Endocarditis due to *Neisseria mucosa* after tongue piercing

H. Tronel[1], *H. Chaudemanche*[2], *N. Pechier*[3], *L. Doutrelant*[3] *and B. Hoen*[4,*]

[1]Laboratoire de Biologie, [2]Service de Médecine Interne, Center Hospitalier Louis Pasteur, 39108 Dole, France, [3]Service de Cardiologie and [4]Service de Maladies Infectieuses et Tropicales, University Medical Center, F-25030 Besançon Cedex, France

*Tel: +33 381 218533 Fax: +33 381 218551 E-mail: bruno.hoen@ufc-chu.Univ-fcomte.fr

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Non-gonococcal non-meningococcal *Neisseria* sp. are normal inhabitants of the upper respiratory tract in human beings. Although these bacteria are regarded as having low pathogenicity, *Neisseria mucosa* has been reported to cause serious infections such as endocarditis [1–5]. We report a case of infective endocarditis due to *N. mucosa* that probably resulted from tongue piercing.

A 20-year-old woman was admitted to the emergency room with a 2-week history of fatigue, myalgia, arthralgia and intermittent fever. She had no medical history but, 1 month prior to admission, she underwent tongue piercing with the insertion of two metal barbells transfixing her tongue. Three days before admission, she removed one of them because of persistent pain and significant swelling of the tongue. Physical examination revealed a persisting wound of the tongue at the place where the barbell was previously inserted. Laboratory data showed elevated white blood cell count (22 × 109/L) and C-reactive protein (88 mg/L). She was offered in-patient care but refused it. She was readmitted 1 week later with high-grade fever (temperature 39 °C) and purpuric lesions in her fingers. Her arterial blood pressure was 130/70 mmHg and heart rate was 110/min. Heart and lung examination were normal. She was admitted to the Cardiology Department with a presumptive diagnosis of infective endocarditis. Laboratory findings were as follows: white blood cell count, 10 × 109/L; erythrocyte sedimentation rate, 88 mm/h; and C-reactive protein, 85 mg/L. Within 24 h the first three blood cultures were positive with Gram-negative diplococci identified as *N. mucosa* using the NEISSERIA 4H test (Sanofi Diagnostics Pasteur, Marnes-la-Coquette, France). This identification was subsequently confirmed by the Centre National de Référence des méningocoques et *Neisseria* apparentées, Institut Pasteur, Paris, France. The organism was susceptible to amoxicillin, cefotaxime, ceftriaxone and ciprofloxacin, but showed intermediate susceptibility to penicillin G (MIC 0.38 mg/L). The test for β-lactamase production was negative. By the time the results of blood cultures became available, the patient had escaped from hospital. She returned 48 h later with persistent high-grade fever and the systolic ejection murmur of mitral regurgitation. Transthoracic echocardiography disclosed grade II mitral incompetence. Two more blood cultures were drawn and were also positive for *N. mucosa*. Intravenous antibiotics were started with ceftriaxone 1 g twice daily and ciprofloxacin 200 mg twice daily. The next day, transesophageal echocardiography revealed a large vegetation on the anterior leaflet of the mitral valve. During the procedure the metal barbell of the tongue was removed for culture and the patient's tongue and throat were also swabbed for culture. None of these samples, obtained after antibiotic therapy was started, grew *Neisseria*.

On the third day of treatment, the patient became afebrile. A transesophageal echocardiogram performed on the 9th hospital day still showed the large vegetation previously observed on the anterior leaflet of the mitral valve. The patient remained afebrile during her subsequent hospital course, and eight blood cultures drawn between the 3rd and 21st day of treatment remained negative.

Antibiotic therapy was discontinued after 28 days of treatment and the patient was discharged 4 days later. At that time, the patient remained afebrile, the erythrocyte sedimentation rate and C-reactive protein had fallen to 13 mm/h and 3 mg/L, respectively. A new transesophageal echocardiogram showed a twofold reduction of the vegetation size, with stable grade II mitral regurgitation. Two months later, the patient had experienced no relapse and was doing well.

The case we report here fulfillls the Duke criteria for clinically definite endocarditis [6], and is the first report of *N. mucosa* infective endocarditis associated with tongue piercing. Although cultures of the removed barbell and patient's tongue and throat failed to yield *N. mucosa*, we believe that the wound of the tongue was the most likely source for *N. mucosa*. *N. mucosa* is an extremely rare cause of endocarditis. Our search of the literature led us to identify only 19 cases of endocarditis caused by *N. mucosa*. Among these, eight cases had no prior valvular or congenital heart disease [1,2,4,5]. The mitral valve was the most frequently involved valve [1,2,4,5].

Since *N. mucosa* may have varying susceptibility to penicillin [2], fluoroquinolones such as ciprofloxacin have been used [3].

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Body piercing, even when intraoral sites are involved, is generally not regarded as a risk factor for endocarditis. However, tongue piercing has been reported to cause significant local infectious complications [7]. In addition, other sites of body piercing have been associated with various infectious complications [8], including rare cases of endocarditis, as illustrated by two cases that occured in teenagers, one caused by *Staphylococcus aureus* after nasal piercing [9], and the other caused by *Streptococcus mitis* after ear piercing [10].

With the growing fashion of body piercing, clinicians should be prepared to take care of patients with infectious – sometimes serious – complications, resulting from these procedures and devices.

REFERENCES

1. Bricaire F, Frottier J, Bure A *et al*. Endocardites à *Neisseria* saprophytes. *Sem Hop (Paris)* 1982; 58: 263–7.
2. Ingram RJH, Cornere B, Ellis-Pegler RB. Endocarditis due to *Neisseria mucosa:* two case reports and review. *Clin Infect Dis* 1992; 15: 321–4.
3. Anderson MD, Miller LK. Endocarditis due to *Neisseria mucosa*. *Clin Infect Dis* 1993; 16: 184.
4. Epelbaum S, Laurent C, Morin G, Laurans G, Piussan Ch. Endocardite à *Neisseria mucosa* compliquée d'anéurysmes intracérébraux. *Arch Fr Pédiatr* 1993; 50: 231–3.
5. Lechowski L, Veyssier P, Maître B, Domart Y, Boulanger J, Darchis JP. Endocardite à *Neisseria mucosa*: à propos d'un cas et revue de la litérature. *Ann Méd Int* 1995; 146: 592–3.
6. Durack DT, Lukes AS, Bright DK and the Duke Endocarditis Service. New criteria for diagnosis of infective endocarditis: utilization of specific echocardiographic findings. *Am J Med* 1994; 96: 200–9.
7. Price SS, Lewis MW. Body piercing involving oral sites. *J Am Dent Assoc* 1997; 128: 1017–20.
8. Tweeten SS, Rickman LS. Infectious complications of body piercing. *Clin Infect Dis* 1998; 26: 735–40.
9. Ramage IJ, Wilson N. Fashion victim: infective endocarditis after nasal piercing. *Arch Dis Child* 1997; 77: 187.
10. Battin M, Fong LV, Monro JL. Gerbode ventricular septal defectfollowing endocarditis. *Eur J Cardiothor Surg* 1991; 5: 613–4.

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Acute pulmonary hypertension following paclitaxel in a patient with AIDS-related primary effusion lymphoma

S. Cherifi[1], P. Hermans[1], S. De Wit[1], B. Cantinieaux[2] and N. Clumeck[1,]*

[1]C.H.U. St Pierre, Division of Infectious Diseases and [2]Laboratory of Haematology, Free University of Brussels, Division of Infectious Diseases rue Haute 322, 1000 Brussels, Belgium

*Tel: + 32 2/535 41 30 Fax: + 32 2/539 36 14 E-mail: maladiesinfectieuses@stpierre-bru.be

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Primary effusion lymphoma (PEL), also known as body-cavity-based lymphoma, was firstly described by Knowles et al. in 1988 [1]. Few data exist on the clinical management of this peculiar tumor but, when reported, its short-term prognosis is commonly poor [2]. Paclitaxel is currently used for treating different malignancies and preliminary data are available in patients with non-Hodgkin's lymphomas and AIDS-related Kaposi's sarcoma (KS) [3]. Pulmonary hypertension could be a rare drug-related adverse event occurring in one patient with lung cancer treated with paclitaxel [4]. In addition, pulmonary hypertension has been reported in AIDS patients in different conditions: intravenous drug abuse, collagen vascular disease, hemophilia, heart disease, significant underlying lung disease, pulmonary thromboembolism or portal hypertension. In the absence of established etiology, the syndrome is known as primary pulmonary hypertension [5].

We report the case of a 33-year-old HIV-positive man with an 8-month history of highly agressive KS with cutaneous, pulmonary and lymphatic involvement. The patient was a homosexual man from Burundi, known to be HIV-positive for 4 years. He had been treated with six courses of adriamycin, bleomycin and vincristine every 2 weeks with an excellent clinical response, followed by a maintenance treatment with bleomycin for 4 months. Triple therapy for HIV was initiated in May 1998 with stavudine, lamivudine and indinavir. An empyema was treated by drainage and antibiotherapy in July 1998 and histology revealed inflammatory cells without neoplastic cells, with a positive culture for *Streptococcus pyogenes*.

As KS lesions rapidly progressed with a relapsing episode of pleural effusion, the patient was given paclitaxel (60 mg) on 20 January 1999. His CD4 lymphocyte count was 73 cells/mm^3 with a plasma viral load of 445 000 copies RNA/mL. Four days later, he presented with acute respiratory distress syndrome and hemodynamic shock. Blood pressure was 70/50 mmHg, pulse was 140 beats/min and respiratory rate 30/min. He had pulsus paradoxus. Pulmonary auscultation revealed bilateral hypoventilation, especially of the right lung. Echocardiography showed severe pericarditis, with signs of tamponade.

Pericardiocentesis was immediately performed, removing 550 mL of bloody pericardial effusion. Further analysis of both pleural and pericardial effusions established the diagnosis of PEL with a monoclonal population of large immunoblast-like cells with negative B- and T-cell-associated antigens, but CD45-positive and CD30-positive, with clonal rearrangements of the heavy-chain immunoglobulin gene and β-chain of the T-cell receptor as confirmed by Southern blot hybridization. These cells were positive for Epstein–Barr virus and human herpesvirus-8 as confirmed by PCR amplification. One week later, the pericarditis resolved and a chest radiographic image showed clear lung fields without any effusions. Computed tomography (CT) of the chest revealed no pulmonary infiltrates.

Chemotherapy was re-instituted with an increased dose of 75 mg/m^2 of paclitaxel (129 mg) on 2 February 1999. Worsening of dyspnea occurred on the 4th day after paclitaxel infusion and cardiac echography showed tricuspid insufficiency with pulmonary artery hypertension estimated at 40–45 mmHg, and marked right ventricular and atrial dilatation. Left ventricular function was normal. No known cause of pulmonary hypertension could be found.

There was no evidence of congenital heart disease, vascular disease or cardiomyopathy. There was no suspicion of collagen vascular disease, chronic active hepatitis, or portal hypertension on clinical grounds or from laboratory results. The patient gave no history of ingestion of anorectic agents. He suffered from KS, but a recent CT scan of the chest confirmed a successful response to chemotherapy (bleomycin). Primary pulmonary hypertension, a very unusual syndrome, appears unlikely as it is usually not transient.

Fifteen days after the administration of paclitaxel, echocardiography showed no signs of pericardial or pleural effusion, grade 1/4 tricuspid insufficiency with a moderately dilated right ventricule, and the previously described pulmonary hypertension had resolved. No specific treatment had been administered. Only supportive measures were administered (diuretics and oxygen).

After a transient clinical improvement, the patient developed severe thrombocytopenia which postponed any myelosuppressive

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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.

REFERENCES

1. Knowles DM, Inghirami G, Ubriaco A, Dalla-Favera R. Molecular genetic analysis of three AIDS-associated neoplasms of uncertain lineage demonstrates their B-cell derivation and the possible pathogenic role of the Epstein–Barr virus. *Blood* 1989; 73: 792–9.
2. Cesarman E, Chang Y, Moore PS, Said JW, Knowles DM. Kaposi's sarcoma-associated herpesvirus-like DNA sequences in AIDS-related body-cavity-based lymphomas. *N Engl J Med* 1995; 332: 1186–91.
3. Younes A. Paclitaxel-based treatment of lymphoma. *Semin Oncol* 1999; 26 (1 (Suppl. 2): 123–8.
4. Bonomi P, Faber LP, Warren W *et al.* Postoperative bronchopulmonary complications in stage III lung cancer patients treated with preoperative paclitaxel-containing chemotherapy and concurrent radiation. *Semin Oncol* 1997; 24: S12–3–9.
5. Mesa RA, Edell ES, Dunn WF, Edwards WD. Human immunodeficiency virus infection and pulmonary hypertension: two new cases and a review of 86 reported cases. *Mayo Clin Proc* 1998; 73: 37–45.

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

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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 β-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 β-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]. β-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 β-lactamase bands. ESBL *bla* genes were amplified by using specific primers for bla_{TEM} [15] and bla_{SHV} [16]. In addition, forward primer 5′-GCT GAT-GAG CGC TTT GCG-3′ (nucleotide positions 193–210 of $bla_{CTX-M-1}$) and reverse primer 5′-TTA CAA ACC GTT GGT-GAC G-3′ (last 19 nucleotides of $bla_{CTX-M-1}$, 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 β-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 β-lactamase producers (Figure 1). The greater efficacy of these cephalosporins compared to ceftazidime could be related to the specific ESBL

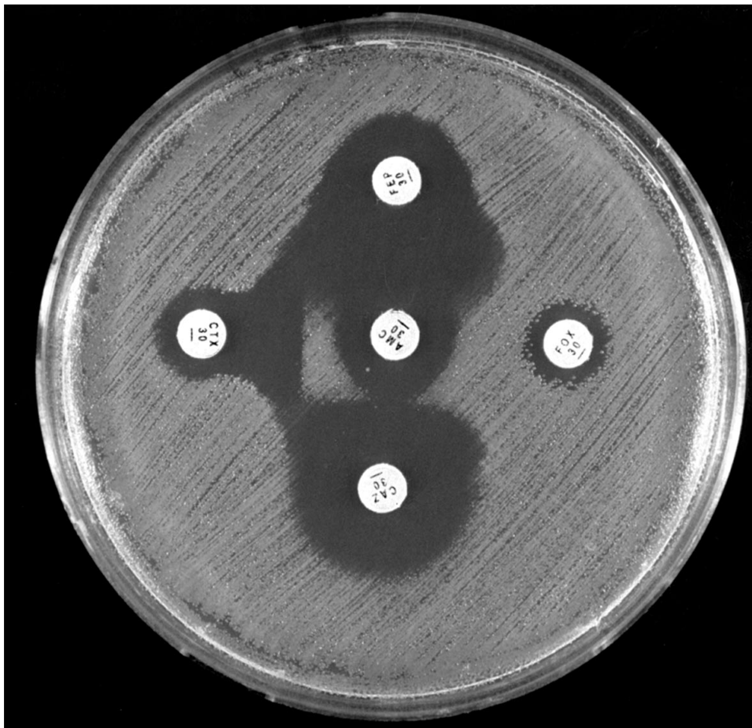

Figure 1 Double disk synergy test of an ESBL-producing *Enterobacter cloacae* isolate (top, cefepime; right, cefoxitin; bottom, ceftazidime; left, cefotaxime; centre, amoxicillin/clavulanate).

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

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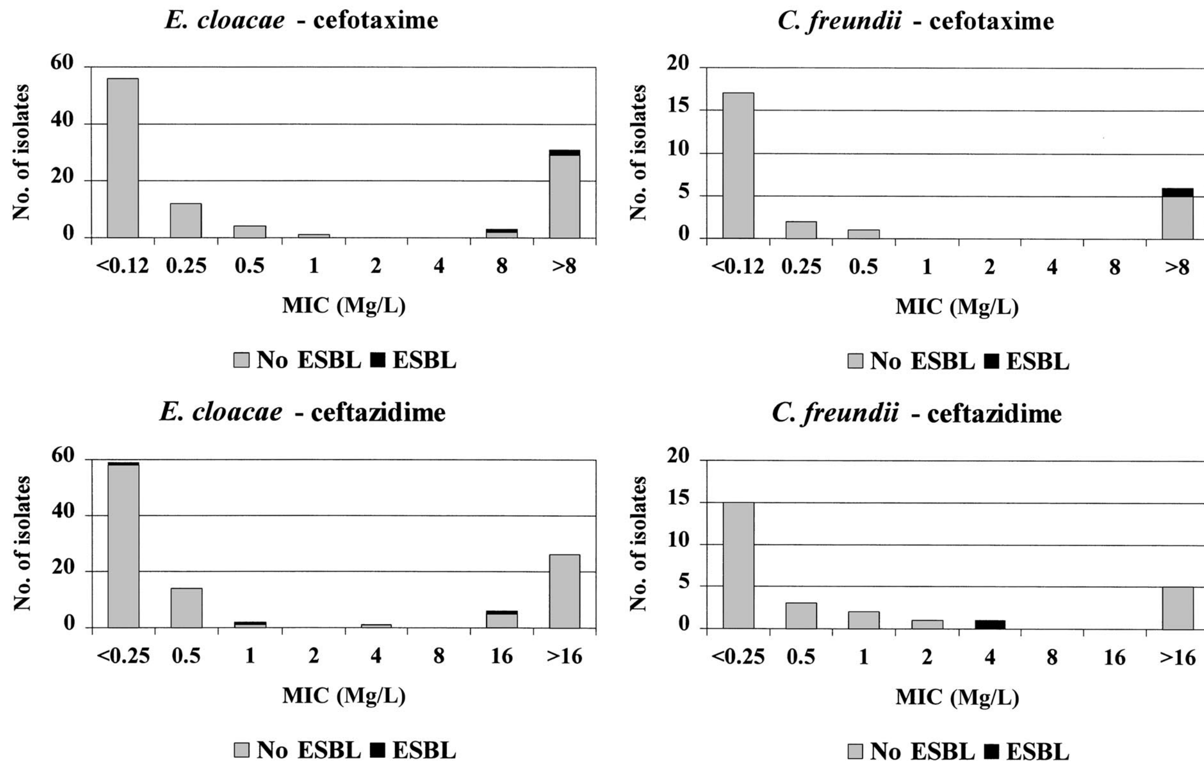

Figure 2 Cefotaxime and ceftazidime MIC distributions against *Enterobacter cloacae* and *Citrobacter freundii* isolates.

produced. In fact, all ESBL-producing isolates were PCR positive for bla_{CTX-M} and not for bla_{TEM} or bla_{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 β-lactamase of pI 8.1. The ESBL gene was amplified by PCR using specific primers for bla_{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

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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.

REFERENCES

1. Livermore DM. β-Lactamases in laboratory and clinical resistance. *Clin Microbiol Rev* 1995; 8: 557–84.
2. Jacoby GA, Medeiros AA. More extended spectrum β-lactamases. *Antimicrob Agents Chemother* 1991; 35: 1697–704.
3. Gianneli D, Tzelepi E, Tzouvelekis LS, Mentis AF, Nikolopoulou C. Dissemination of cephalosporin-resistant *Serratia marcescens* strains producing a plasmidic SHV type beta-lactamase in Greek hospitals. *Eur J Clin Microbiol Infect Dis* 1994; 13: 764–7.
4. Coudron PE, Moland ES, Sanders CC. Occurrence and detection of extended-spectrum beta-lactamases in members of the family *Enterobacteriaceae* at a veterans medical center: seek and you may find. *J Clin Microbiol* 1997; 35: 2593–7.
5. Gniadkowski M, Schneider I, Palucha A, Jungwirth R, Mikiewicz B, Bauernfeind A. Cefotaxime-resistant *Enterobacteriaceae* isolates from a hospital in Warsaw, Poland: identification of a new CTX-M-3 cefotaxime-hydrolyzing β-lactamase that is closely related to the CTX-M-1/MEN-1 enzyme. *Antimicrob Agents Chemother* 1998; 42: 827–32.
6. Pitout JD, Thomson KS, Hanson ND, Ehrhardt AF, Coudron P, Sanders CC. Plasmid-mediated resistance to expanded-spectrum cephalosporins among *Enterobacter aerogenes* strains. *Antimicrob Agents Chemother* 1998; 42: 596–600.
7. Silva J, Aguilar C, Becerra Z, López-Antunano F, García R. Extended-spectrum beta-lactamases in clinical isolates of enterobacteria in Mexico. *Microb Drug Resist* 1999; 5: 189–93.
8. Tzelepi E, Giakkoupi P, Sofianou D, Loukova V, Kemeroglou A, Tsakris A. Detection of extended-spectrum β-lactamases in clinical isolates of *Enterobacter cloacae* and *Enterobacter aerogenes*. *J Clin Microbiol* 2000; 38: 542–6.
9. Bush K. Is it important to identify extended-spectrum β-lactamase-producing isolates? *Eur J Clin Microbiol Infect Dis* 1996; 15: 361–4.
10. Cantón R, Pérez-Vázquez M, Oliver A *et al*. Evaluation of the Wider system, a new computer-assisted image-processing device for bacterial identification and susceptibility testing. *J Clin Microbiol* 2000; 38: 1339–46.
11. National Committee for Clinical Laboratory Standards. *Performance Standards for Antimicrobial Susceptibility Testing; eleventh informational supplement. M100-S11.* Wayne, PA: NCCLS, 2001.
12. Jarlier V, Nicolas MH, Fournier G, Philippon A. Extended spectrum β-lactamases conferring transferable resistance to newer β-lactam agents in *Enterobacteriaceae*: hospital prevalence and susceptibility patterns. *Rev Infect Dis* 1988; 10: 867–78.
13. Leich C, Boonlayangoor S. β-lactamases tests. In: Isenberg HD, ed. *Clinical Microbiology Procedures Handbook*, Vol. 1. Washington, DC: American Society for Microbiology, 1992: 5.3.1–5.3.8.
14. Huovinen S. Rapid isolectric focusing of plasmid-mediated β-lactamases with Pharmacia PhastSystem. *Antimicrob Agents Chemother* 1988; 32: 1730–2.
15. Mabilat C, Goussard S, Sougakoff W, Spencer RC, Courvalin P. Direct sequencing of the amplified structural gene and promoter for the extended-broad-spectrum beta-lactamase TEM-9 (RHH-1) of *Klebsiella pneumoniae*. *Plasmid* 1990; 23: 27–34.
16. Nuesch-Inderbinen MT, Hachler H, Kayser FH. Detection of genes coding for extended-spectrum SHV beta-lactamases in clinical isolates by a molecular genetic method, and comparison with the E test. *Eur J Clin Microbiol Infect Dis* 1996; 15: 398–402.
17. Oliver A, Pérez-Díaz JC, Coque TM, Baquero F, Cantón R. Nucelotide sequence and characterization of a novel cefotaxime-hydrolysing β-lactamase (CTX-M-10) isolated in Spain. *Antimicrob Agents Chemother* 2001; 45: 616–20.
18. Tzouvelekis LS, Tzelepi E, Tassios PT, Legakis NJ. CTX-M-type beta-lactamases: an emerging group of extended-spectrum enzymes. *Int J Antimicrob Agents* 2000; 14: 137–42.
19. Sabate M, Tarrago Navarro F, Miro E, Verges C, Barbe J, Prats G. Cloning and sequencing of the gene encoding a novel cefotaxime-hydrolyzing β-lactamase (CTX-M-9) from *E. coli* in Spain. *Antimicrob Agents Chemother* 2000; 44: 1970–3.
20. Nordmann P, Guibert M. Extended spectrum-betalactamases in *Pseudomonas aeruginosa*. *J Antimicrob Chemother* 1998; 42: 128–31.

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