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Salmonella enterica Serovar Virchow with CTX-M-Like β-Lactamase in Spain

ENCARNA SIMARRO,[1] FERRÁN NAVARRO,[2] JOAQUÍN RUIZ,[1]*
ELISENDA MIRÓ,[2] JOAQUÍN GÓMEZ,[3]
AND BEATRIZ MIRELIS[2]

Departamentos de Microbiología[1] y de Infecciosas,[3] del Hospital Virgen de Arrixaca, 30120 Murcia, and Departament de Microbiologia, Hospital de la Santa Creu i Sant Pau, Universitat Autònoma, 08025 Barcelona,[2] Spain

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Four *Salmonella enterica* serovar Virchow strains resistant to broad-spectrum cephalosporins were isolated from patients with gastroenteritis in 1997 and 1998 in Murcia and Barcelona, Spain. The isolates expressed a β-lactamase with a pI of about 8 and a positive PCR when specific primers for CTX-M-9 were used. These results suggest the presence of a CTX-M-9 β-lactamase in these strains.

Extended-spectrum β-lactamases (ESBLs) were first described in *Klebsiella pneumoniae* about 20 years ago (13) and now have been isolated in several species throughout the world. However, in *Salmonella* ESBLs have been detected only recently, mainly in the serovar Typhimurium.

Most ESBLs are derivatives of TEM-1, TEM-2, or SHV-1 enzymes, but there is an increasing number of reports of β-lactamases belonging to other families, like OXA or CTX-M, although their frequencies are still low.

This paper describes four strains of ESBL-producing *Salmonella enterica* serovar Virchow isolated in our laboratories in Murcia and Barcelona in 1997 and 1998. The β-lactamase belongs to the CTX-M family.

The four strains were isolated from stool samples of four patients with gastroenteritis. Three of these patients were seen at the Hospital Virgen de Arrixaca in Murcia, while the fourth was assisted at the Hospital de la Santa Creu i Sant Pau in Barcelona. Two of them were children 1 and 3 years old. One of these children required hospitalization. The other two patients were adults and were treated in the community. None of the four patients were apparently related; they lived in different towns and were seen on widely separate dates.

The isolates were identified biochemically by the API 20 identification system (bioMérieux S.A., Marcy-l'Etoile, France). The serogroup was determined in our laboratories and the serotype and phage type were determined in the Servicio de Enterobacterias del Centro Nacional de Microbiología, Instituto Carlos III, Majadahonda, Madrid, Spain. All four strains were identified as *S. enterica* serovar Virchow phage type 19. The epidemiological relationship of the three isolates from Murcia (isolates 91, 95, and 163) was investigated by pulsed-field gel electrophoresis (20) using the enzyme *Xba*I. The restriction patterns were identical except for one band of difference in one strain (Fig. 1). Although it is possible that a single strain had been responsible for all the cases, we do not know how discriminatory this technique is for *S. enterica* serovar Virchow.

Susceptibility was determined by disk diffusion following the National Committee for Clinical Laboratory Standards recommendations (16). MICs were determined by E-test (AB Biodisk, Solna, Sweden) according to the manufacturer's recommendations. *Escherichia coli* ATCC 25922 and *E. coli* ATCC 35218 were used as control strains for susceptibility studies.

The four isolates were resistant to ampicillin, trimethoprim-sulfamethoxazole, tetracycline, and streptomycin, and two isolates were resistant to kanamycin. All four strains were susceptible to gentamicin, tobramycin, quinolones, and chloramphenicol. The presence of an extended-spectrum β-lactamase was suggested by the synergies detected between clavulanic acid and cefotaxime, ceftazidime, or aztreonam in a disk-diffusion assay with a typical deformation of the inhibition zone, as well as by a decreased susceptibility to third-generation cephalosporins. The MICs of the β-lactams are shown in Table 1.

The β-lactamase extracts and isoelectric focusing were performed as described by Barthélémy et al. (1), with a pH gradient from 4 to 11 (Servalyt 4-9 T, 9-11 T; Serva, Heidelberg, Germany). The enzyme activities were revealed by the iodometric method. The acrylamide gel was overlaid with agar gel containing iodine and ceftriaxone in order to detect activity against ceftriaxone. Afterward, a gel containing iodine with penicillin G was applied to detect those β-lactamases without ceftriaxone activity. The four isolates produced two β-lactamases, one with an isoelectric point (pI) of 5.4 and the other with a pI of about 8. The latter β-lactamase showed activity against ceftriaxone, whereas the pI-5.4 β-lactamase did not reveal any activity. From the total DNA obtained directly from a colony of each of these four strains, a positive PCR was obtained when a pair of specific primers for TEM-type β-lactamases were used as previously described (19). These results suggest that the pI-5.4 β-lactamase is consistent with TEM-1.

The fact that the strains were more resistant to cefotaxime than to ceftazidime, the presence of a β-lactamase with a pI of about 8, and negative PCR results when specific primers for SHV-type β-lactamases were used (17) suggested the existence of a β-lactamase belonging to the recently reported CTX-M type and more probably the CTX-M-9 type (18). To confirm this possibility, a PCR assay was performed using the primers 5′-GTG ACA AAG AGA GTG CAA

* Corresponding author. Mailing address: Departamentos de Microbiologia, Hospital Virgen de Arrixaca, Jaime I 1, 3 izda, E-3008, Murcia, Spain. Phone: 34-68-369789. Fax: 34-68-369678. E-mail:microbiologia@ctv.es.

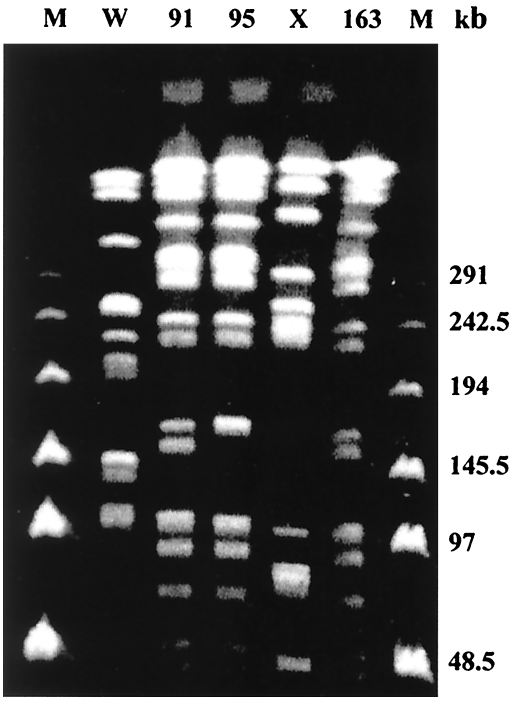

FIG. 1. Restriction pattern by pulsed-field electrophoresis of *S. enterica* serovar Virchow strains 91, 95, and 163. W, *E. coli*; X, *S. enterica* serovar Enteritidis; M, marker.

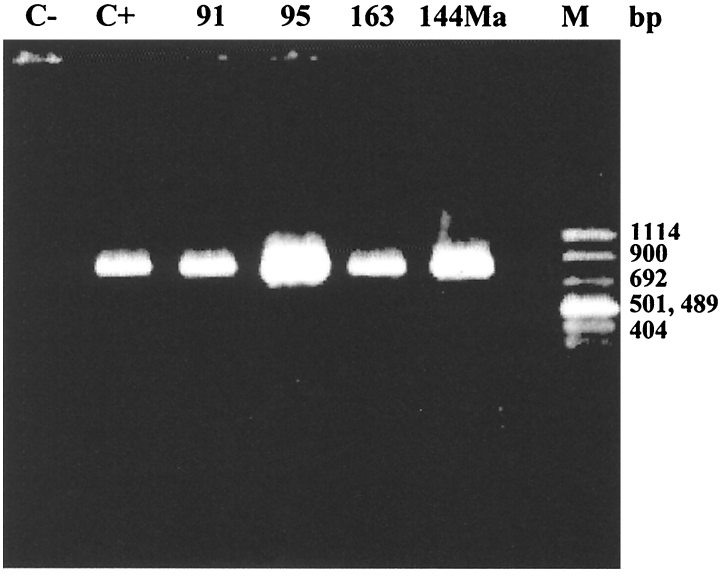

FIG. 2. PCR products of the four *S. enterica* serovar Virchow strains (91, 95, 163, and 144Ma) by using CTX-M-9 primers (C− and C+). M, marker.

CGG-3′ and 5′-ATG ATT CTC GCC GCT GAA GCC-3′, which comprise the positions 4 to 24 and 860 to 840, respectively, with respect to the CTX-M-9 translational starting point (GenBank accession number AF174129). PCR was performed with the four isolates, and an 856-bp fragment was obtained (Fig. 2).

The transconjugants MSP498, MSP499, MSP500, and MSP501 were obtained for the four isolates, 91, 95, 163, and 144Ma, respectively, presenting a similar resistance pattern (Table 1) and expressing β-lactamases with pIs of 5.4 and about 8, similar to the donors.

CTX-M-type β-lactamase-producing strains have been identified incidentally in different geographical areas: CTX-M-1 in Germany in 1989 (4); MEN-1 in France from an Italian patient in 1989 (2, 6); CTX-M-2 in Argentina in 1990 (3, 5); CTX-M-3 in Poland in 1996 (11); CTX-M-4 in Russia in 1996 (8, 10); CTX-M-5 in Latvia in 1991 (7); CTX-M-5 in Greece in 1996 (9, 20); CTX-M-6 in Greece in 1997 (9, 20); Toho-1 (1993) and Toho-2 (1995) in Japan (12, 14); CTX-M-7 in Brazil (GenBank accession number AF189721); and CTX-M-9 in Barcelona (18). In addition to these initial findings, the CTX-M-type β-lactamases have been described in several species of the *Enterobacteriaceae* family in widely differing geographical areas, with all of the β-lactamases being plasmid encoded. The divergence of the amino acid sequences (between 70 and 98% sequence homology), as well as the temporal and geographical dispersion of strains carrying this β-lactamase, makes any assumption about the origin of this plasmid-mediated β-lactamase difficult.

Unlike the other CTX-M-type β-lactamases described to date in *Salmonella*, including CTX-M-2 (3, 5), CTX-M-4 (8, 10), CTX-M-5 (7), CTX-M-5 (9, 21), and CTX-M-6 (9, 21), which all present high resistances to cefotaxime, our isolates require much more moderate MICs (between 4 and 8 μg/ml).

The first and, to date, the only report of an extended-spectrum β-lactamase of *Salmonella* in Spain was that described by Morosini et al. (15). It was the TEM-27 β-lactamase and was isolated in a nosocomial outbreak produced by *S. enterica* se-

TABLE 1. MICs of β-lactams for *S. enterica* serovar Virchow strains 91, 95, 163, and 144Ma and the respective transconjugants (MSP498, MSP499, MSP500, and MSP501)

β-lactam agent	MIC (μg/ml) required							
	91	MSP498	95	MSP499	163	MSP500	144Ma	MSP501
Amoxicillin-clavulanic acid (2:1)	4	1.5	4	2	4	2	4	2
Cefuroxime	>256	48	>256	>256	>256	48	>256	128
Cefotaxime	4	1	8	4	4	1	6	1.5
Cefotaxime-clavulanic acid (2:1)	0.023	≤0.016	0.023	≤0.016	0.032	≤0.016	0.023	≤0.016
Ceftazidime	1	0.094	1	0.19	1	0.064	0.75	0.125
Ceftazidime-clavulanic acid (2:1)	≤0.064	≤0.064	≤0.064	≤0.064	≤0.064	≤0.064	≤0.064	≤0.064
Cefepime	2	0.094	2	0.5	1	0.094	1	0.19
Aztreonam	0.5	0.032	1	0.5	0.5	0.047	1	0.125

rovar Othmarschen. Our isolates represent the first description of a β-lactamase of the CTX-M family in salmonellae in Spain and also the first description of an ESBL in *S. enterica* serovar Virchow.

The establishment and spread of *S. enterica* serovar Virchow organisms resistant to therapeutically important broad-spectrum β-lactams are cause for concern.

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