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Early Dissemination of CTX-M-Derived Enzymes in South America

In a recent publication by Bonnet et al. regarding a new CTX-M-derived enzyme, a short history of this group of enzymes is provided, stating that "initially found in Europe, microorganisms producing these enzymes have now been observed over a wide geographic area. . ." (3). We want to point out that although the description of the first members of enzymes was achieved in Europe, they had been widely distributed in South America at least since 1989. Three different groups of investigators reported, in a national meeting, the explosive dissemination of extended-spectrum cephalosporin-resistant salmonella, initially in neonatology units in La Plata and Buenos Aires city hospitals (A. Picandet, S. Giugno, M. I. Caffer, and G. Schembri, Abstr. II Congreso Internacional de SADEBAC, Antimicrobianos '90, abstr. A-16, 1990; H. Lopardo, M. I. Caffer, N. Fernandez, et al., Abstr. II Congreso Internacional de SADEBAC, Antimicrobianos '90, abstr. A-15, 1990; E. Maiorini, A. Procopio, S. Furmanski, et al., Abstr. II Congreso Internacional de SADEBAC, Antimicrobianos '90, abstr. A-17, 1990). An outbreak in a single hospital was presented at the 17th Congress of Chemotherapy, Berlin, 1991 (H. Lopardo, N. Fernandez, M. Fernandez Cobo, et al., abstr. 2088, 1991).

The presence of a new enzyme in the implicated microorganisms was pointed out in a communication to the 1992 ASM General Meeting (A. Rossi, M. Woloj, G. Gutkind, et al., Abstr. 92nd Gen. Meet., abstr. A-135, 1992), and a brief historic perspective of their dissemination was reported in a paper that finally appeared in 1995 (7). In that paper, information relevant to the initial isolates, dissemination of this resistance marker within different salmonella serovars, and some biochemical characteristics of the enzymes present was displayed. In brief, they made their appearance in a really explosive way, initially in a single hospital in La Plata, from there to neonatology units in pediatric hospitals in Buenos Aires, and from them it was disseminated to Paraguay, Misiones (in northern Argentina), and Uruguay. However, formal sequencing of the structural gene of the enzyme responsible for this outbreak (and naming of the enzyme) was achieved by a more efficient collaboration of Bauernfeind and his group with Casellas and his group (1–2).

Since then, its presence has been suggested or demonstrated in different microorganisms, such as *Escherichia coli* (M. Radice, A. Rossi, M. Venuta, H. Lopardo, and G. Gutkind, XVI Congreso Chileno de Microbiologia, p. 31, 1994; (5a), *Shigella sonnei* (5), and *Proteus mirabilis* (M. Quinteros, M. Mollerach, M. Radice, et al., Abstr. 39th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 893, 1999; M. Radice, M. Quinteros, M. Matteo, et al., Abstr. 9th Int. Congr. Infect. Dis., abstr. 16028, 2000) (as typical non-AmpC producers), *Morganella morganii* (reference 44 and P. Power, M. Radice, C. Barberis, et al., Abstr. 98th Gen. Meet. Am. Soc. Microbiol. 1998, abstr. V-125, 1998), *Citrobacter freundii*, *Serratia marcescens*, and *Enterobacter aerogenes*, among other "AmpC-inducible" enterobacteria (M. Quinteros, M. Radice, P. Power, et al., Abstr. II Int. Congr. β-Lactamases, abstr. A-27, 1999), *Vibrio cholerae* (reference 6 and M. Galas, A. Petroni, R. Melano, et al., Abstr. 38th Intersci. Conf. Antimicrob. Agents Chemother., abstr. C-174, 1998), and *Aeromonas hydrophyla* (M. Quinteros, M. Radice, P. Power, et al., Abstr. 9th Int. Congr. Infect. Dis., abstr. 15884, 2000).

A discussion on the ability of different susceptibility testing systems has been presented also (M. Quinteros, M. Matteo, P. Power, et al., Abstr. VIII Congreso Argentino de Microbiologia, abstr. A-44, 1998; M. Quinteros, M. Mollerach, M. Radice, et al., Abstr. 39th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 893, 1999; S. Kauffman, M. Quinteros, M. Radice, et al., Abstr. 9th Int. Congr. Infect. Dis., abstr. 15883, 16028, and 16031, 2000). A more straightforward recommendation was presented almost a decade ago by the Subcomisión de Antimicrobianos, Sociedad Argentina de Bacteriologia Clinica, Asociación Argentina de Microbiología, for changing on a national scale the interpretation of NCCLS breakpoints (while keeping all the other NCCLS method recommendations), stating that any enterobacteria (except for those in which AmpC is typically inducible) with inhibition zones lower than 26 mm around 30-μg cefotaxime disks or with a MIC higher than 1 mg/liter should be considered as potentially resistant to all extended-spectrum cephalosporins. It would be interesting to learn if this new CTX-M-derived enzymes explosion would have been so easily detected if NCCLS recommendations had not moved the breakpoint to 27 mm.

Being able to detect it does not prevent its dissemination or allows its control: CTX-M-2 is present in almost 75% of the extended-spectrum β-lactamase (ESBL)-producing enterobacteria submitted to our lab, coincident to an epidemiological study in Buenos Aires hospitals through a network of microbiological laboratories (Quinteros et al., unpublished data) being PER-2 (another nonclassical enzyme), the second most prevalent ESBL. Why are these enzymes and not the classical TEM- or SHV-derived enzymes (or any other classical family) are the most prevalent ESBLs in our region, as A. Medeiros has been asking Argentinean microbiologists in each of his visits? There is no formal answer to date.

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Authors' Reply

We recently published a paper in this journal on the structure and properties of a novel extended-spectrum β-lactamase, CTX-M-16 (7). The enzyme differs from CTX-M-9 by a single amino acid substitution, Asp-240→Gly. The most interesting feature was the increased hydrolytic activity of the novel enzyme for ceftazidime. This finding shows the evolutionary potential of the spectrum of CTX-M activity.

In our article (7), the aim was not to trace the history of the dissemination of CTX-M enzymes; however, in the introduction we did mention that "initially found in Europe, CTX-M-producing strains have now been observed over a wide geographic area." G. Gutkind and coworkers have contested the phrase "initially found in Europe" and state that CTX-M β-lactamases "had been widely distributed in South America at least since 1989" and possibly before appearing in Europe.

The CTX-M-1 (2) and MEN-1 (1) β-lactamases, which were the first fully characterized CTX-M reported, were initially detected from strains isolated in Europe. The first came from Germany and the second was isolated in France from a patient who was an Italian national.

At an International Congress on Infectious Diseases meeting held in Montreal in 1990, one of us (R. Labia) met A. Bauernfeind, who was presenting a poster about an enzyme which was then named CTX-M-1 (A. Bauernfeind, S. Schweighart, and H. Grimm, Int. Congr. Infect. Dis., abstr. 570, p. 17, 1990), and they discussed the similarity of their enzymes, CTX-M-1 and MEN-1, which they were studing. They exchanged strains and observed that the enzymes were identical. One sequence was published in 1992 (1) and the other was published in 1996 (4), but both strains had been isolated at the beginning of 1989 (5). In 1992, Bauernfeind et al. published a preliminary paper on a CTX-M-2-producing *Salmonella enterica* serovar Typhimurium strain (pI 7.9) isolated "in the beginning of August 1990" in Argentina without further details of its origin (3). In 1995, Rossi and coworkers described cefotaxime-resistant *Salmonella* spp. strains isolated in Argentina in 1991 (17). They described unidentified β-lactamases of alkaline pI (7.4, 8.1) which hydrolyze cefotaxime. These enzymes could be CTX-M extended-spectrum β-lactamases (ESBLs), and it would be interesting to characterize their β-lactamase-encoding genes.

In Japan, Matsumoto et al. had already described an FEC-1 enzyme in 1988, which induced higher resistance levels to cefotaxime than to ceftazidime (15). The enzyme had a pI and kinetic constants similar to those of CTX-M ESBLs. In our opinion, this work may have been the first report of a CTX-M-type enzyme. Currently, Japan is a large reservoir of CTX-M enzymes (13, 14, 19), as are South America 6; M. Galas, F. Pasteran, R. Melano, A. Petroni, G. Lopez, A. Corso, A. Rossi, and WHONET collaborative group, Abstr. 38th Intersci. Conf. Antimicrob. Agents Chemother., abstr. E-109, p. 201, 1998) and Eastern Europe (8–12, 16, 18).

A considerable delay is usually observed between the isolation of a strain and the publication of its characteristics. Thus, we agree that CTX-M β-lactamases may well have been present in Argentina in 1989, or even before, and were quite likely present in other countries as well.

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