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

Antonino Nastasi* and Caterina Mammina†

*University of Florence, Florence, Italy, and †University of Palermo, Palermo, Italy

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, ceftotaxime, 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 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, GGCACTTCAAGCAGCAAG 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 sulI; beta-lactam resistance genes oxa2, pse1, and tem; aminoglycoside resistance genes aac(3)-Ia, aac(3)-IIa, aac(6')-Ib, ant(3")-Ia, adaA2 [also named ant(3")-Ib]; and trimethoprim resistance gene dhfrI (15). The presence of the pasppflo-like (flor) and tetG 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,
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 pse1 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 tetG 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.

### 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 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)             |
| Livingston | 2 (2.9)           |

### 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) of recipient | Resistance pattern of recipient | Integron sizes (kb) of E. coli |
|----------|------------------------------|--------------------|-----------------------|---------------------------------|--------------------------------|--------------------------------|
| Derby    | 1997 Human                   | Ap Cm Sm Su Tc Tp F Na | 120p                  | 2.0                            | Ap Cm Sm Su Tc Tp F Na         | 2.0                            |
| Newport  | 1997 Human                   | Ap Cm Sm Su Tc Tp F Na | 120                   | 2.0                            | Ap Cm Sm Su Tc Tp F Na         | 2.0                            |
| Paratyphi B | 1997 Tropical fish          | Ap Cm Sm Su Tc F Na  | 120                   | 1.8                            | Ap Cm Sm Su Tc F Na            | 1.8                            |
| Saintpaul | 1997 Poultry                | Ap Cm Sm Su Tc F Na  | 120                   | 1.8                            | Ap Cm Sm Su Tc F Na            | 1.8                            |
| Sandiego | 1997 Poultry                | Ap Cm Sm Su Tc F Na  | 120                   | 1.8                            | Ap Cm Sm Su Tc F Na            | 1.8                            |
| Anatum   | 1998 Food (not specified)   | Ap Cm Sm Su Tc Tp F Na | 120                  | 1.8                            | Ap Cm Sm Su Tc Tp F Na         | 1.8                            |
| Blockley | 1998 River water             | Ap Cm Sm Su Tc Tp F Na | 120                  | 1.8                            | Ap Cm Sm Su Tc Tp F Na         | 1.8                            |
| Brandenburg | 1998 Human                   | Ap Cm Sm Su Tc Tp F Na | 120                  | 1.8                            | Ap Cm Sm Su Tc Tp F Na         | 1.8                            |
| Livingston | 1998 River water            | Ap Cm Sm Su Tc Tp F Na | 120                  | 1.8                            | Ap Cm Sm Su Tc Tp F Na         | 1.8                            |
| Ohio     | 1998 River water             | Ap Cm Sm Su Tc Tp F Na | 120                  | 1.8                            | Ap Cm Sm Su Tc Tp F Na         | 1.8                            |
| Ohio     | 1998 Swine                   | Ap Cm Sm Su Tc Tp F Na | 120                  | 1.8                            | Ap Cm Sm Su Tc Tp F Na         | 1.8                            |
| Panama   | 1998 Swine                   | Ap Cm Sm Su Tc Tp F Na | 120                  | 1.8                            | Ap Cm Sm Su Tc Tp F Na         | 1.8                            |
| Saintpaul | 1998 Human                  | Ap Cm Sm Su Tc Tp F Na | 120                  | 1.8                            | Ap Cm Sm Su Tc Tp F Na         | 1.8                            |
| Anatum   | 1999 Sewage                  | Ap Cm Sm Su Tc Tp F Na | 120                  | 1.8                            | Ap Cm Sm Su Tc Tp F Na         | 1.8                            |
| Anatum   | 1999 River water             | Ap Cm Sm Su Tc Tp F Na | 120                  | 1.8                            | Ap Cm Sm Su Tc Tp F Na         | 1.8                            |
| Blockley | 1999 Human                   | Ap Cm Sm Su Tc Tp F Na | 120                  | 1.8                            | Ap Cm Sm Su Tc Tp F Na         | 1.8                            |
| Brandenburg | 1999 Tropical fish          | Ap Cm Sm Su Tc Tp F Na | 120                  | 1.8                            | Ap Cm Sm Su Tc Tp F Na         | 1.8                            |
| Brandenburg | 1999 Tropical fish          | Ap Cm Sm Su Tc Tp F Na | 120                  | 1.8                            | Ap Cm Sm Su Tc Tp F Na         | 1.8                            |
| Bredeney | 1999 Sewage                  | Ap Cm Sm Su Tc Tp F Na | 120                  | 1.8                            | Ap Cm Sm Su Tc Tp F Na         | 1.8                            |
| Derby    | 1999 Sewage                  | Ap Cm Sm Su Tc Tp F Na | 120                  | 1.8                            | Ap Cm Sm Su Tc Tp F Na         | 1.8                            |
| Heidelberg | 1999 Human                  | Ap Cm Sm Su Tc Tp F Na | 120                  | 1.8                            | Ap Cm Sm Su Tc Tp F Na         | 1.8                            |
| Stanley  | 1999 Tropical fish           | Ap Cm Sm Su Tc Tp F Na | 120                  | 1.8                            | Ap Cm Sm Su Tc Tp F Na         | 1.8                            |

*Outbreak strain.

*Numbers in bold indicate the approximate molecular size of self-transferable resistance plasmids.

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**Dispatches**
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-DT104 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.

Dr. Nastasi is professor of hygiene at the Department of Public Health of the University of Florence, Italy. His research interests include epidemiology and surveillance of infectious diseases.

Dr. Mammina is a physician at the Department of Hygiene and Microbiology of the University of Palermo, Italy. Her work focuses on epidemiologic investigation of infectious diseases by molecular typing.

**References**

1. Threlfall EJ, Rowe B, Ward LR. A comparison of multiple drug resistance in salmonellas from humans and food animals in England and Wales, 1981 and 1990. Epidemiol Infect 1993;111:189-97.
2. Tassios PT, Chadjichristodoulou C, Lambiri M, Kansouzidou-Kanakoudii A, Sarandopoulou Z, Kourea-Kremastinou J, et al. Molecular typing of multidrug-resistant *Salmonella* blockley outbreak isolates from Greece. Emerg Infect Dis 2000;6:604.
3. Glynn MK, Bopp C, Dewitt W, Dabney P, Mokhtar M, Angulo FJ. Emergence of multidrug-resistant *Salmonella enterica* serotype Typhimurium DT104 infections in the United States. N Engl J Med 1998;338:1333-8.
4. Mølbak K, Baggesen DL, Aarestrup FM, Ebbesen JM, Engberg J, Frydenahl K, et al. An outbreak of multidrug-resistant, quinolone-resistant *Salmonella enterica* serotype Typhimurium. N Engl J Med 1999;341:1420-5.
5. Recchia GD, Hall RM. Gene cassettes: a new class of mobile elements. Microbiology 1995;141:3015-27.
6. Tosini F, Visca P, Luzzi I, Dionisi AM, Pezzella C, Petrucca A, et al. Class I integron-borne multiple-antibiotic resistance carried by IncF1 and IncL/M plasmids in *Salmonella enterica* serotype Typhimurium. Antimicrob Agents Chemother 1998;42:3053-8.
7. Jones ME, Peters E, Weersink AM, Fluit A, Verhoef J. Widespread occurrence of integrons carrying multiple antibiotic resistance in bacteria [letter]. Lancet 1997;349:1742-3.
8. Rankin SC, Coyne MJ. Multiple antibiotic resistance in *Salmonella enterica* serotype enteritis [letter]. Lancet 1998;351:1429-5.
9. Brown AW, Rankin SC, Platt DJ. Detection and characterization of integron in *Salmonella enterica* serotype Enteritidis. FEBS Microbiol Lett 2000;191:145-9.
10. Glynn MK, Ribot EM, Barrett TJ. Multidrug-resistant *Salmonella enterica* serotype Typhimurium infections [letter]. N Engl J Med 1998;339:922.
11. Kaufman F. Serological diagnosis of *Salmonella* species. Copenhagen (Denmark): Munksgaard; 1972.
12. National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial disk susceptibility tests for bacteria that grow aerobically. Approved standard M7-A4. Villanova (PA): The Committee; 1997.

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

| Serotype   | sulI | pse1 | tem | oxa2 | aadA2 | ant(3')-Ia | aac(3)-IA | dfrA1 | tetG | pasppflo-like (flor) |
|------------|------|------|-----|------|-------|------------|-----------|-------|------|----------------------|
| Derby      | +    | +    |     |      |       |            |           |       |      |                      |
| Newport    | +    | +    |     |      |       |            |           |       |      |                      |
| Paratyphi B| +    | +    | +   |      |       |            |           |       |      |                      |
| Saintpaul  | +    | +    |     |      |       |            |           |       |      |                      |
| Sandiego   | +    | +    |     |      |       |            |           |       |      |                      |
| Anatum     | +    | +    |     |      |       |            |           |       |      |                      |
| Blockley   | +    | +    |     |      |       |            |           |       |      |                      |
| Brandenburg| +    | +    | +   |      |       |            |           |       |      |                      |
| Livingstone| +    | +    |     |      |       |            |           |       |      |                      |
| Ohio       | +    | +    |     |      |       |            |           |       |      |                      |
| Ohio       | +    | +    |     |      |       |            |           |       |      |                      |
| Panama     | +    | +    |     |      |       |            |           |       |      |                      |
| Saintpaul  | +    | +    |     |      |       |            |           |       |      |                      |
| Anatum     | +    | +    |     |      |       |            |           |       |      |                      |
| Blockley   | +    | +    |     |      |       |            |           |       |      |                      |
| Brandenburg| +    | +    |     |      |       |            |           |       |      |                      |
| Brandenburg| +    | +    |     |      |       |            |           |       |      |                      |
| Bredeney   | +    | +    |     |      |       |            |           |       |      |                      |
| Derby      | +    | +    |     |      |       |            |           |       |      |                      |
| Heidelberg | +    | +    |     |      |       |            |           |       |      |                      |
| Stanley    | +    | +    |     |      |       |            |           |       |      |                      |

*Outbreak strain.*
13. Datta N. Transmissible drug-resistance in an epidemic strain of *Salmonella typhimurium*. J Hyg 1997;78:297-300.

14. Birnboim HC, Doly J. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucleic Acids Res 1979;7:1513-23.

15. Lévesque C, Piché L, Larose C, Roy PH. PCR mapping of integrons reveals several novel combinations of resistance genes. Antimicrob Agents Chemother 1995;39:185-91.

16. Ng LK, Mulvey MR, Martin I, Peters GA, Johnson W. Genetic characterization of antimicrobial resistance in Canadian isolates of *Salmonella* serovar Typhimurium DT104. Antimicrob Agents Chemother 1999;43:3018-21.

17. Casin I, Breuil J, Brisabois A, Mouri F, Grimont F, Collatz E. Multidrug-resistant human and animal *Salmonella* typhimurium isolates in France belong predominantly to a DT104 clone with the chromosome- and integron-encoded beta-lactamase PSE-1. J Infect Dis 1999;179:1173-82.

18. Angulo FJ, Griffin PM. Changes in antimicrobial resistance in *Salmonella enterica* serovar Typhimurium [letter]. Emerg Infect Dis 2000;6:436-7.