Presence of Class I Integrons in Multidrug-Resistant, Low-Prevalence *Salmonella* Serotypes, Italy

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In 1997 to 1999, we detected class I integrons in multidrug-resistant isolates of *Salmonella enterica* serovars Anatum, Blockley, Brandenburg, Bredeney, Derby, Heidelberg, Livingstone, Newport, Ohio, Panama, Paratyphi B, Saintpaul, Sandiego, and Stanley.

Bacterial resistance to antimicrobial agents is a serious problem worldwide. Of particular concern is the increasing frequency of multidrug resistance within *Salmonella* strains isolated from zoonotic foodborne infections (1,2). This aspect has been extensively investigated in *Salmonella enterica* serovar Typhimurium in relation to the worldwide spread of multidrug-resistant (MDR) strains of definitive phage type (DT) 104, with chromosomally integrated genes coding for resistance to ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline (3,4).

Recently, a basic role in dissemination and evolution of antimicrobial resistance in MDR *S.* Typhimurium DT104 (MDR-DT104) and many other organisms has been attributed to integrons, gene expression elements that potentially account for rapid and efficient transmission of drug resistance because of their mobility and ability to collect resistance gene cassettes (5,6). These elements have been described in a wide range of pathogenic organisms (7), including *S.* Typhimurium and *S.* Enteritidis (8,9); reports of these integrons in other *Salmonella* serotypes are anecdotal (10).

Although *S.* Enteritidis and *S.* Typhimurium account for approximately 45% and 25%, respectively, of the strains of *Salmonella* identified at the Centre for Enteric Pathogens of southern Italy, other serotypes, such as Brandenburg, Derby, Livingstone, and Thompson, are frequently identified from various sources, exhibiting sometimes unusually wide patterns of antibiotic resistance. We investigated the presence of class I integrons in MDR strains of *Salmonella* serotypes other than *S.* Typhimurium and *S.* Enteritidis, identified in 1997 to 1999, to obtain information on the presence of these elements in low-prevalence serotypes and to determine their association with multidrug-resistance phenotypes.

The Study

Seventy-four strains of *Salmonella* (of serotypes other than *S.* Enteritidis and *S.* Typhimurium) resistant to three or more antibacterial drugs were identified from January 1997 to December 1999. Isolates were from human and nonhuman sources. Sixty-two isolates were available for further investigation. Identification was performed by the API 20E system (Biomerieux, Marcy l'Etoile, France) and serotyping (11) by commercially obtained antisera (Sanofi Diagnostics Pasteur, Marnes-La Coquette, France).

Susceptibility to ampicillin, amoxicillin-clavulanic acid, cefotaxime, chloramphenicol, ciprofloxacin, gentamicin, nalidixic acid, nitrofurantoin, sulfonamides, streptomycin, tetracycline, and trimethoprim was tested by disk-diffusion assay, according to National Committee for Clinical Laboratory Standard Guidelines (12).

The rifampin-resistant strain of *Escherichia coli* K12J5 Rif_r was used as the recipient in conjugation experiments (13). Transconjugants were selected on Luria-Bertani agar containing 250 µg/mL of rifampin plus 50 µg/mL of ampicillin or sulfonamides or 30 µg/mL of chloramphenicol, streptomycin, tetracycline, or trimethoprim.

Plasmid DNA was extracted by the procedure of Birnboim and Doly (14), electrophoresed on 0.7% agarose, and stained with ethidium bromide simultaneously with reference size plasmids (39R861, MIP 233, R27, and R477).

Screening of isolates for presence of class I integrons was performed by a high-stringency protocol with oligonucleotide primers specific for the sequence of the published 5'-CS and 3'-CS regions adjacent to the site-specific recombinational insertion sequence (15). Primer sequences were: 5'-CS, GGCATCCAAG-CAGCAAG and 3'-CS, AAGCAGACTTGACCTGA (15).

Further polymerase chain reaction (PCR) analysis was performed on the 26 isolates harboring class I integrons to better characterize the antibiotic resistance genes associated with the integron structure. This was done by using primers located at the beginning extremities of the inserted resistance genes in combination with that specific for the 5'-CS conserved segment. The following sequences were tested: sulfonamide resistance gene *sul*I; beta-lactam resistance genes *oxa*2, *pse*1, and *tem*; aminoglycoside resistance genes *aac*(3)-Ia, *aac*(3)-IIa, *aac*(6')-Ib, *ant*(3")-Ia, *aad*A2 [also named *ant*(3")-Ib]; and trimethoprim resistance gene *dhfr*I (15). The presence of the *pasppflo*-like (*flor*) and *tet*G genes, conferring resistance to chloramphenicol, florfenicol, and tetracycline in MDR-DT104, was also investigated by using PCR primers specific for these sequences (16).

From 1997 to 1999, 18 *Salmonella* serotypes were identified, including isolates resistant to three or more antibacterial drugs: Anatum, Blockley, Brandenburg, Bredeney, Derby, Hadar, Heidelberg, Livingstone, Muenchen,

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Newport, Ohio, Panama, Paratyphi B, Saintpaul, Sandiego, Stanley, Thompson, and Virchow. Seventy-four multidrug-resistant isolates were identified, which accounted for 10.0% of the strains belonging to the serotypes under study. The proportion of isolates with a pattern of resistance to three or more drugs is summarized (Table 1); 26 isolates belonging to 14 serotypes contained class I integrons (Table 2). Screening for the presence of plasmid DNA detected no plasmids in 14 strains and plasmids, between 35 and 140 megadaltons in size, in the remaining 12. Three isolates of serotype Brandenburg clustered as an epidemic, according to epidemiologic data and shared identical plasmid DNA and integron profiles. Transfer of plasmids was associated with transmission to *E. coli* of the complete or partial resistance pattern (Table 2). In all but one case, PCR analysis with the 5'CS and 3'CS primers confirmed the presence of integrons in the recipient cells.

Heterogeneous integron-associated resistance genes were present in the isolates under study, despite the extensive similarities of the antibiotic resistance phenotypes (Table 3). Strains belonging to serotypes Ohio, Panama, and Saintpaul carried the *ant*(3")-Ia and *pse*I gene cassettes previously described in two different chromosome-located integrons in MDR-DT104 but inserted in a single integron transferable by conjugation. The integron-associated aminoglycoside resistance genes *aac*(3)-IIa and *aac*(6')-Ib were not detected in the strains tested.

Both *tet*G and *flor* resistance determinants, known to characterize MDR-DT104 strains (16), were found in one strain of Paratyphi B isolated from tropical fish imported from Singapore.

Table 1. Proportion of low-prevalence *Salmonella* serotypes resistant to three or more antibacterial drugs

Serotype	No (% of isolates)
Sandiego	3 (33.3)
Blockley	22 (31.9)
Heidelberg	6 (21.4)
Thompson	11 (20.4)
Stanley	2 (16.7)
Saintpaul	2 (12.5)
Muenchen	1 (11.1)
Brandenburg	6 (10.7)
Anatum	5 (10.0)
Hadar	1 (7.7)
Ohio	2 (7.1)
Bredeney	2 (5.7)
Paratyphi B	1 (5.0)
Newport	1 (4.5)
Panama	1 (4.3)
Virchow	3 (3.3)
Livingstone	2 (2.9)

Conclusions

The emergence of multidrug resistance in *Salmonella* serotypes is causing growing concern because of the high potential of human involvement through food and animal contact. We have detected integrons in MDR-resistant isolates of *Salmonella* identified in southern Italy in the last 3 years. Our findings confirmed not only that integrons are not confined to *S.* Typhimurium DT104 but also that they can be found in many less- prevalent serotypes with extensive reservoirs, encompassing animal species (swine, poultry, domestic pets) and environmental sites (rivers, sewage effluents). A further concern is the presumed location of integrons on the chromosome, detected in isolates of nine different serotypes. This resistance gene location has proved to be very efficient in acquiring and establishing resistance traits and in supporting spread of *S.* Typhimurium DT104 through the food chain in western countries (17).

Table 2. Phenotypic and molecular characteristics of multidrug-resistant and class I integron carrying strains of *Salmonella*

Serotype	Year and source of isolation	Resistance pattern	Plasmid pattern (mDa)	Integron sizes (kb)	Resistance pattern of recipient *Escherichia coli*	Integron sizes (kb) of *E. coli*
Derby	1997 Human	Ap Cm Sm Su Tc Tp Gm	**120**[b]	2.0	Ap Cm Sm Su Tc Tp	2.0
Newport	1997 Human	Ap Cm Sm Su Tc Tp Gm	**120**	2.0	Ap Cm Sm Su Tc Tp	2.0
Paratyphi B	1997 Tropical fish	Ap Cm Sm Su Tc		1.2, 1.0		
Saintpaul	1997 Poultry	Ap Cm Sm Su Tc Tp	**120**	1.8	Ap Cm Sm Su Tc Tp	1.8
Sandiego	1997 Poultry	Ap Cm Sm Su Tc Tp Kf		1.4		
Anatum	1998 Food (not specified)	Ap Sm Su Tc Tp F		1.8		
Blockley	1998 River water	Cm Sm Su Tc Tp F Na		1.8, 1.0		
Brandenburg[a]	1998 Human	Cm Sm Su Tc Tp	**120**	1.0	Cm Sm Su Tc Tp	1.0
Livingstone	1998 River water	Ap Cm Sm Su Tc Tp	**120**	1.8	Ap Cm Sm Su Tc Tp	1.8
Ohio	1998 River water	Ap Cm Sm Su Tc Tp	**120**	1.8	Ap Cm Sm Su Tc Tp	1.8
Ohio	1998 Swine	Ap Sm Su Tc Tp	**120**	1.6	Ap Sm Su Tc Tp	1.6
Panama	1998 Swine	Ap Cm Sm Su Tc Tp	**120**	1.8	Ap Cm Sm Su Tc Tp	1.8
Saintpaul	1998 Human	Ap Cm Sm Su Tc Tp Kf	**140**	1.8	Ap Cm Sm Su Tc Tp	1.8
Anatum	1999 Sewage	Ap Sm Su Tc Tp		1.8		
Anatum	1999 River water	Ap Su Tc Tp F		1.8		
Anatum	1999 River water	Su Tc Tp Na		< 0.1		
Blockley	1999 Human	Cm Sm Su Tc F Na		< 0.1		
Brandenburg	1999 Tropical fish	Cm Su Tc Tp		0.8, 0.2, <0.1		
Brandenburg	1999 Tropical fish	Cm Sm Su Tc Tp		1.8		
Brandenburg	1999 Poultry	Cm Sm Su Tc Tp Gm F Na	**120**	1.8	Cm Sm Su Tc Tp	1.8
Bredeney	1999 Sewage	Sm Su Tc		1.8, 1.0		
Derby	1999 Sewage	Ap Sm Su Tc	**60,** 35	1.0	Ap	
Heidelberg	1999 Human	Ap Sm Su Tc		1.8, 1.0, 0.2		
Stanley	1999 Tropical fish	Cm Sm Su Tc Tp		1.8, 1.0		

[a]Outbreak strain.
[b]Numbers in bold indicate the approximate molecular size of self-transferable resistance plasmids.

Table 3. Resistance genetic sequences identified in class I integron-carrying multidrug-resistant strains of *Salmonella*

	Integron									
Serotype	sulI	pse1	tem	oxa2	aadA2	ant(3")-Ia	aac(3)-IA	dhfrI	tetG	pasppflo-like (flor)
Derby	+		+							
Newport	+		+							
Paratyphi B	+			+					+	+
Saintpaul	+	+				+		+		
Sandiego	+					+		+		
Anatum	+	+								
Blockley	+							+		
Brandenburg[a]	+				+			+		
Livingstone	+	+								
Ohio	+	+						+		
Ohio	+	+				+		+		
Panama	+	+				+		+		
Saintpaul	+	+				+		+		
Anatum	+		+					+		
Anatum	+		+							
Anatum	+									
Blockley	+									
Brandenburg	+									
Brandenburg	+						+			
Brandenburg	+					+		+		
Bredeney	+									
Derby	+		+		+					
Heidelberg	+	+								
Stanley	+						+			

[a]Outbreak strain.

We also recognized in different serotypes a pattern of resistance similar to the five-drug pattern typical of DT104, a phenomenon reported by Glynn et al. (10). The heterogeneous distribution and organization of resistance genes within several low-prevalence serotypes of *Salmonella* suggest the possible emergence of MDR-DT104-like patterns in serotypes other than *S.* Typhimurium that share a similar selective pressure because of intensive use of antimicrobial agents in farming. Moreover, *tet*G and *flor* resistance sequences in one *S.* Paratyphi B isolate from Singapore tropical fish suggest that the use of antimicrobial agents in aquaculture in Asia is contributing to the emergence and spread of multidrug resistance within fish pathogens and, subsequently, MDR-DT 104 strains (18).

The association between emergence of MDR *Salmonella* strains and excessive use of antibiotics in animal husbandry (as growth promoters and for disease prevention and therapy) is receiving increasing attention in developed countries. The presence of integrons in zoonotic serotypes such as Blockley, Brandenburg, Derby, or Saintpaul, which in southern Italy are epidemiologically linked to farming practices, underscores the public health problem of antibiotic resistance diffusion.

Surveillance and monitoring of antimicrobial-drug resistance, including screening for class I integrons as likely indicators of evolution of drug resistance mechanisms and acquisition of new resistance traits, are necessary steps in planning effective strategies for containing this phenomenon within foodborne infectious organisms.

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