Multidrug and Broad-Spectrum Cephalosporin Resistance among *Salmonella enterica* Serotype Enteritidis Clinical Isolates in Southern Italy

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From 1992 to 1997, only six sporadic isolates of *Salmonella enterica* serotype Enteritidis from patients with cases of gastroenteritis in southern Italy exhibited resistance to broad-spectrum cephalosporins. Five isolates produced SHV-12, and one isolate encoded a class C β-lactamase. The *bla*<sub>SHV-12</sub> gene was located in at least two different self-transferable plasmids, one of which also carried a novel class 1 integron.

*Salmonella enterica* serotype Enteritidis is one of the dominant serotypes causing human disease in Europe (6). Most infections caused by serotype Enteritidis and other non-typhoidal salmonellae result in self-limiting diarrhea and do not require antimicrobial treatment. However, invasive infections are fairly common in children, for which cases the broad-spectrum cephalosporins are the antibiotics of choice.

During the period 1990 to 1998, the Center for Enteric Pathogens in Palermo, Italy, typed approximately 1,000 salmonella isolates annually, 20% of which belonged to serotype Enteritidis. Of these, approximately 45% were of human origin (13). These had originated primarily from the two epidemiological sentinel hospitals the “G. Di Cristina” pediatric hospital of Palermo and the “Pugliese” hospital of Catanzaro. Phage type PT4 was predominant, represented by 70 to 80% of all isolates, depending on the year. Susceptibility testing, performed according to NCCLS standards by a disk diffusion method (14), showed resistance to broad-spectrum cephalosporins for only six isolates throughout the whole period. Five of these (S76, S78, S79, S86, and S88) belonged to phage type PT4, while the lysis pattern of the sixth (S87) did not conform to a standard type.

Five isolates (S78, S79, S86, S87, and S88) were resistant to ampicillin, ceftazidime, cefotaxime, and aztreonam. They were also positive in the double-disk synergy test (DDST) (10), indicating production of an extended-spectrum β-lactamase (ESBL). The sixth isolate, S76, was resistant to cefoxitin and amoxicillin-clavulanate but negative in the DDST, thus exhibiting a class C β-lactamase phenotype (Table 1).

It was possible to transfer β-lactam resistance from the five DDST-positive isolates to *Escherichia coli* by conjuration (19), along with all other resistance markers (Table 1).

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described so far. The integrons were located on the \textit{bla}_{SHV-12}^{\text{TEM}}-lactamase-carrying plasmids, as demonstrated by Southern blot hybridization on plasmid DNA, using an \textit{intI1}-specific probe (Fig. 2D). The three remaining isolates were negative for the presence of class 1 integrons by both PCR and hybridization with \textit{intI1} probe.

Molecular typing by pulsed-field gel electrophoresis (PFGE) of XbaI-restricted genomic DNA (8) showed that all clinical strains carrying the \textit{bla}_{SHV-12}^{\text{TEM}} gene were highly related at the chromosomal level. Their PFGE patterns differed by three to four DNA fragments, classifying them in five subtypes (data not shown). Nevertheless, isolates S78 and S79 had been identified in Sicily 2 years apart and were epidemiologically unrelated. Isolates S86, S87, and S88, however, had been recovered from patients in three gastroenteritis cases that had occurred in Catanzaro, Calabria, during a very brief interval of time. Given the similarity of their PFGE profiles, these isolates may represent a clonal outbreak, though clinical records did not indicate any epidemiological association. The cephalosporinase-producing S76 isolate exhibited a distinct PFGE type.

The present findings constitute further evidence regarding the increasing frequency of isolation of cephalosporin-resistant strains among epidemiologically important \textit{Salmonella} serotypes. Most other studies so far have focused on \textit{S. enterica} serotype Typhimurium strains that had acquired plasmids encoding various ESBL types such as TEM, SHV, CTX-M, and PER (20, 22). Recently, serotype Typhimurium strains producing cephalosporinases similar to the chromosomal enzymes of \textit{C. freundii} have also been reported in the United States (5, 23). However, \beta\text{-lactamase-mediated resistance to newer cephalosporins is much more rare in serotypes other than Typhimurium. Class A ESBLs have also been encountered in \textit{K. pneumoniae} isolates from hospitals throughout Italy (11, 15). A similar hypothesis could also be formulated for isolate S76, which produced a class C \beta\text{-lactamase, since enterobacterial clinical isolates with plasmid-mediated cephalosporinases have been repeatedly reported in European countries and the United States (16, 23).

Production of newer cephalosporin-hydrolyzing \beta\text{-lactamases by strains belonging to a predominant phage type of serotype Enteritidis is a disturbing development. Further dissemination of such strains may drastically reduce therapeutic options for severe salmonella infections in children. In addi-
FIG. 2. Restriction patterns of plasmids isolated (Concert Purification Midi kit; Life Technologies, Milan, Italy) from five E. coli transconjugant clones and serotype Enteritidis isolate S76. (A) Plasmid fragments were separated by electrophoresis on 0.8% agarose gels and transferred onto positively charged nylon membranes (Boehringer-Mannheim GmbH, Mannheim, Germany). Molecular weight markers (1-kb ladder) are in lane M. (B to D) Southern blot hybridization was performed according to standard protocols (19) with positively charged nylon membranes (Boehringer-Mannheim GmbH, Mannheim, Germany). Molecular weight markers (1-kb ladder) are in lane M. (B to D) Southern blot hybridization was performed according to standard protocols (19) with positively charged nylon membranes (Boehringer-Mannheim GmbH, Mannheim, Germany). Molecular weight markers (1-kb ladder) are in lane M.

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