

# Characterization of multi-drug resistance in *Salmonella* strains isolated from animals

Cristina Romani<sup>(a)</sup>, Aurora Aleo<sup>(b)</sup>, Nathalie Pellissier<sup>(c)</sup>,  
Alessandro Viganò<sup>(c)</sup> and Mirella Pontello<sup>(c)</sup>

<sup>(a)</sup>Dipartimento di Sanità Pubblica, Università degli Studi, Florence, Italy

<sup>(b)</sup>Dipartimento di Igiene e Microbiologia "G. D'Alessandro" Università degli Studi, Palermo, Italy

<sup>(c)</sup>Dipartimento di Sanità Pubblica, Microbiologia, Virologia, Università degli Studi, Milan, Italy

**Summary.** Spread of antibiotic resistances through agro-food chain remains an actual question for both researchers and public health operators. Our study investigates antimicrobial resistance in *Salmonella* spp. strains isolated from avicultural and swine samples (prevalence: 2% and 5.5% respectively). Strains belonging to different serotypes-pulsetypes have resulted multi-resistant in 76% of cases (avicultural strains: 13/23, swine strains: 73/90). Multi-drug resistant strains (MDR) have been studied for transferability by conjugation – observed in 25.6% cases – and for genetic determinants of resistances: 73.3% MDR strains harbour plasmids; class-1 integrons have been identified in 14 strains. Comparing our findings to available scientific literature, we discuss Salmonellosis resistance pattern according to the food chain and serotype.

**Key words:** *Salmonella*, antibiotic resistance, plasmids, integrons.

**Riassunto** (*Poliresistenza in sierotipi di Salmonella isolati da animali*). La diffusione delle antibiotico-resistenze attraverso le filiere agroalimentari è un fenomeno che permane al centro dell'attenzione sia per i ricercatori che per gli operatori di sanità pubblica. Il nostro studio ha esaminato i caratteri di resistenza in stipiti di *Salmonella* spp. isolati dalle filiere avicole (2% di campioni positivi) e suinicola (5,5%). Gli stipiti appartenenti a diversi sierotipi-pulsotipi sono risultati poliresistenti nel 76% dei casi (13/23 e 73/90 ceppi rispettivamente nelle due filiere). Sugli stipiti multiresistenti (MDR) si è indagata la trasferibilità – ottenuta nel 25,6% dei casi – e le basi genetiche delle resistenze: 73,3% degli stipiti MDR veicolano plasmidi; in 14 casi sono presenti integroni di classe 1. I dati ottenuti sono confrontati con quelli riferiti in letteratura, in funzione del tipo di filiera esaminata e dei sierotipi isolati.

**Parole chiave:** *Salmonella*, antibiotico-resistenza, plasmidi, integroni.

## INTRODUCTION

Non-typhoidal salmonellosis continue to be a relevant foodborne disease in industrialised countries in spite of the implementation of surveillance and control activities by producers and public health operators. Non-typhoidal salmonellosis are infections typically occurring in industrialised countries and have a strong relevance in term of costs related to monitoring, epidemiological investigation, laboratory tests, veterinary and medical treatment, lost working days and withdrawn and destroyed foods. In United States, 1.4 million episodes of salmonellosis and 400 deaths occur every year, meaning expenditure for an amount of US\$ 2.3 and 3.6 billion [1-6]. In addition antibiotic resistance for *Salmonella* spp. is widespread. Massive use of antibiotics in zootechny for salmonellosis prevention and therapy may have contributed to diffusion of resistances. Animal reservoir (all the vertebrates) is the natural habitat of *Salmonella* spp. About 90% of non-typhoidal salmonellosis is related to consump-

tion of food of animal origin, in particular products derived from avicultural and swine sectors [7, 8].

Antibiotic resistance is a significant example of fast bacterial adaptation to a new ecosystem. Antibiotic resistance may be explained through resistance genes acquisition by horizontal transfer or point mutations, whose change the pre-existent genome [9]. The main cell-to-cell transfer mechanism is the bacterial conjugation, a process that is generally plasmid-encoded and it is not species-specific. Mobile DNA elements play an important role in the spread and transmission of resistance determinants among *Enterobacteriaceae* strains and particularly *Salmonella* serotypes, while the presence of integrons enhances the persistence of genes encoding for the resistances when antimicrobial selective pressure is absent [7, 10, 11].

The aims of this study are to estimate prevalence of antibiotic resistance in *Salmonella* spp. strains isolated from avicultural and swine samples, to investigate *in vitro* transferability by conjugation and to study molecular bases of antibiotic resistance.

## MATERIALS AND METHODS

### Bacterial strains

Isolation, identification and serotyping of animal strains have been performed from 01.06.2003 to 31.05.2005 by the Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, starting from microbiological examination of different samples collected from avicultural and swine sectors for diagnosis or monitoring screening purposes.

### Antibiotic susceptibility

We studied strains susceptibility to 12 antibiotics: nalidixic acid (Na), ampicillin (Am), cefalotin (Cf), cefotaxime (Ctx), ciprofloxacin (Cip), chloramphenicol (C), gentamycin (Gm), streptomycin (S) sulfamide (Su), tetracycline (Te), trimethoprim (Tmp), rifampicin (Ra) - using disk diffusion method (Kirby-Bauer) and NCCLS interpretation guidelines. Multi-drug resistant (MDR) strains, defined as resistant to two or more antibiotics or only to nalidixic acid, have been analysed performing different techniques described in the following paragraphs.

### Pfge

Pulsetyping of *Salmonella* strains has been performed according to the PulseNet protocols [12], using the *XbaI* and *BlnI* (primary and secondary, respectively) restriction enzymes and *S. Braenderup* H9812 as internal reference strain.

### Bacterial conjugation

We investigate transferability of antibiotic resistance from MDR *Salmonella* strains (donors) to the recipient *Escherichia coli* K12J5 (lactose+; Ra-resistant) following conjugation method described by

Arvanitidou *et al.* [13]. Ampicillin, tetracycline, streptomycin, and chloramphenicol have been selected to verify resistance transfer on Mac Conkey Agar, in view of high frequency transfer of these antibiotics, as it is reported in the literature.

### Plasmid DNA analysis and integrons detection

MDR *Salmonella* strains and transconjugant *E. coli* have been analysed to determine their plasmid profile. Plasmid DNA has been extracted by alkaline lysis as described by Birnboim [14]. Briefly, after cell lysis, there is a selective renaturation of plasmid DNA followed by a serial process including centrifugation, precipitation, suspension and RNA-ase contact. The last step is the migration on agarose gel in order to obtain plasmid pattern and molecular weight in comparison with 4 reference plasmids of 98, 42, 23,9 and 4,6 MDa, all of them extracted from reference strain *E. coli* 39R861.

Integrons have been detected as proposed by Lévesque *et al.* [15]: genes encoding for the antibiotic resistances, located between conserved segments in integrons, have been searched by PCR using the proper oligonucleotide probes.

## RESULTS

In this study 113 *Salmonella* strains have been included: 23 and 90 strains have been isolated from avicultural and swine sector for a prevalence (on the total of analysed samples) of 2% and 5.5%, respectively. In addition to an untypeable E-serogroup strain, 20 different serotypes have been identified: only 3 (Typhimurium, Anatum, Bredeney) are common among both the sectors; globally the most fre-

**Table 1** | Susceptibility, mono- and poly-resistance of *Salmonella* serotypes: the presence of plasmids and transconjugants *E. coli* is indicated only for the multi-drug resistant (MDR) strains

Serotype	N. of strains				MDR strains	
	Total	Susceptible	Mono- resistant	Poly- resistant	Harbouring plasmids	transconjugant <i>E. coli</i>
Typhimurium	38	0	3	35	23	7
4,5:i:-	19	0	0	19	18	1
Derby	15	3	2	10	8	3
Bredeney	8	2	0	6	2	1
Heidelberg	5	0	0	5	5	5
Anatum	6	0	1	5	5	4
Altona	4	3	0	1	0	0
Virchow	3	1	2	0	0	0
Give	2	2	0	0	0	0
Agona	2	2	0	0	0	0
Enteritidis	2	2	0	0	0	0
Other serotypes*	9	2	2	5	2	1
<b>TOTAL</b>	<b>113</b>	<b>17</b>	<b>10</b>	<b>86</b>	<b>63</b>	<b>22</b>
Avicultural sector	23	6	4	13	7	6
Swine sector	90	11	6	73	56	16

\**Gallinarum*, *Schwarzengrund*, *Infantis*, *Brandenburg*, *Braenderup*, *Blockley*, *Choleraesuis*, *Goldcoast*, and untypeable *E* serogroup

quent serotype is Typhimurium (34%), followed by its monophasic variant *S. enterica* subsp. *enterica* 4,5:i:- (17%).

As shown in Table 1, 17 strains (15%) result fully susceptible to the tested antibiotics, 10 (9%) show one resistance (to ampicillin or tetracycline) and 86

(76%) are multi-drug resistant. Among avicultural strains and particularly from poultry, 26% strains are susceptible; a frequency higher than in swine isolates (12%). Antibiotic resistance are more frequent and wider in swine strains than in avicultural ones. Tetracycline, ampicillin, streptomycin, and

**Table 2 | Plasmid profiles and resistances transfer in MDR *Salmonella* strains: serotypes Typhimurium and monophasic variant**

Serotype (N)	<i>Salmonella</i> : resistance pattern*	<i>Salmonella</i> : plasmids (MDa)	<i>E. coli</i> : resistance pattern	<i>E. coli</i> : plasmids (MDa)
Typhimurium (23)	AmCfCGmSSuTeTmp	4; 2.2; 1.2		
	<b>NaAmCfGmSSuTeTmp</b>	60; 34; 9	AmSuTeTmp	34
	AmCGmSSuTeTmp	120; 18; 6.9 98; 13; 1.9; 1.2		
	<b>NaAmGmSSuTeTmp</b>	60; 45; 27; 6 60; 38	AmSuTeTmp AmSuTeTmp	45 38
	AmCfSSuTeTmp	60; 20; 13; 4		
	AmCSSuTeTmp	90; 40; 3; 2.7; 2.3; 1.5 60		
	AmCSSuTe	60		
	NaAmSSuTe	9.5; 4.6 1.5; 1.3 14; 1.5 3; 1.4		
	AmSSuTe	5.1; 3.3 4.6; 2.3 43; 22; 18; 2.8; 2.5 13 45; 4.2 3.5; 1.3	Am Am	43; 2.8 45
	<b>GmSSuTe</b>	60; 50; 8; 2.8; 1.8	SSuTe	50; 2.8
	NaAmSSu	4; 2.1		
	<b>AmTe</b>	60; 27; 22; 2.7	AmTe	27
	4.5:i:- (18)	NaAmCGmSSuTeTmp	25; 2; 1.8; 1.7 14; 3; 1.5 14; 3; 1.5	
AmCGmSSuTeTmp		27; 2; 1.8; 1.7 14; 3; 1.5 4; 2.2; 1.2		
AmCfSuTeTmp		42		
<b>AmSSuTeTmp</b>		60; 54; 46; 16.5; 15; 3.5 14; 1.5	AmSuTe	60; 54
CSSuTeTmp		24; 2; 1.8; 1.7		
NaAmSSuTe		4.1; 3.3 14; 3; 1.5 14; 3; 1.5 14; 3; 1.5		
AmSSuTe		18; 4.2		
AmSuTeTmp		14; 1.5		
NaGmSuTe		12.5; 3; 1.5 14; 3; 1.5		

(N): number of strains harbouring plasmids.

\* in bold the resistances transferred to *E. coli*.

**Table 2bis** | Plasmid profiles and resistances transfer in MDR *Salmonella* strains: serotypes other than *Typhimurium* and monophasic variant

Serotype (N)	<i>Salmonella</i> : resistance pattern*	<i>Salmonella</i> : plasmids (MDa)	<i>E. coli</i> : resistance pattern	<i>E. coli</i> : plasmids (MDa)
Derby (8)	NaCfCGmSSuTeTmp	32; 3.4; 3.1; 1.8		
	NaAmCSSuTeTmp	32; 3.4; 3.1; 1.8		
	<b>AmSSuTeTmp</b>	40; 7.1; 5.9; 1.9; 1.7; 1.5	Am	40
	CSSuTeTmp	30; 3.4; 3.1; 1.8		
	NaAmSSuTe	3.8; 2.4		
	<b>AmSSuTe</b>	60; 4	AmSu	60; 4
		60; 4	AmSu	60
Bredeney (2)	<b>CGmSSuTeTmp</b>	98; 55; 4.7	CTmp	98
	AmSSuTeTmp	18		
Heidelberg (5)	<b>NaAmGmSSuTe</b>	49; 28; 19	AmSSuTe (a); AmSTe (b)	49; 28 (a); 49; 28 (b)
	<b>NaAmSSuTe</b>	27; 21	AmSTe	27
	<b>NaAmSTe</b>	24; 19	AmSTe	24
		29; 21.5 60; 31; 22	AmSTe AmSTe	29 60; 31; 22
Anatum (5)	NaAmCSSuTeTmp	28; 7		
	<b>AmSSuTeTmp</b>	27; 4	AmTmp (a); AmSSuTeTmp (b)	27 (a); 27; 4 (b)
	<b>AmSSuTe</b>	32; 7	Am	32
		26; 5 22; 5.9	Am Am	26 22
Blockley (1)	NaSTe	1.8		
Choleraesuis (1)	<b>AmSSuTe</b>	28; 4.6; 1.8	AmSSuTe	28

(N): number of strains harbouring plasmids.  
\* in bold the resistances transferred to *E. coli*.

sulfamides are less active than other antibiotics (resistant strains are respectively 81%, 63%, 71% and 71% of the total) Mono-resistance is shown mainly towards the tetracycline; none of the strains are resistant to cefotaxime and ciprofloxacin. Most of the strains are multi-resistant, such as *S. Typhimurium* and *S. 4,5:i-* (35/38 and 19/19, respectively) and exhibit a total of 30 different resistance patterns; the most frequent patterns are AmSSuTe (19.7%) and NaAmSSuTe (11.6%). Looking at *S. Typhimurium* and its monophasic variant, 21 resistance patterns are combined with 16 different PFGE profiles; two pulsetypes, namely TA2 and TC3 are common to both serotypes and include 47.3% of all these isolates with 18 and 9 strains, respectively for *S. Typhimurium* and its monophasic variant. The remaining serotypes are characterised by 15 resistance patterns and grouped into 14 different pulsetypes.

Results of conjugation with *E. coli* K12J5, plasmid profiles of *Salmonella* (63 isolates harbour plasmids) and transconjugant *E. coli* strains (22 positive strains) are reported in Tables 2 and 2-bis. Acquisition of resistance phenotype by *E. coli* occurs in 22 out of 86 cases;

twice after conjugation *E. coli* strains characterised by two different resistance patterns have been isolated.

In conjugation process, resistance to ampicillin and tetracycline are respectively transferred in 20 out of 67 (29.9%) and 13 out of 82 (15.9%) cases. The most frequent transferred patterns (54.5%) are resistance to ampicillin and tetracycline, but transfer of whole pattern is recorded only in 3 out of 12 cases. All *S. Heidelberg* strains and most of *S. Anatum* strains result positive to conjugation. Antibiotic resistances of *S. Typhimurium* and its variant *S. 4,5:i-* have less frequently transferred (20.6% and 5.3%).

Overall result of conjugation tests combined with MDR *Salmonella* strains harbouring one or more plasmids show different features with respect to the serotype:

- S. Typhimurium* and monophasic variant: *S. Typhimurium* strains harbouring plasmids (23 out of 35) have partially or totally transferred resistances only in 7 cases (12.9%); we detected integrons in 6 cases. A 60-MDa serotype-specific virulence plasmid not transferred and not harbouring resistance determinants has been

identified in 8 cases. Partial transfer of resistances by conjugation has been pointed out for only one monophasic variant strain that shows a large plasmid pattern; integrons have not been detected in *S. 4,5:i:-*.

- b) *Other serotypes harbouring plasmids and mainly conjugation-positive*
- Heidelberg: every strains of this serotype transfer resistances, that are located on different-size plasmids;
  - anatum: 4 out of 5 strains transfer, mainly resistance to ampicillin;
  - choleraesuis (one strain): we identified a 28-MDa plasmid harbouring tetra-resistance AmSSuTe and transferred to *E. coli*;
- c) *Other serotypes harbouring plasmids and mainly conjugation-negative*
- Derby: 3 out of 8 strains transfer resistances and show plasmid-located class 1 integrons;
  - Bredeney: only 1 out of 6 strains transfer CTm resistance pattern, that are rare located on a 98-MDa plasmid;
  - blockey: strain with NaSTe resistance pattern has not transferred any antibiotic resistance to *E. coli*.

Class 1 integrons have been detected in 15.1% of 86 MDR strains belonging to 5 different serotypes (Table 3). There are 11 different resistance patterns associated to integrons and related to 13 strains: only in 3 cases the same molecular-weight integrons have been reported for transconjugants *E. coli*.

## DISCUSSION AND CONCLUSIONS

Spread of antibiotic resistances among strains isolated, from human, animal sources and food prod-

ucts is well documented. Maintaining and improving surveillance system should be a public health priority in order to control and contain antibiotic resistances [16, 17]. In addition, among zoonotic agents, *Salmonella* spp. is considered a valid indicator to estimate prevalence of resistances. Various Authors underline the differences according to the *Salmonella* serotype, the assayed source (animal, food, etc.) and the geographical area [6, 10, 16, 18-22, 24-29]. We compared our results with Literature data (Table 4):

- in terms of the distribution of resistance determinants among *Salmonella* strains, high variability of patterns with respect to serotypes and sectors is confirmed. Frequency of antibiotic resistance is higher in swine strains than avicultural strains;
- resistance prevalence in *Salmonella* strains isolated from animal sources are higher than frequencies reported by other Authors. Differences may not be exclusively related to different selection criteria for sampling;
- our data confirm that *S. Typhimurium* is the serotype less susceptible to antibiotics in agreement with data from a Spanish study [24]. Emergence of 4,5:i:- variant was notified for the first time (1997) in Spain, where 100% strains of this serotype showed to be antibiotic resistant. Additionally 90% of the Spanish resistant sample were found to be multi-resistant;
- ampicillin, streptomycin, tetracycline, and sulfamides are the less effective drugs in our study, as reported from Literature. Resistance to chloramphenicol in our strains is less spread in comparison with other reports; resistance to chloramphenicol is related to 28 isolates belonging to the following serotypes: Typhimurium, monophasic

**Table 3** | Class-1 integrons in *Salmonella* strains and transconjugant *E. coli* related to resistance patterns and plasmid profile

<i>Salmonella</i> : serotype	<i>Salmonella</i> : resistance pattern (N)	Class-1 integrons		
		<i>Salmonella</i>	Transconjugant <i>E. coli</i>	<i>Salmonella</i> : plasmids (MDa)
Typhimurium	AmCGmSSuTe (1)	1.2-1.0 kb (4)	None	None
	AmCSSuTe (2)		None	None
	NaAmSSuTe (1)		None	14; 1.5
	AmCSSuTeTmp (1)	1.0 kb (1)	None	None
	GmSSuTe (1)	1.6 kb (1)	None	60; 50; 8; 2.8; 1.8
Derby	AmSSuTeTmp (1)	0.7 kb (1)	0.7 kb	40; 7.1; 5.9; 1.9; 1.7; 1.5
	AmSSuTe (2)	1.0 kb (3)	None	60; 4
	SSuTe (1)		1.0 kb	60; 4
			None	1.8; 1.5
Bredeney	AmSSuTeTmp (1)	1.6 kb (1)	None	18
Heidelberg	NaAmGmSSuTe (1)	1.0 kb (1)	1.0 kb	49; 28; 19
Altona	AmCSSuTe (1)	1.2-1.0 kb (1)	None	None

In brackets the number of strains characterised by the resistance pattern (column 2) and integrons (column 3).

**Table 4** | Antibiotic resistance in *Salmonella* strains: review of literature data

Geographical area	Isolation source	<i>Salmonella</i> <sup>(a)</sup> serotype	Prevalence of resistant strains <sup>(b)</sup>	Most frequent resistances or resistance patterns	Reference
England and Wales	Animal	Tm (28.053)	Tm: 42.6-92.4%	Tm: Am, Te, S, Su	Jones (2002)
	Environment	Dublin (6.548)	Dublin: 0.3-1.8%		
	Fodder	Other (74.524)	Other: 11.8-45.5% <sup>(c)</sup>		
Spain	Animal	Tm (104) Other (370)	61.7% 81.5%	Am, S, Te Su, Te	Usara (2002)
USA	Imported food	Weltevreden (24) Other (163)	All the serotypes: 8%	Te (4.3%)	Zhao (2003)
Europe	Animal	Tm (108) Other (163)	(d)	Am (16.1-54.7%) Te (34.9-100%) <sup>(c)</sup>	Bywater (2004)
USA	Swine	Tm	(d)	AmKmStSuTe (44.6%) AmCmStSuTe (36.2%)	Gebreyes (2002)
USA	Swine	Tm (24) Other 6 serotypes (17)	All the serotypes: MDR 98%	All the serotypes: Su (95%); Te (88%) Tm DT104: AmCSSuTe (100%)	White (2003)
USA	Imported food	Weltevreden (41) Other (167)	All the serotypes: 11%	Te (9%)	Zhao (2006)
UK	Human Animal	35 serotypes (397)	All the serotypes: 45%	Am (22.9%) C (21.4%)	Randall (2004)
France	Human	Tm (320)	78.5%	Te (71%); Su (68%); Amx (64.7%); S (64.5%); Sp (59%); C (59%); AmSSpSuCte (48.8%)	Weill (2006)
Ireland	Human, food, animal	Tm (67)	88% poly-resistant	ACSSuT (77.6%)	Gorman (2004)
Holland	Human, animal	≥25 serotypes (237) <sup>(e)</sup>	All the serotypes: 46% resistant, 29% poly-resistant	Su (29%); Am (21%); Te and S (17%)	Vo Anit (2006)
Portugal	Human, food, environment	All the serotypes (1183)	Na; Te; S; Su; Am: 17-31%	None	Antunes (2006)
Italy <sup>(f)</sup>	Human, animal	Tm	(d)	AmCSSpSuT (75.9%)	Carattoli (2002)
Italy (Lombardia)	Animal	21 serotypes (113) Tm and variant (54)	All the serotypes: 85.0% (MDR 76.1%) Tm and monophasic variant: 100% (MDR 94.7%)	AmSSuTe (19.7%)	Our study

Tm: abbreviation for *S. Typhimurium*

<sup>(a)</sup> On brackets the number of strains, when it is indicated

<sup>(b)</sup> The prevalence of resistant strains (mono- and poly-resistant) is referred to all the analysed serotypes or for the main serotypes, when it is possible

<sup>(c)</sup> Range period indicated 1988-1999: for *S. Typhimurium* the minimum value in 1992 and maximum in 1995; for *S. Dublin* the minimum value in 1990 and 1996, and the maximum in 1997; for other serotypes the minimum value in 1988 and maximum in 1995

<sup>(d)</sup> The overall prevalence and/or the prevalence by serotypes is not reported; for the European review (Bywater) the prevalence is associated to the kind of animal and by country (for Am maximum value in France and minimum in Holland; for Te maximum value in Spain and minimum in UK)

<sup>(e)</sup> The total of serotypes is not achievable; the authors report that the resistant strains belong to 25 serotypes

<sup>(f)</sup> Strains coming from collection of the Istituto Superiore di Sanità-Roma and Istituto Zooprofilattico Sperimentale- Padova

variant, Derby, Altona, Anatum, Goldcoast, and Bredeney. In our investigation 21 strains belonging to 5 different serotypes are gentamycin-resistant: this phenotype is less frequently spread than previously reported. Only three strains (*S. Typhimurium*, one strains of both monophasic variant and *S. Derby*) showed resistance to cefalotin; moreover we have not found strains resistant to cefotaxime and ciprofloxacin, rarely reported from other authors [11, 18, 30, 31];

- finally, resistance patterns most frequently observed in our study and involving the resistance to ampicillin, streptomycin, tetracycline, and sulfamides are partially overlapped with ones referred by other Authors: the penta-resistance AmCSSuTe in many cases is extended also to the spectinomycin (Sp) - not tested in our study - and is prevalent worldwide above all but not exclusively in the clone *Typhimurium* DT104 [32, 33]. Conjugation tests have shown a limited transferability of resistances.



When resistances have been transferred to *E. coli*, often partially, different molecular-weight plasmids have been detected. Particularly focusing the attention on S. Typhimurium and its monophasic variant, the conjugation occurred only in 7/35 and 1/19 cases, respectively: this figure suggests that genes encoding for the resistances are prevalently chromosomal-located. It has to be pointed out that 4 strains of MDR S. Typhimurium – 3 without plasmids – have shown a resistance pattern common to DT104 clones, those are well known to be characterised by chromosomal location of resistances [34-36].

In our study other MDR strains (serotypes Heidelberg, Anatum, Derby) harbouring 1,5-98 MDa plasmids transfer by conjugation resistances with a frequency (60%) higher than Typhimurium and its monophasic variant (14.8%); transferability is particularly efficient with respect to ampicillin and tetracycline resistances.

Transfer of gentamycin and cefalotin resistances to *E. coli* never occurred: according to Gebreyes *et al.* [20], gentamycin resistance could be associated to a conjugative plasmid harbouring resistance determinants to Am, Km, S, Su; an extended plasmid-mediated resistance spectrum to cephalosporines (ESC) is also referred by Miriagou [30].

Resistance to chloramphenicol – observed in 28 out of 86 MDR strains – has been transferred to *E. coli* only from one penta-resistant strain of *S. Bredeney* (CGmSuTeTmp) in parallel with a 98-MDa plasmid harbouring also resistance to trimethoprim; 21 strains of *S. Typhimurium* and its monophasic variant have never transferred the chloramphenicol-resistance in agreement with data from scientific literature [32].

Non-transferability – by conjugation – of genes encoding for the resistance related to extra-plasmid location is well documented in the Literature [8, 9, 19, 22, 24, 26, 27, 33]. Class-1 integrons are generally located on the *Salmonella* Genomic Island 1 (SGI1) and strongly associated to widespread and penta-resistant (AmCSSuTe) clone Typhimurium DT104 [34-36]. In our study integrons have been detected in a limited number of strains, namely 6 penta- or hexa-resistant *S. Typhimurium*. Randall *et al.* [23] and Vo *et al.* [25] have reported MDR *Salmonella* strains harbouring class-1 integrons only in 20.4% and 15.2% cases respectively; in the same studies and in Italian one by Carattoli *et al.* [19], 1 and 1,2 kb class-1 integrons – as we have detected in our strains – are mainly associated to *S. Typhimurium* DT104. Nevertheless these Author report that in Italy strains harbour a defective SGI1, due to lost of genes encoding for chloramphenicol-florphenicol and tetracycline resistances: this consideration could explain low frequency of chloramphenicol-resistance observed in our prevalent serotypes (*S. Typhimurium* and *S. 4,5:i:-*). At the same time, although susceptibility to kanamycin have not been tested, it is likely that our prevalent pattern (AmSSuTe in 9 out of 38

strains) meets the penta-resistance AmKmSSuTe associated to phagetype DT193, regarded as precursor of phagetype DT104: in Lombardia Region we have documented spread of DT193 clone during an outbreak caused by a swine food product (salami) [20, 37].

Comparing our findings with wide existent literature on antibiotic resistance of *Salmonella enterica*, an extremely complicated and variable figure emerges: i) differences between serotypes, geographical areas and analysed sources are reported; ii) only for a restricted number of cases, resistance determinants are located on plasmids transferable by conjugation; iii) except for the non-transferable penta-resistance pattern NaAmSSuTe related to the monophasic variant, in view of different observed resistance patterns, variable presence of both plasmids and transferable plasmids, as well rare detection of class-1 integrons is difficult to identify resistances specifically associated to certain serotypes, iiiii) besides data on antibiotic resistance and its molecular determinants, if the distribution of pulsetypes is considered, some serotypes (Heidelberg, Bredeney, *S. 4,5:i:-*) appear to be more homogeneous (monoclonal spread?) than other ones, particularly *S. Typhimurium* (34 strains associated to 12 different pulsetypes: XT A2 is the most prevalent among them).

In conclusion, spread of antibiotic resistance in animal reservoir and from here to human through the food chain, results very dynamic, intricate, and highly variable with respect of microorganism “type”, in terms of sero/phage/pulse/antibio-type. This phenomenon is probably influenced by selective pressure derived from large use of antibiotics in zootechny. Compared to results of previous studies – performed in other geographical areas – our study contributes to point out similarity and dissimilarity regarding frequency of resistances in different circulating serotypes, and genetic determinants of resistances, whose can be located on a great number of mobile elements (plasmids, transposons, integrons) transferable by both horizontal and vertical processes, as it is widely reported [9, 20, 23, 25, 38].

In the last 20 years emergence of MDR strains in animals and huge number of outbreaks associated to MDR strains, first of all belonging to *Salmonella* serotypes, have occurred [39-42]. Therefore in order to contribute to the global effort of containment of antimicrobial resistance in foodborne pathogens, it is essential to enhance surveillance of antibiotic resistances and their molecular bases, integrating information about human infections with available data in veterinary.

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