drug administration. Recurrence of bilateral pleural effusion was evident on chest radiography. He died of respiratory failure secondary to intractable lymphoma-related pleural effusions 1 month following diagnosis of PEL.

To our knowledge, this is the first reported case of pulmonary hypertension secondary to paclitaxel administration for PEL with pleural and pericardial involvement in an HIV-infected patient. Further clinical data on treatment of PEL are needed, as well as a better understanding of the pathogenesis of this rare condition.

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# Prevalence of extended-spectrum β-lactamases in group-1 β-lactamase-producing isolates

*C. Varela, A. Oliver, T. M. Coque, F. Baquero and R. Cantón**

Servicio de Microbiología, Hospital Ramón y Cajal, 28034-Madrid, Spain

*Tel: +34 91 3368330 Fax: +34 91 3368809 E-mail: rcanton@hrc.insalud.es

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Resistance to extended-spectrum cephalosporins and aztreonam emerges in *Enterobacter* spp., *Citrobacter freundii, Serratia* spp., *Morganella morganii* and *Pseudomonas aeruginosa* due to a mutation in a chromosomal gene that normally prevents high-level expression of this organism's chromosomal β-lactamase [1]. This mutation results in high-level production of the chromosomal Bush Group 1 β-lactamase (AmpC). A different mechanism of resistance to extended-spectrum cephalosporins has also been recognized in these species. This involves the acquisition of plasmid encoding extended-spectrum β-lactamases (ESBLs). These enzymes, which have been mainly described in *Escherichia coli* and *Klebsiella pneumoniae*, usually derive from the classical TEM-1, TEM-2 and SHV-1 β-lactamases [1,2].

The occurrence of ESBLs in Enterobacteriaceae that possess group 1 β-lactamase is increasingly reported worldwide, particularly in *Enterobacter* spp. [3–8]. In these species, the detection of ESBL-producing isolates by methods based on the inhibitory effects of clavulanic acid could be difficult, because of the superimposed resistance phenotype due to ESBLs and AmpC hyperproduction, and it is dependent on the level of chromosomal enzyme production [9]. From a clinical point of view, the discrimination between ESBL and overproduced group 1 β-lactamases in these species may not be critical. Nevertheless, the detection of such 'hidden' ESBLs is still of epidemiologic importance for the hospital environment, because it implies the possibility of plasmid transmission among different isolates in addition to patient-to-patient transmission of the ESBL-producing strains.

In order to investigate the prevalence of ESBLs among group 1 β-lactamase-producing isolates, 277 Enterobacteriaceae strains (107 *Enterobacter cloacae,* 34 *Enterobacter aerogenes,* 51 *Serratia* spp., 26 *C. freundii*, 54 *M. morganii* and five *Providencia stuartii*) and 193 *P. aeruginosa* strains were consecutively and prospectively collected during a 6-month period in a Spanish

teaching hospital (January to June, 1999). Only one isolate per patient and bacterial species was selected, to avoid repetition of strains. Preliminary identification and antimicrobial susceptibility testing were performed by using the semi-automated microdilution Wider System (Francisco Soria Melguizo, SA Madrid, Spain) [10]. Moreover, the NCCLS agar dilution method [11] was used to determine the MIC values for different $\beta$-lactam antibiotics. The inoculum effect on antibiotic MICs was determined using a $10^3$ CFU/mL inoculum (low inoculum) and a $10^7$ CFU/mL inoculum (high inoculum). The corresponding manufacturers provided all antibiotics as powders. The double disk synergy (DDS) test was performed to assess ESBL expression [12]. The strains that showed synergy between oximino-cephalosporins or aztreonam and clavulanic acid (DDS-positive test) were considered to produce ESBL enzymes. Induction of the AmpC $\beta$-lactamase was also determined by a double disk test [13]. Conjugation experiments were performed with nalidixic acid-resistant *E. coli* BM21 strain as the recipient. Transconjugants were selected on MacConkey agar (Difco, Detroit, MI, USA) containing nalidixic acid (64 mg/L) and cefotaxime (2 mg/L). ESBL production in the transconjugants was confirmed with the DDS test. Isoelectric focusing was performed by electrophoresis of ultrasonic cell extracts on polyacrylamide gels containing ampholytes with pHs that ranged from 3 to 9 in a PhastSystem apparatus (Pharmacia Biotech, Uppsala, Sweden) [14]. $\beta$-Lactamases with known pIs (TEM-1, 5.4; TEM-4, 5.9; TEM-3, 6.3; SHV-2, 7.6; SHV-4, 7.8; CTX-M-10, 8.1; SHV-5, 8.2) were focused in parallel as controls. Gels were stained with a 0.2 mg/mL nitrocefin solution (Oxoid, Basingstoke, UK) to identify the $\beta$-lactamase bands. ESBL *bla* genes were amplified by using specific primers for $bla_{\mathrm{TEM}}$ [15] and $bla_{\mathrm{SHV}}$ [16]. In addition, forward primer 5′-GCT GAT-GAG CGC TTT GCG-3′ (nucleotide positions 193–210 of $bla_{\mathrm{CTX\text{-}M\text{-}1}}$) and reverse primer 5′-TTA CAA ACC GTT GGT-GAC G-3′ (last 19 nucleotides of $bla_{\mathrm{CTX\text{-}M\text{-}1}}$, including the stop codon) specific to CTX-M-1 and related enzymes (CTX-M-3 and CTX-M-10) were used [5,17,18].

Eighteen per cent (51 of 277) of Enterobacteriaceae and 15.5% (30 of 193) of *P. aeruginosa* isolates were resistant to third-generation cephalosporins. The highest values were observed among *Enterobacter* spp. (24.1%) and *C. freundii* (23.0%) isolates. Only three *E. cloacae* isolates (2.1% of all *Enterobacter* isolates tested) and one *C. freundii* (3.8%) isolate showed a DDS-positive test and were considered to produce ESBL enzymes. None of the *Serratia* spp., *M. morganii*, *Providencia* spp. and *P. aeruginosa* isolates displayed a DDS-positive test. It is remarkable that ceftazidime–clavulanate synergy, commonly used in commercial systems to detect ESBL-producing strains, was less suitable for detecting these $\beta$-lactamases, as a lower DDS-positive result was observed with this cephalosporin. In contrast, cefotaxime–clavulanate and cefepime–clavulanate synergies were more useful for detecting ESBLs among group 1 $\beta$-lactamase producers (Figure 1). The greater efficacy of these cephalosporins compared to ceftazidime could be related to the specific ESBL

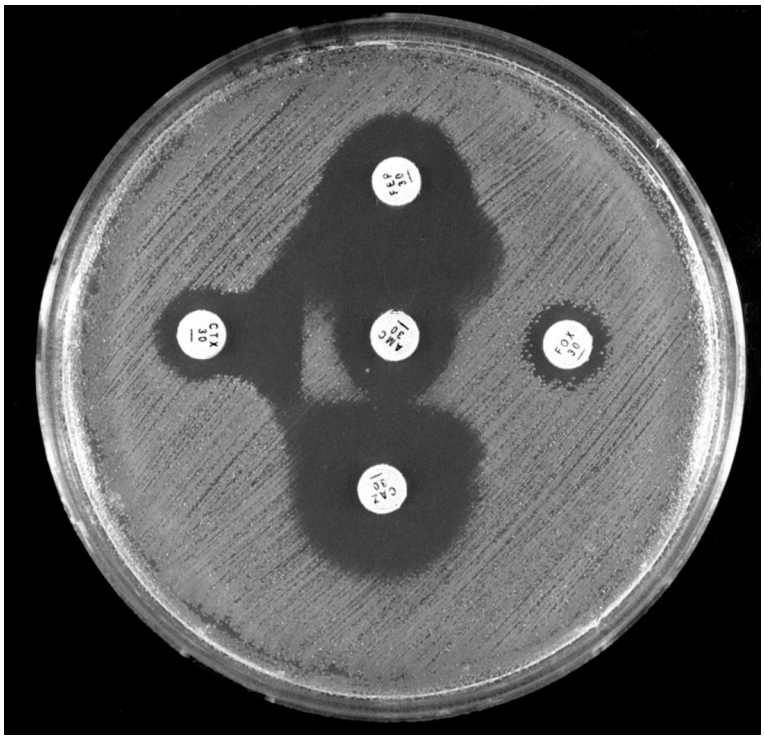


**Figure 1** Double disk synergy test of an ESBL-producing *Enterobacter cloacae* isolate (top, cefepime; right, cefoxitin; bottom, ceftazidime; left, cefotaxime; centre, amoxicillin/clavulanate).

**Table 1** MICs of $\beta$-lactam and non-$\beta$-lactam antibiotics against ESBL-producing isolates and their corresponding transconjugants, and the effect of different inoculum size on MIC values of several $\beta$-lactam antibiotics

| | MIC (mg/L) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Antibiotic (inoculum size, CFU/mL)** | ***E. cloacae* 99043823** | ***E. coli* TC-99043823** | ***E. cloacae* 99074125** | ***E. coli* TC-99074125** | ***E. cloacae* 99071078-1** | ***E. coli* TC-99071078-1** | ***C. freundii* 99071078-2** | ***E. coli* TC-99071078-2** |
| Amoxicillin ($10^5$) | >128 | >128 | >128 | >128 | >128 | >128 | >128 | >128 |
| Amoxicillin/clavulanate ($10^5$) | 64 | 8 | 32 | 4 | 32 | 16 | 64 | 8 |
| Ticarcillin ($10^5$) | >128 | >128 | >128 | >128 | >128 | >128 | >128 | >128 |
| Piperacillin ($10^5$) | 128 | 64 | >128 | >128 | >128 | >128 | >128 | >128 |
| Piperacillin/tazobactam | | | | | | | | |
| ($10^3$) | 0.5 | 2 | 2 | 2 | 4 | 2 | 2 | 2 |
| ($10^5$) | 0.5 | 4 | 2 | 4 | 4 | 4 | 2 | 2 |
| ($10^7$) | 2 | 8 | 4 | 8 | 8 | 8 | 4 | 8 |
| Cefuroxime ($10^5$) | 256 | >256 | >256 | >256 | >256 | >256 | >256 | >256 |
| Cefotaxime | | | | | | | | |
| ($10^3$) | 2 | 4 | 8 | 4 | 64 | 32 | 32 | 16 |
| ($10^5$) | 8 | 8 | 16 | 8 | 128 | 64 | 64 | 64 |
| ($10^7$) | >256 | >256 | 256 | 256 | >256 | >256 | >256 | >256 |
| Ceftazidime | | | | | | | | |
| ($10^3$) | 0.1 | 1 | 0.5 | 0.5 | 8 | 1 | 4 | 2 |
| ($10^5$) | 0.2 | 1 | 1 | 0.5 | 16 | 2 | 4 | 2 |
| ($10^7$) | 1 | 4 | 2 | 2 | 32 | 4 | 8 | 4 |
| Cefepime | | | | | | | | |
| ($10^3$) | 0.2 | 1 | 2 | 4 | 16 | 4 | 4 | 4 |
| ($10^5$) | 0.5 | 2 | 2 | 4 | 32 | 8 | 8 | 8 |
| ($10^7$) | >256 | >256 | 256 | >256 | >256 | >256 | 256 | >256 |
| Aztreonam | | | | | | | | |
| ($10^3$) | 0.2 | 4 | 2 | 4 | 16 | 16 | 8 | 8 |
| ($10^5$) | 0.5 | 4 | 4 | 4 | 32 | 32 | 16 | 16 |
| ($10^7$) | 32 | 128 | 128 | 128 | >256 | >256 | >256 | >256 |
| Imipenem ($10^5$) | 0.5 | 0.1 | 0.5 | 0.2 | 0.1 | 0.1 | 0.2 | 0.1 |
| Gentamicin ($10^5$) | ≤1 | ≤1 | ≤1 | ≤1 | ≤1 | ≤1 | ≤1 | ≤1 |
| Tobramycin ($10^5$) | ≤1 | ≤1 | ≤1 | ≤1 | ≤1 | ≤1 | ≤1 | ≤1 |
| Amikacin ($10^5$) | ≤4 | ≤4 | ≤4 | ≤4 | ≤4 | ≤4 | ≤4 | ≤4 |
| Co-trimoxazole ($10^5$) | ≤1/19 | ≤1/19 | ≤1/19 | ≤1/19 | ≤1/19 | ≤1/19 | ≤1/19 | ≤1/19 |

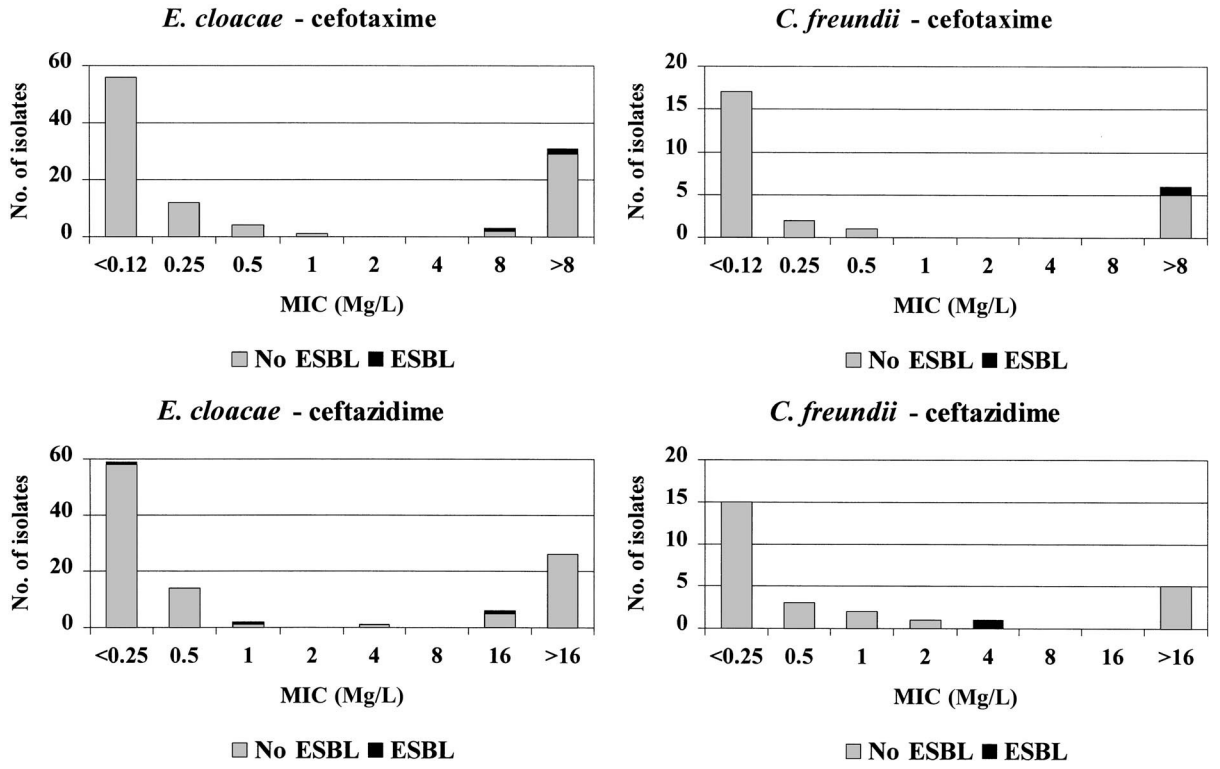


**Figure 2** Cefotaxime and ceftazidime MIC distributions against *Enterobacter cloacae* and *Citrobacter freundii* isolates.

produced. In fact, all ESBL-producing isolates were PCR positive for $bla_{\text{CTX-M}}$ and not for $bla_{\text{TEM}}$ or $bla_{\text{SHV}}$ genes. In addition, cefepime, a fourth-generation cephalosporin, in contrast to those of the third generation, retains activity against AmpC-derepressed Enterobacteriaceae isolates [1], and it could be more useful for detecting ESBL production in these isolates [8]. Ceftazidime was specifically less affected in all four isolates with ESBL enzymes. This was also observed with the transconjugant isolates and the inoculum effect experiments (Table 1). As previously noted [8], closer application of cefepime and clavulanate disks, 20–25 mm instead of 30 mm, enhanced the detection of ESBL-producing isolates, as a clear synergy inhibition zone was observed (Figure 1).

On the other hand, the disk induction approximation test demonstrated that all ESBL-producing isolates were inducible, and no AmpC hyperproduction was noted. It is interesting to note that, excluding all four ESBL-producing isolates, 11.7% of third-generation cephalosporin-resistant Enterobacteriaceae isolates still demonstrated a positive induction test. This discordance, which could reflect an AmpC partial depression or the absence of a major porin, has been extensively described in *P. aeruginosa* isolates [1].

It is worth noting that all ESBL-producing isolates have a transferable $\beta$-lactamase of pI 8.1. The ESBL gene was amplified by PCR using specific primers for $bla_{\text{CTX-M}}$ genes for the CTX-M-1 and related enzymes, which may correspond to the CTX-M-10 newly described by our group [17]. This CTX-M enzyme is widely distributed in our hospital, as nearly 50% of our ESBL-producing *E. coli* strains carried the same enzyme of pI 8.1 (data not shown). CTX-M ESBL is a heterogeneous family, which is endemic in South America and is now increasingly being recognized in Spain and other European countries [5,17–19].

All ESBL-producing isolates were resistant according to NCCLS criteria [11] to amoxicillin, amoxicillin–clavulanate, ticarcillin, piperacillin, cefuroxime, and cefotaxime, but susceptible to piperacillin–tazobactam (Table 1). In the absence of DDS and disk induction approximation tests, the latter combination could be essential to discriminate between AmpC hyperproduction and ESBL synthesis, as the former but not the latter commonly confers resistance to this combination [1]. Considering the ceftazidime MICs, it is remarkable that the *E. cloacae* ESBL-producing isolates fall completely into the ceftazidime NCCLS susceptible category (MIC 0.2–4 mg/L) (Figure 2) [11]. Following the NCCLS criteria, change to the resistance category is only considered when ESBLs are detected in *Klebsiella* spp. or *E. coli* isolates, but not when these enzymes are detected in other Enterobacteriaceae. However, this change, justified for the TEM- and SHV-type ESBL producers, could be unreliable for ceftazidime in the presence of CTX-M-type

enzymes. In fact, remarkable increases in cefepime, aztreonam and cefotaxime MICs were observed with the increase of the inoculum size in all wild-type isolates and the corresponding transconjugants, but were barely observed with ceftazidime (Table 1), as this antibiotic is a poor substrate for CTX-M enzymes [5,17–19]. As previously pointed out [17], the report of isolates harboring CTX-M β-lactamases as susceptible or resistant to ceftazidime can only be resolved with clinical observations.

Interestingly, and in contrast to the majority of ESBL-producing isolates [1,2], the association between aminoglycoside and/or co-trimoxazole resistance and ESBL production was not found (Table 1).

It is not surprising that none of the *P. aeruginosa* isolates produced ESBLs, as these enzymes are less frequently observed in these isolates than in Enterobacteriacaeae. To date, TEM-, SHV- and OXA-type ESBLs have been identified in *P. aeruginosa*, and most isolates have been recovered in Turkey [20]. On the other hand, the prevalence of ESBLs among group 1 β-lactamase-producing Enterobacteriaceae during the studied 6-month period in our hospital was low (1.4%). Nevertheless, this result represented 7.8% of all third-generation cephalosporin-resistant group 1 β-lactamase-producing Enterobacteriaceae. Considering the prevalence of ESBLs in *E. cloacae*, three of 107 isolates (2.8%), this value was similar to that previously observed in other studies [4,7] but lower than that found by Tzelepi et al [8], who recently observed 32% of *E. cloacae* strains with ESBLs over a 3-month period. The prevalence of these organisms in our hospital seems to have been quite stable during the last decade, as only 11 *E. cloacae* and three *E. aerogenes* isolates with ESBLs have been detected during this period. The possibility of clonal relatedness of all these isolates requires further study. Nevertheless, *E. cloacae* isolates were recovered from unrelated patients attending different units. From a clinical point of view, the discrimination between ESBLs and over-produced group 1 β-lactamases may not be critical, but it is important for epidemiologic purposes. The possibilities of plasmid transmission among different isolates and patient-to-patient transmission of ESBL-producing isolates support the continuous monitoring of these isolates. This approach could enhance the epidemiologic importance of detecting these enzymes among group 1 β-lactamase-producing isolates.

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