ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Feb. 2005, p. 866–867 Vol. 49, No. 2
0066-4804/05/$08.00+0 doi:10.1128/AAC.49.2.866–867.2005

Escherichia coli Producing an ACC-1 Class C β-Lactamase Isolated in Barcelona, Spain

Class C β-lactamases, primarily chromosomally encoded (AmpC), were first described to occur in a plasmid in 1988. Since then, these plasmid-mediated β-lactamases have been identified throughout the world. In the Mediterranean area, most belong to a homogeneous group, CMY-2 to CMY-5 and LAT-1 to LAT-4, related to the chromosomally encoded AmpC-type β-lactamase of *Citrobacter freundii* (9).

In Spain, the first plasmidic AmpC enzyme described was FOX-4, found in an *Escherichia coli* strain isolated in the Canary Islands in 2000 (2). A CMY-2 enzyme was later detected in *Proteus mirabilis*, *Klebsiella oxytoca*, and *Salmonella enterica* serovars Mikawasima and Montevideo in Barcelona (Catalonia) and Guipuzkoa (Basque Country) (7). To date, no other *ampC* genes have been described in Spain.

We report the first plasmid-encoded ACC-1 β-lactamase, related to the chromosomally encoded AmpC-type β-lactamase of *Hafnia alvei* (5), isolated in Barcelona from an *E. coli* strain.

The *E. coli* strain (U49G11) was isolated from the urine of a 55-year-old female with Hodgkin's disease treated with chemotherapy and allogeneic peripheral stem cell transplantation. During her illness, she presented with various infections, including oral candidiasis, human herpesvirus 1 infection, disseminated human herpesvirus 5 (cytomegalovirus) infection, urinary sepsis due to *E. coli*, and, 1 month later, a *Klebsiella pneumoniae* bacteremia of unknown origin. Several antimicrobial agents were administered, including meropenem, cefepime, and cefuroxime as β-lactams. Nevertheless, during the month prior to the isolation of the U49G11 strain, she did not receive antimicrobial therapy.

U49G11 showed a particular β-lactam-resistant phenotype, which suggested the presence of an AmpC-type enzyme. Using the disk diffusion test according to NCCLS guidelines (6), this strain was resistant to ampicillin, cefazolin, cefuroxime, cefotaxime, ceftazidime, amoxicillin-clavulanate, kanamycin, tobramycin, tetracycline, and trimethoprim-sulfamethoxazole and was susceptible to cefoxitin, cefepime, aztreonam, imipenem, gentamicin, quinolones, chloramphenicol, and nitrofurantoine. The MICs of cefoxitin, cefotaxime, ceftazidime, and cefepime, determined by Etest (AB Biodisk, Solna, Sweden), were 8, >16, >32, and 0.75 mg/liter, respectively. When clavulanate was associated with these cephalosporins, no synergistic effect was observed. The resistance pattern was successfully transferred by conjugation (frequency of 10^{-6}) on solid media using *E. coli* HB101 (Nal^r) as the recipient strain (7). Transconjugants have the same resistance pattern (although MICs of cephalosporins for them are lower) as that of the donors except where trimethoprim-sulfamethoxazole is concerned.

The analytical isoelectric focusing (7) performed on the donor and transconjugant strains showed a band with a pI of 7.7, which, together with the susceptibility to cefoxitin, suggested the presence of an ACC-type enzyme. A positive PCR was obtained by using the ACCMF and ACCMR primers, which amplify ACC-type enzymes (8). In order to sequence the gene, an additional PCR was performed using the ACC-1up (5′-TGC GTA AAA AAA TGC AGA A-3′) and ACC-1dn (5′-CTA CTT ATT CCC TTC CA-3′) primers. The deduced amino acid sequence of the obtained product showed 100% homology with the ACC-1 enzyme (1).

ACC-1 was first described in Germany (1) and later in France during a multiresistant-*K. pneumoniae* outbreak in an intensive care unit following the admission of a patient transferred from Tunisia (5); it has also been shown to be present in *E. coli* and *P. mirabilis* (3). Studies at Sfax Hospital (Tunisia) reveled the ACC-1 enzyme in *K. pneumoniae*, *P. mirabilis*, and *S. enterica* serovars Livingstone and Mbandaka (4, 10).

This is the first time that the AAC-1 enzyme has been reported in Spain, now the fourth country in the world where strains carrying this enzyme have been isolated. Three of these four countries (Spain, France, and Tunisia) are in the Mediterranean area. Such findings suggest that the ACC-1 enzyme is spreading in Europe, especially in the Mediterranean area.

REFERENCES

1. **Bauernfeind, A., I. Schneider, R. Jungwirth, H. Sahly, and U. Ullmann.** 1999. A novel type of AmpC β-lactamase, ACC-1, produced by a *Klebsiella pneumoniae* strain causing nosocomial pneumonia. Antimicrob. Agents Chemother. **43:**1924–1931.
2. **Bou, G., A. Oliver, M. Ojeda, C. Monzón, and J. Martínez-Beltrán.** 2000. Molecular characterization of FOX-4, a new AmpC-type plasmid-mediated β-lactamase from an *Escherichia coli* strain isolated in Spain. Antimicrob. Agents Chemother. **44:**2549–2553.
3. **Girlich, D., A. Karim, C. Spicq, and P. Nordmann.** 2000. Plasmid-mediated cephalosporinase ACC-1 in clinical isolates of *Proteus mirabilis* and *Escherichia coli.* Eur. J. Clin. Microbiol. Infect. Dis. **19:**893–895.
4. **Makanera, A., G. Arlet, V. Gautier, and M. Manai.** 2003. Molecular epidemiology and characterization of plasmid-encoded β-lactamases produced by Tunisian clinical isolates of *Salmonella enterica* serotype Mbandaka resistant to broad-spectrum cephalosporins. J. Clin. Microbiol. **41:**2940–2945.
5. **Nadjar, D., M. Rouveau, C. Verdet, J.-L. Donay, J.-L. Herrmann, P. H. Lagrange, A. Philippon, and G. Arlet.** 2000. Outbreak of *Klebsiella pneumoniae* producing transferable AmpC-type β-lactamase (ACC-1) originating from *Hafnia alvei.* FEMS Microbiol. Lett. **187:**35–40.
6. **National Committee for Clinical Laboratory Standards.** 2004. Performance standards for antimicrobial susceptibility testing. Document M100-S14, 14th informational supplement. NCCLS, Wayne, Pa.
7. **Navarro, F., E. Pérez-Trallero, J. M. Marimon, R. Aliaga, M. Gomariz, and B. Mirelis.** 2001. CMY-2-producing *Salmonella enterica*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Proteus mirabilis* and *Escherichia coli* strains isolated in Spain (October 1999–December 2000). J. Antimicrob. Chemother. **48:**383–389.
8. **Pérez-Pérez, F. J., and N. D. Hanson.** 2002. Detection of plasmid-mediated AmpC β-lactamase genes in clinical isolates by using multiplex PCR. J. Clin. Microbiol. **40:**2153–2162.
9. **Philippon, A., G. Arlet, and G. A. Jacoby.** 2002. Plasmid-determined AmpC-

type β-lactamases. Antimicrob. Agents Chemother. **46:**1–11.
10. **Rhimi-Mahjoubi, F., M. Bernier, G. Arlet, Z. B. Jemaa, P. Jouve, A. Hammami, and A. Philippon.** 2002. Mise en évidence de la céphalosporinase plasmidique ACC-1 dans différentes entérobactéries (*Klebsiella pneumoniae*, *Proteus mirabilis*, *Salmonella*) isolées dans un Hôpital Tunisien (Sfax 1997–2000). Pathol. Biol. **50:**7–11.

Elisenda Miró
Beatriz Mirelis
Ferran Navarro*
Servei de Microbiologia
Hospital de la Santa Creu i Sant Pau
Universitat Autònoma de Barcelona
Av. Sant Antoni M. Claret, 167
08025 Barcelona, Spain

Lurdes Matas
Montserrat Giménez
Cinta Rabaza
Servei de Microbiologia
Hospital Germans Trias i Pujol
Universitat Autònoma de Barcelona
Badalona, Spain

*Phone: 34 932 919 071
Fax: 34 932 919 070
E-mail: fnavarror@hsp.santpau.es