium 104	60 (14)	54	53	0	1	55	55	57	53	8	4
Typhimurium U302	6 (0)	4	4	0	0	4	6	6	6	3	0
Typhimurium 193	11 (0)	6	1	0	1	0	4	6	6	4	5
Typhimurium other	18 (2)	6	6	0	0	7	7	8	9	4	6
Virchow	12 (5)	1	1	0	0	0	0	3	1	3	9
Others[b]	42 (14)	3	4	1	4	5	9	20	11	12	18
Total	397 (55)	91	85	2	14	81	119	127	108	45	219

[a]Resistance classed as strains with MICs of >16 mg/L for AMP (ampicillin), CHL (chloramphenicol), GEN (gentamicin), KAN (kanamycin), STR (streptomycin), TET (tetracycline) and TMP (trimethoprim), >64 mg/L for SPT (spectinomycin) and >1024 mg/L for SDZ (sulfadiazine).
[b]Others included strains for which three or less of each serotype were tested, e.g. *S.* Agama, Agona, Albany, Blockley, Cubana, Derby, Emek, Gold coast, Haart, Haifa, Kedougou, Kisangani, Liverpool, Manhattan, Muechen, Ohio, Reading, Saint-paul, Stanley, structure only, Thompson and Urettevreden. This group also includes strains that were not ascertained a particular serotype, e.g. structure only (n = 10).

There was good correlation between the presence of resistance genes and corresponding resistance phenotypes suggesting resistance genes, when present, were usually expressed. MICs of sulfadiazine against strains harbouring the *sul1* or *sul2* genes were >2048 mg/L. MICs of all the other antibiotics tested were 32 to >128 mg/L for strains harbouring resistance genes specific for the antibiotic, with the exception of 13/59 strains with the *strA* gene that were streptomycin-susceptible (Table 3). All strains that were ampicillin-resistant contained either the *bla(Carb$_2$)* and/or the *bla(Tem)* gene and the two gentamicin-resistant strains contained the *aadB* gene. The *aadA1* and *aadA2* probes cross-hybridized and using the combined probe and PCR methodology described, it was shown that 15 strains were positive for *aadA1* alone, 68 strains were positive for *aadA2* alone and three strains were positive for both genes (Table 4).

There were several occasions in which a strain was resistant to an antibiotic but the identity of the gene conferring resistance was not ascribed. For example, 24 of 85 chloramphenicol-resistant strains did not contain *cat1, cat2* or *floR* genes, one of 14 kanamycin-resistant strains did not contain the *aphA1-IAB* gene, five of 81 spectinomycin-resistant strains did not contain the *aadA1* or *aadA2* genes, 16 of 119 streptomycin-resistant strains did not contain *aadA1, aadA2* or *strA* genes, seven of 127 sulfadiazine-resistant strains did not contain *sul1* or *sul2* genes, 21 of 108 tetracycline-resistant strains did not contain *tetA(A), tetA(B)* or *tetA(G)* genes and 38 out of 45 trimethoprim-resistant strains did not contain the *dhfr1* gene.

Distribution of antibiotic resistance genes between different serotypes

The genes *bla(Carb$_2$), floR* and *tetA(G)* were associated with *S.* Typhimurium strains (Table 4) and one strain of *S.* Emek that contained the *floR* and *tetA(G)* genes (Table 4, 'Others'). The *aadA2* and *sul1* genes were found mainly in *S.* Typhimurium isolates with both genes in ~85% of DT104, in ~65% of U302 and ~20% non-DT104 and U302 *S.* Typhimurium, but these genes were also found in other serotypes (Table 4). The *tetA(G)* gene was the most prevalent tetracycline resistance determinant in *S.* Typhimurium DT104 and U302 strains, whereas the *tetA(A)* and *tetA(B)* genes were relatively common in other serotypes and phage types (Table 4). Ampicillin-resistant strains that were not positive for *bla(Carb$_2$)* were positive for *bla(Tem)*, although there were two instances of *S.* Typhimurium DT104 positive for both *bla(Carb$_2$)* and *bla(Tem)*. Of the three streptomycin resistance genes tested for (*aadA1, aadA2, strA*), the *aadA2* gene was the prevalent resistance determinant for *S.* Typhimurium DT104, the *strA* gene was the prevalent resistance determinant for non-Typhimurium strains and the *aadA1* gene was only detected in non-*S.* Typhimurium isolates (Table 4). There were instances of the *aadA1* and *aadA2*, the *aadA1* and *strA*, and the *aadA2* and *strA* genes occurring together (data not shown).

The *dhfr1* gene was present in only seven strains and was not present in any of the trimethoprim-resistant *S.* Typhimurium isolates.

Table 3. MIC range for strains with specific resistance genes

Resistance gene	Mechanism of resistance	Resistant to	MIC range for organisms with gene (mg/L)
aadA1	streptomycin/spectinomycin adenyltransferase	SPT, STR	32–>128
aadA2	streptomycin/spectinomycin adenyltransferase	SPT, STR	32–>128
aadB	aminoglycoside transferase	GEN	32–>128
aphAI-IAB	aminoglycoside phosphotransferase	KAN	32–>128
bla(Carb$_2$)	β-lactamase	AMP	128–>128
bla(Tem)	β-lactamase	AMP	128–>128
cat1	chloramphenicol acetyl-transferase	CHL	>128
cat2	chloramphenicol acetyl-transferase	CHL	>128
dhfr1	dihydrofolate reductase	TMP	128–>128
floR	efflux	CHL	64–>128
strA	streptomycin phosphotransferase	STR	8–>128
sul1	dihydropteroate synthase	SDZ	>2048
sul2	dihydropteroate synthase	SDZ	>2048
tetA(A)	efflux	TET	128–>128
tetA(B)	efflux	TET	>128
tetA(G)	efflux	TET	32–>128

See Table 2 for abbreviations used for antibiotics.

Table 4. Number of strains within each serotype with specific resistant genes

		Number of strains containing resistance genes[a]														
Serotype	No. tested	A1	A2	Ab	Ap	Bc	Bt	C1	C2	D	F	St	S1	S2	Ta	Tb	Tg
Binza	15	2	1	0	0	0	0	0	0	0	0	3	0	2	0	1	0
Dublin	30	1	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0
Enteritidis	63	1	0	0	2	0	3	0	0	1	0	0	2	3	0	0	0
Fischerkietz	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hadar	19	1	0	0	1	0	5	0	0	0	0	7	0	0	0	0	0
Heidelberg	10	2	0	0	0	0	2	0	0	0	0	0	2	0	0	0	0
Indiana	5	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0
Livingstone	11	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Mbandaka	11	2	0	0	1	0	1	0	0	1	0	2	2	1	2	1	0
Montevideo	32	1	0	0	1	0	2	0	0	0	0	2	0	2	0	1	0
Newport	23	1	0	0	1	0	4	1	0	1	0	6	1	6	0	0	0
Senftenberg	24	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0
Typhimurium 104	60	0	53	0	1	52	5	0	1	0	48	10	53	8	0	1	44
Typhimurium U302	6	0	4	0	0	4	0	0	0	0	4	5	4	5	1	0	4
Typhimurium 193	11	0	0	0	1	1	5	0	0	0	0	5	0	5	1	0	0
Typhimurium other	18	0	6	0	0	7	1	0	0	0	3	5	6	5	0	2	4
Virchow	12	0	0	0	0	0	1	1	0	2	0	1	2	1	0	0	0
Others[b]	42	3	4	1	4	0	2	0	0	2	1	12	7	13	3	0	1
Total	397	15	68	2	13	64	31	3	1	7	56	59	80	53	7	7	53

[a]A1, *aadA1*; A2, *aadA2*; Ab, *aadB*; Ap, *aphAI-IAB*; Bc, *bla(Carb$_2$)*; Bt, *bla(Tem)*; C1, *cat1*; C2, *cat2*; D, *dhfr1*; F, *floR*; St, *strA*; S1, *sul1*; S2, *sul2*; Ta, *tetA(A)*; Tb, *tetA(B)*; Tg, *tetA(G)*.
[b]Others, see Table 2.

The *aphaA1-IAB* gene was detected in most of the kanamycin-resistant strains of different serotypes and the *aadB* gene was detected in the two gentamicin-resistant strains. The *cat* genes were only found in *S.* Dublin, Newport, Typhimurium DT104 and Virchow and only one strain in each of the four serotypes was positive (Table 4).

The more common resistance genes such as *aadA2, bla(Carb$_2$), bla(Tem), floR, tetA(G), strA* and *sul1* were present in *Salmonella* strains from cattle, the environment, humans, pigs, poultry and sheep with the exception that the *bla(Tem)* gene was not found in isolates from cattle (data not shown).

Table 5. Strains positive for class 1 integrons, approximate size of integron PCR products[a] and resistance genes within or associated with integrons

Serotype	Resistance profile[b]	No. with profile[c]	Resistance genes[d] associated with resistance profile	Integron PCR size (kb)[e]	Resistance genes within integrons			
					A2	Bc	S1	Resistance genes associated with *Xba*I fragment (size kb) containing *sul1*[f]
Emek	CHL, SDZ, TET, TMP	1	D,F,S1,Tg	1.2	NP[g]	NP	+	D+F+Tg+ (>12)
Haifa	SDZ, TET, TMP	2	S1,Ta	0.7	NP	NP	+	
Heidelberg	AMP, STR, SPT, SDZ	1	A1,Bt,S1	1	+	NP	+	
Heidelberg	SPT, STR, SDZ	1	A1,S1	1	+	NP	+	
Mbandaka	STR, SDZ, TET, TMP	1	A1,D,SI,Ta	1.5	+	NP	+	
Newport	–	2	–	0.7	NP	NP	NP	
Newport	AMP, CHL	1	Bt	0.7	NP	NP	NP	
Newport	STR, SDZ	2	St,S1,S2	0.7	NP	NP	+	St–S2– (various)
Newport	AMP, CHL, KAN, STR, SDZ, TET, TMP	1	A1,Ap,Bt,D,C1, S1,S2,St	0.7 and 1.5	+	NP	+	D+St+S2+ (>12)
Ohio	SPT, STR, SDZ, TMP	1	A2,S1,S2,St	2	+	NP	+	St–S2– (various)
Stanley	SPT, STR, SDZ, TET, TMP	1	A1,A2,S1	1	+	NP	+	
Typhimurium DT104	AMP, SDZ	2	Bc,S1	1.2	NP	+	+	
Typhimurium DT104	STR, SDZ	2	A2,S1	1	+	NP	+	
Typhimurium DT104	AMP, CHL, SPT, STR, SDZ, TET	43[h]	A2,Bc,Bt,F,S1, S2,St,Tg	1, 1.2	+	+	+	F+Tg+ (12) St– (>12)
Typhimurium DT104	AMP, CHL, KAN, SPT, STR, SDZ, TET	1	A2,Ap,Bc,F,S1,Tg	1, 1.2	+	+	+	F+Tg+ (12)
Typhimurium DT104	AMP, CHL, SPT, STR, SDZ, TET, TMP	8[h]	A2,Bc,Bt,C2,F,S1, S2,St,Tg	1, 1.2	+	+	+	F+Tg+ (12) S2–St– (various)
Typhimurium U302	AMP, CHL, SPT, STR, SDZ, TET	1	A2,Bc,F,S1,Tg	1, 1.2	+	+	+	F+Tg+ (12)
Typhimurium U302	AMP, CHL, SPT, STR, SDZ, TET, TMP	3[h]	A2,Bc,F,S1,S2,St,Tg	1, 1.2	+	+	+	F+Tg+ (12) S2–St– (various)
Typhimurium other	AMP, CHL, SPT, STR, SDZ, TET	3[h]	A2,Bc,F,S1,Tg	1, 1.2	+	+	+	F+Tg+
Typhimurium other	AMP, CHL, SPT, STR, SDZ, TET, TMP	3[h]	A2,Bc,F,S1,S2,St,Tg	1, 1.2	+	+	+	F+Tg+ (12) S2–St– (various)
Virchow	SDZ, TMP	1	D,S1	1.2	NP	NP	+	D+

[a]Presence of class 1 integrons was determined by PCR using integron primer set A and sizes refer to approximate size of PCR product using this primer set.
[b]See Table 2 for abbreviations used for antibiotics.
[c]Only one strain for each profile was tested for the presence of resistance genes, integrons and relationship of resistance genes to integrons.
[d]See Table 4 for abbreviations used for resistance genes.
[e]Approximate size of integrons on basis of comparison with DNA ladder.
[f]Only the genes *dhfr1, floR, strA* and *sul2* were tested for association with *Xba*I fragment that hybridized with *sul1*. +, gene co-located on *sul1* fragment; –, gene not co-located on *sul1* fragment.
[g]NP, gene not present in genome of organism tested.
[h]Where there is more than one strain per serotype with a given resistance profile, not all strains necessarily have all genes shown.

Multiple antibiotic resistance

For strains which had class 1 integrons, multiple antibiotic resistance (resistance to at least three and up to seven antibiotics) was associated with the serotypes *S.* Emek, Haifa, Heidelberg, Mbandaka, Newport, Ohio, Stanley, Typhimurium DT104, U302 and other phage types of Typhimurium and Virchow (Table 5). For strains that did not have class 1 integrons, multi-resistance was associated with the serotypes *S.* Binza, Dublin, Enteritidis, Hadar, Manhattan, Mbandaka, Montevideo, Newport, Typhimurium DT193 and Virchow (Table 6). The most common DT104 resistance profile was AMP-CHL-SPT-STR-SDZ-TET. A strain of *S.* Newport that was resistant to AMP-CHL-KAN-STR-SDZ-TET-TMP and had two integrons (~0.5 and 1.5 kb) was not resistant to cefoxitin (results not shown).

Integrons and resistance genes

Of the 397 strains tested, 81 were positive for class 1 integrons primarily associated with *S.* Typhimurium DT104, U302 and other *S.* Typhimurium but also with several other serotypes (Table 5).

Table 6. Multi-resistance[a] in strains without class 1 integrons

Resistance to[b]	Serotypes with profile (no. with profile)	Resistance genes[c] involved
AMP, STR, TET	Hadar (5), Montevideo (1)	A1,Bt,St,Ta
AMP, STR, SDZ	Newport (1)	Bt,St
AMP, STR, TET, KAN	Hadar (1)	Ap,Bt,Ta
AMP, SPT, STR, TET, KAN	Montevideo (1)	A1,Ap,Bt,St,Tb
AMP, CHL, SDZ, TET, TMP	Virchow (1)	Bt,C1,D,S1
AMP, SPT, STR, SDZ, TET	Mbandaka (1)	A1,Bt,S1,Ta
AMP, CHL, STR, SDZ, TET	Typhimurium DT193 (1)	Bc,S2,Ta
AMP, KAN, STR, SDZ, TET	Typhimurium DT193 (1)	Ap,Bt,St,Ta
AMP, STR, SDZ, TET, TMP	Typhimurium DT193 (2)	Bt,S2,St,Ta
CHL, SPT, STR, SDZ	Dublin (1)	A1,S1
CHL, KAN, SPT, STR, SDZ, TET	Manhattan (1)	A1,Ap,S2,St
SPT, STR, SDZ	Enteritidis (1)	A1,S1
SPT, STR, SDZ, TET, TMP	Binza (1)	A1,A2,S2,St,Tb
STR, SDZ, TET	Manhattan (1)	S2,St,Ta
STR, SDZ, TET, TMP	Mbandaka (1)	St,Ta
SDZ, TET, TMP	Mbandaka (1)	S2,St,Tb

[a]Multi-resistance, resistant to three or more antibiotics using criteria described.
[b]See Table 2 for abbreviations used for antibiotics.
[c]See Table 4 for abbreviations used for resistance genes. Where there is more than one strain per serotype with a given resistance profile, not all strains necessarily have all genes shown.

Where they occurred, the *aadA2, bla(Carb$_2$)* and *sul1* genes were found to be within integrons using the methods described (Table 5 and Figure 1). Results showed that the *floR, dhfr1* and *tetA(G)* genes were co-located with the *sul1* gene on *Xba*I fragments, suggesting that these genes were co-located with the integrons (Table 5 and Figures 1 and 2). The *strA* and *sul2* genes did not appear to be co-located with the integrons except possibly in one of the *S.* Newport strains (Table 5).

Discussion

The emergence of MDR *S.* Typhimurium, Paratyphi and Agona suggests that this multi-antibiotic-resistant phenotype may emerge in other *S. enterica* serotypes.[1,13] It may also be that some of the resistance genes in the ACSSuT-resistant *S.* Typhimurium DT104 strains can transfer to other *S. enterica* serotypes. Although there was evidence for all or some of the ACSSuT resistance profile being in a range of serotypes other than *S.* Typhimurium DT104 and U302, the *bla(Carb$_2$), floR* and *tetA(G)* genes were exclusively associated with *S.* Typhimurium with the exception of one strain of *S.* Emek which harboured the *florR* and *tet(G)* resistance genes. The *aadA2* gene

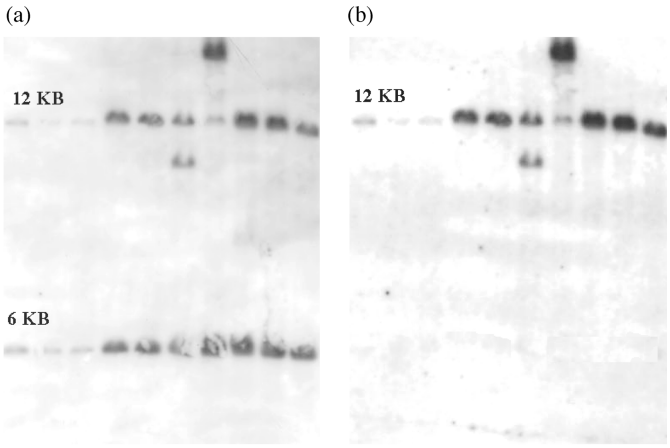

Figure 2. Southern hybridization of genomic DNA from 10 strains of *S.* Typhimurium (DT104, U302 and other phage types) digested to completion with *Xba*I and probed with *sul1* (a) and *tetA(G)* (b). (a) With *sul1*, two hybridizing fragments were seen. The one of 12 kb is associated with *sul1* contained in the InC integron[7] and the other fragment is associated with *sul1* on the InD integron.

was found mainly in *S.* Typhimurium strains as was the *sul1* gene, but the *sul1* gene was also found in several other serotypes apart from *S.* Typhimurium. Other resistance genes such as *aadA1, aphA-IAB, cat1, cat2, dhfr1, strA, sul2, tetA(A)* and *tetA(B)* were found in a wide range of serotypes, although the *aadA1* and *dhfr1* genes were not found in any *S.* Typhimurium strains tested. Multi-resistance was shown to be associated with strains positive and negative for class 1 integrons. The involvement of integrons in multi-resistance in *S.* Typhimurium DT104 isolates has been studied extensively and such integrons are reported to be 1 and 1.2 kb in size.[1,2,4,6,7,10,16] In *S.* Typhimurium DT104, the *aadA2* and *sul1* genes are located on the InC integron whereas the *bla(Carb$_2$)* or *pse1* gene and *sul1* gene are located on the InD integron and the region between these two integrons contains the *floR* and *tetA(G)* genes.[2] Our studies confirmed the presence of *aadA2* and or *bla(Carb$_2$)* and or *sul1* within the class 1 integrons of all strains tested including those integron-positive non-Typhimurium strains, with the exception of two *S.* Newport strains and these warrant further investigation. Additionally, the *dhfr1, floR* and *tetA(G)* genes were found to be associated with an *Xba*I fragment containing *sul1* for all strains tested, suggesting that these genes were also associated with class 1 integrons. With the exception of the *S.* Newport strain, these data indicated that the *strA* and *sul2* genes were associated with plasmids. The *S.* Newport strain that was resistant to seven antibiotics and which contained eight resistance genes and two integrons (approximate sizes of 0.5 and 1.5 kb) warrants further investigation. However, this strain, which was of porcine origin, was not resistant to cefoxitin and as such does not warrant the concern currently shown for cefoxitin-resistant strains of *S.* Newport.[17] In the USA, multi-resistant strains of *S.* Newport with the ACSSuT resistance profile have increasingly been isolated from clinical disease in humans and animals.[17] Some strains also show resistance to third generation cephalosporins such as ceftriaxone and this is of particular concern as it is one of the antibiotics for treating *Salmonella* in children.[17]

It was interesting to note that some strains harboured two different resistance genes for the same antibiotic, such as *aadA2* and *strA*. It is possible that for class 1 integron-positive strains, one gene was

associated with the integron, but that the strain also harboured a plasmid containing the other resistance gene. However, in some instances, strains negative for class 1 integrons also contained two resistance genes for the same antibiotic. These alternative possibilities are worthy of further investigation.

Previous workers have shown that non-*S.* Typhimurium isolates (such as *S.* Agona, Enteritidis, Chomedey, Djugu, Infantis and Oranienburg) can have integrons ranging in size from 0.65 to 2.7 kb and these integrons were associated with the presence of various resistance genes including *aadA1, aadA2, aadA5, aadB, bla(Carb$_2$)* (also known as *pse1*), *catB3, oxa1, dhfrA1, dhfrA12, dhfrA17* and *dfrXIII*.[18–20] This study confirms the presence of resistance genes associated with integrons in additional non-Typhimurium serotypes to those reported above including *S.* Emek, Haifa, Heidelberg, Mbandaka, Newport, Ohio, Stanley and Virchow.

Multi-resistance in strains that did not have class 1 integrons was associated with the serotypes *S.* Binza, Dublin, Enteritidis, Hadar, Manhattan, Mbandaka, Montevideo, Newport and Typhimurium DT193. These strains sometimes contained the *aadA2* and *sul1* genes common to *S.* Typhimurium DT104 and U302 strains. As the *sul1* gene is the backbone of class 1 integrons,[2,3] it was of some concern that six strains negative for class 1 integrons were positive for the *sul1* gene and these strains warrant further investigation. However, resistance determinants for ampicillin, chloramphenicol and tetracycline in class 1 integron-negative strains were *bla(Tem), cat1, tetA(A)* and *tetA(B)* instead of the *bla(Carb$_2$), floR* and *tetA(G)* genes. Additionally, the *aadA1, sul2* and *strA* genes were often found in these strains rather than the *aadA2* and *sul1* genes. Analysis of *Xba*I fragments suggested that the *sul2* and *strA* genes were plasmid located, possibly.

The trimethoprim-resistant strains that lacked the *dhfr1* gene usually contained the *sul2* gene, and this may suggest that a trimethoprim resistance gene other than *dhfr1* was co-located on a plasmid with the *sul2* and *strA* genes. However, it is possible that integrons other than class 1 integrons were also involved in multi-resistance and a number of different class A dihydrofolate reductase genes within gene cassettes have been described by others.[5]

Overall the data did not suggest any difference in the distribution of resistance genes between human and animal isolates of *Salmonella.* As *Salmonella* is a zoonotic organism, it is to be expected that many of the strains isolated from humans originated from animals. As such, it would be expected that in general the same resistance genes would be found in strains from both animals and humans.

It has been shown that antibiotic resistance genes can be silent in *Salmonella*. For example, three *Salmonella enterica* isolated from retail ground meat samples were susceptible to streptomycin even though they harboured the *aadA2* gene.[20] In this study, 13/59 strains that contained the *strA* gene were susceptible to streptomycin. It would be of interest to determine the genetic basis for susceptibility and to evaluate the risk that might be associated with reversion to resistance. It is possible that these strains did not contain the *strB* gene which is also required for resistance, but this was not established.

Overall, the data indicate that transfer of the resistance genes *bla(Carb$_2$), floR* and *tetA(G)* from strains such as *S.* Typhimurium DT104 or U302 to other serotypes is rare on the basis of the strains studied in this panel. However, the data illustrate the involvement of a range of different resistance genes in both class 1 and non-class 1 integron associated resistance. Whilst resistance genes occurred in a wide range of *Salmonella* serotypes, results illustrated the specificity of some genes to either *S.* Typhimurium or non-*S.* Typhimurium serotypes. Finally, the presence of class 1 integrons is described in serotypes for which it is believed class 1 integrons have not previously been reported and the location of some resistance genes within or associated with the integrons is verified.

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