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Note

Evolution of Resistance among Clinical Isolates of *Acinetobacter* over a 6-Year Period

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Abstract The aim of this report was to study the evolution of susceptibilities of 1532 clinical isolates of *Acinetobacter* recovered over a period of 6 years. The minimal inhibitory concentrations (MICs) of 15 antimicrobial agents were determined for all the isolates. The respective percentages of resistant strains in the years 1991 and 1996 were as follows: ciprofloxacin, 54.4% and 90.4%; tobramycin, 33% and 71.8%; amikacin, 21% and 83.7%; ampicillin plus sulbactam, 65.7% and 84.1%; ceftazidime, 57.4% and 86.8%; ticarcillin, 70% and 89.4%; trimethoprim plus sulfamethoxazole, 41.1% and 88.9%; and imipenem, 1.3% and 80%. The MIC90s of ciprofloxacin, sparfloxacin, biapenem, meropenem, imipenem, cefepime, cefpirome, and rifampicin against 250 imipenem-resistant *Acinetobacter* strains were >32, >32, 128, >256, 256, >256, 256, and 16 mg/l, respectively. With serious infections, it was necessary to resort to the use of colistin, the only antibiotic active in vitro.

Introduction

Toward the end of the 1970s, several reports revealed an increase in the frequency of nosocomial infections caused by bacteria of the genus *Acinetobacter* [1]. Since then, numerous outbreaks caused by these microorganisms have been described in hospital patients, especially in high-risk areas, such as intensive care units (ICUs), burn wards, and spinal-cord-injury units [2–3].

Since the beginning of the 1990s, numerous infections due to multiresistant *Acinetobacter calcoaceticus/Acinetobacter baumannii* complex strains have occurred in our hospital, causing serious problems when a course of treatment must be chosen. The aim of this study was to investigate the in vitro activity of 15 antimicrobial agents against 1532 clinical isolates over a 6-year period.

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Materials and Methods

The "Virgen de la Arrixaca" University Hospital consists of 1000 beds, 800 of which are in the general hospital, where there is an ICU of 33 beds divided into six wards. The remaining 200 beds are located in the maternity and children's hospital.

During the period from 1991 to 1996, we studied all the *Acinetobacter* strains from clinical sources recovered in our laboratory. Only one strain per patient was studied. Isolates were identified as belonging to the *Acinetobacter calcoaceticus/Acinetobacter baumannii* complex on the basis of Gram stain, motility testing, negative oxidase reaction, positive catalase, API NE (API System; bioMeriéux, France), growth at 37 °C, 41 °C, and 44 °C, and production of acid from glucose [4].

Antimicrobial susceptibility tests were carried out using a commercial standardized microtiter broth dilution method (Pasco; Difco, USA). By the disk diffusion method on Mueller-Hinton agar, we tested susceptibility against imipenem and colistin.

The minimal inhibitory concentrations (MICs) of the following antimicrobial agents were determined for 250 imipenem-resistant strains by means of the agar dilution method [5]: ciprofloxacin, sparfloxacin, biapenem, meropenem, imipenem, cefepime, cefpirome, and rifampicin. *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as controls. A total of 30 imipenem-resistant *Acinetobacter* strains (1 strain per patient) from the ICU, isolated between 1993 and 1996, were randomly selected to obtain the highest diversity between them and were further characterized by pulsed-field gel electrophoresis of total genomic DNA after digestion with *Apa*I, as described previously [6], to establish a relationship between the isolates.

Results and Discussion

During the 6-year period of the study, the total number of patients in the general hospital was 106 591, 8640 of whom were ICU patients. *Acinetobacter* strains were

isolated from 1532 (1.43%) patients, 549 (35.8%) from the ICU and 983 (64.2%) from other services in the general hospital. No strains were isolated from patients in the maternity and children's hospital. Table 1 shows the origin of the samples and the distribution on a yearly basis.

The activities of ciprofloxacin, ofloxacin, gentamicin, tobramycin, amikacin, ampicillin plus sulbactam, ceftazidime, piperacillin, ampicillin, cefotaxime, aztreonam, tetracycline, ticarcillin, trimethoprim plus sulfamethoxazole, and imipenem against the *Acinetobacter calcoaceticus*/*Acinetobacter baumannii* complex strains over the 6-year period of the study are shown in Figure 1. Susceptibility testing revealed 67 different antibiotypes, 21 of which corresponded to the imipenem-resistant strains.

Rates of resistance to all the antibiotics have gradually been increasing over the years, the highest figures being those obtained during the last year of the study. Tobramycin is the most active antibiotic, although resistance increased from 33% in 1991 to 71.8% in 1996. Thus, it was sometimes administered at high doses to try to control the infection.

Resistance to ampicillin plus sulbactam, a combination shown previously to be effective in vivo as well as in vitro [7–9], due to the action of sulbactam [10], decreased in 1994. This combination was subsequently used in our ICU, but the resistance rates quickly increased, reaching 62.7% and 84.1% in 1995 and 1996, respectively.

Tetracycline had low activity: resistance to this agent progressed rapidly from 79.3% in 1991 to 93.6% in 1993 and 98.3% from 1994 onwards. Given that some authors have found that doxycycline and not tetracycline had activity against *Acinetobacter* spp. [10], we tested this antibiotic by means of the disk diffusion method with 100 imipenem-resistant strains, but in all the cases they were also resistant (data not shown).

Colistin was the only antimicrobial agent with in vitro activity against all strains, in agreement with results published by other authors [7–9]. This agent was administered to four patients with pneumonia (5 mg/kg/day i.v.), three of whom recovered and the other died. Intraventricular colistin was also administered to three other patients with ventriculitis (0.015–0.03 mg/kg/day). In all of these cases the treatment was unsuccessful: one patient died and the other two improved after external ventricular drainage systems were removed. None of the seven patients suffered secondary effects.

The MIC90 of rifampicin was 16 mg/l, similar to data published by Thornsberry et al. [11], but higher than those published by Hogg et al. [12], although the strains in the latter study were more sensitive than ours to the other antimicrobial agents. In that same report, the activity of the combination of colistin and rifampicin was studied, and synergism in vitro was found [12]. We have not tested this with our strains.

In a study of the evolution of resistance, carried out during the years 1984–1988, Joly-Guillou et al. [13] found significant increases for several antibiotics, noting, as we did, the changes in resistance to ceftazidime [43.5% (first year) and 92% (last year)], tobramycin (33% and 84%), amikacin (25.5% and 84%), and quinolones (51% and 92.5%). Only 5.5% of their isolates were resistant to imipenem in 1988, while 80% of our isolates were resistant to this antibiotic in the last year of the period we studied. In another study in a Spanish hospital during the period 1990–1994 [14], an

Table 1 Distribution by clinical sources of 1532 strains of *Acinetobacter* recovered over a period of 6 years

Source	Distribution per year						Total
	1991	1992	1993	1994	1995	1996	
Sputum	15	10	23	19	27	22	116
Bronchial aspirate	21	27	30	67	78	73	296
Occluded telescopated catheter	0	4	3	6	2	1	16
Bronchoalveolar lavage	0	5	2	6	0	1	14
Pleural effusion	1	1	3	3	0	0	8
Cerebrospinal fluid	4	2	2	1	4	2	15
Blood	22	30	33	24	30	28	167
Urine	90	74	119	54	52	50	439
Wounds	47	54	93	44	39	48	325
Catheters[a]	6	0	5	3	2	12	28
Other body fluids	6	6	18	9	10	4	53
Biopsies	0	3	0	0	0	0	3
Others	9	6	10	4	12	11	52
Total	221	222	341	240	256	252	1532

[a] Including six venous catheters

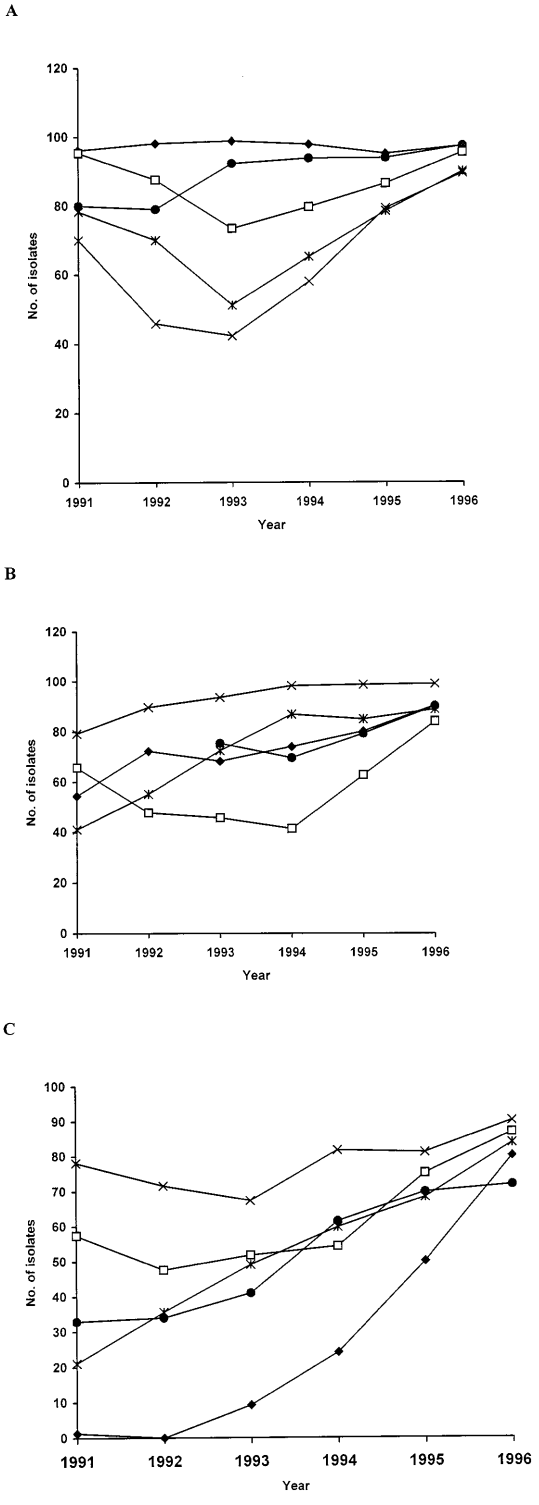

Figure 1 Evolution of resistance rates over the 6-year study period. Number of isolates per year: 1991, 221; 1992, 222; 1993, 341; 1994, 240; 1995, 256; and 1996, 252. **A** The β-lactam agents tested (◆ ampicillin, ● cefotaxime, □ aztreonam, ∗ piperacillin, and × ticarcillin). **B** ◆ ciprofloxacin, ● ofloxacin, □ ampicillin plus sulbactam, × trimethoprim plus sulfamethoxazole, and ∗ tetracycline. **C** ◆ imipenem, ● tobramycin, □ ceftazidime, × amikacin and ∗ gentamicin (the rates of resistance include intermediate and resistant strains)

increase in the resistance to antibiotics such as ceftazidime, amikacin, tobramycin, and ofloxacin was also seen, but carbapenems were not affected (1% resistance).

Although imipenem-resistant isolates (MIC>8 mg/l) have already been described as the cause of small outbreaks [7–9], resistant strains have become predominant (9.3% in 1993 to 80% in 1996) in our hospital, and imipenem is not a good therapeutic choice. The resistance of *Acinetobacter* to imipenem has been associated with the increased use of this antibiotic [8]. The first imipenem-resistant strains of *Acinetobacter* appeared in our ICU in 1993, coinciding with an outbreak of imipenem-resistant *Pseudomonas aeruginosa* and frequent use of the drug since the beginning of the 1990s.

On the basis of pulsed-field gel electrophoresis analysis, there were nine banding patterns from the 30 imipenem-resistant strains, 18 (60%) of the strains belonging to two of the patterns. The remaining patterns included between one (3.3%) and three (10%) strains.

The MIC50s and MIC90s of ciprofloxacin, sparfloxacin, biapenem, meropenem, imipenem, cefepime, cefpirome, and rifampicin against 250 imipenem-resistant strains were >32 and >32, 8 and >32, 32 and 128, 256 and >256, 128 and 256, 128 and >256, 128 and 256, and 4 and 16 mg/l, respectively. In all cases, they were above the breakpoints recommended by the National Committee for Clinical Laboratory Standards [5]. Thus, for example, although the MICs of biapenem were lower than those of imipenem and meropenem, as in other reports [15], they were always above 32 mg/ml. The new fourth-generation cephalosporins (cefepime and cefpirome) were shown to have little activity, their MIC90s being much greater than the breakpoints. The two quinolones tested, ciprofloxacin and sparfloxacin, were active against some of these imipenem-resistant strains, the latter agent having a lower MIC, but only a minority of the strains (5.2%) exhibited this antibiotype pattern.

The high resistance of *Acinetobacter* strains in our hospital has given rise to serious infections, such as pneumonia, septicemia, and meningitis, which can only be treated with colistin. This, in turn, has caused very serious problems due to the lack of availability of this old antibiotic in our country, the side effects it produces, and its reduced in vivo activity.

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