JOURNAL OF CLINICAL MICROBIOLOGY, June 1999, p. 2109–2110 Vol. 37, No. 6
0095-1137/99/$04.00+0
Copyright © 1999, American Society for Microbiology. All Rights Reserved.

Fast-Track Communication

Emergence of Carbapenem-Hydrolyzing Enzymes in *Acinetobacter baumannii* Clinical Isolates

Received 3 March 1999/Accepted 10 March 1999

In recent years, the number of nosocomial infections caused by *Acinetobacter baumannii* has increased significantly (4). Many outbreaks have been reported, especially among patients confined to hospital intensive care units, where the widespread use of antibiotics may select multidrug-resistant strains. The difficulty of treating *A. baumannii* nosocomial infection is associated with the high resistance to a wide range of antimicrobial agents frequently observed in this species (8). Often, imipenem remains one of the few therapeutic alternatives. Fortunately, imipenem resistance is relatively rare among *Acinetobacter* clinical isolates. Carbapenem resistance can arise by a decrease in expression of an outer membrane protein (3) or by alteration in penicillin-binding proteins (5). In general, the emergence of carbapenem-hydrolyzing enzymes has been limited compared to the prevalence of other β-lactamases (1). However, in 1985 in Scotland, an *A. baumannii* strain that produced a plasmid-mediated carbapenemase, ARI-I, was isolated (7), and recently, imipenem-hydrolyzing enzymes have been noted in some *Acinetobacter* isolates in a United Kingdom burns unit (10).

This communication reports the production of imipenem-hydrolyzing enzymes in two *A. baumannii* isolates obtained from urine cultures in 1998 in a Portuguese teaching hospital.

MICs were determined by the E-test method according to the manufacturer's instructions, and resistance was defined according to National Committee for Clinical Laboratory Standards guidelines (6). Crude sonicates of cell suspensions were assayed by spectrophotometry (UV/Vis Perkin-Elmer Lambda 6 spectrophotometer) with 0.1 mM imipenem and 0.1 mM nitrocefin solutions. One unit of β-lactamase activity was defined as the amount of enzyme hydrolyzing 1 nmol of the substrate per min per mg of protein at 30°C in 0.1 M phosphate buffer, pH 7.0. Isoelectric focusing (IEF) was performed in precast polyacrylamide gels, pI 3 to 9 (Pharmacia), by using a PhastSystem apparatus according to the manufacturer's instructions. Prior to staining with nitrocefin, one of the gels was overlaid with 0.1 mM cloxacillin solution, which is known to inhibit Bush group 1 β-lactamases (2), enzymes that are usually produced by *Acinetobacter* spp. (9). Two control strains were used in the experiments: an *A. baumannii* clinical isolate susceptible to imipenem and *A. baumannii* ATCC 19606. In an attempt to detect transfer of imipenem resistance, filter mating experiments were performed at 37°C by using *Escherichia coli* K802N as a recipient cell.

The results are shown in Table 1. The isolates (strains 122FFC and 65FFC) presented similar resistance patterns, with high-level resistance to imipenem and meropenem (MICs of both compounds, >32 mg/liter) and cephalosporins, including a new cephalosporin, cefepime (MICs, >256 mg/liter). They were susceptible to ciprofloxacin (MICs of 1.5 and 0.5 mg/liter, respectively) and to aminoglycosides (data not shown).

IEF revealed a large β-lactamase band with a pI of >8, presumably a chromosomal Bush group 1 enzyme. A sharp β-lactamase band with a pI of >8 was observed after the treatment with cloxacillin, suggesting the production of another β-lactamase not inhibited by cloxacillin.

Imipenem was readily hydrolyzed by crude extracts of imipenem-resistant isolates, but not by extracts of the controls, confirming the presence of an imipenem-hydrolyzing enzyme. The observed carbapenem resistance correlated with hydrolytic activity. A slight improvement of the β-lactamase activity was observed during the measurement of imipenem hydrolysis in the presence of 1 mM $ZnCl_2$ solution. Preincubation of the extract with 1 mM EDTA solution for 10 min at 30°C resulted in a decrease in β-lactamase activity (between 70 and 80% inhibition). All attempts to transfer imipenem resistance to *E. coli* K802N were unsuccessful.

The results obtained suggest the possibility of a metalloenzyme. Neither of the *A. baumannii* carbapenemases reported in the literature are zinc-dependent enzymes (7, 10). This is the first report of an imipenem-hydrolyzing enzyme in this species found in Portugal. The increase in carbapenem therapy might be associated with the emergence of *A. baumannii* strains that produce imipenem-hydrolyzing enzymes, which is a serious

TABLE 1. Characteristics of the isolates of *A. baumannii* studied

Characteristic	Value for isolate:			
	122FFC	65FFC	3625[a]	ATCC 19606
Date isolated (mo/yr)	4/98	9/98		
MIC (mg/liter)				
Amoxicillin	>256	>256	>256	>256
Amoxicillin-clavulanate	8	16	16	4
Piperacillin	48	48	>256	16
Ceftazidime	>256	>256	48	8
Ceftriaxone	>256	>256	>256	32
Cefepime	>256	>256	24	16
Aztreonam	64	64	96	16
Imipenem	>32	>32	2	0.38
Meropenem	>32	>32	ND[b]	ND
Ciprofloxacin	1.5	0.5	ND	ND
Hydrolysis by crude extracts (U/mg of protein)[c]				
Imipenem	247.79	205.5	0.95	0.32
$ZnCl_2$[d]	273.8	229.96	ND	ND
EDTA[e]	37.34	45.32	ND	ND
Nitrocefin	2,087.47	1,871.75	13,452	6.15

[a] Clinical strain from laboratory collection.
[b] ND, not determined.
[c] Nanomoles of substrate hydrolyzed per minute per milligram of protein.
[d] Enzyme activity in the presence of 1 mM $ZnCl_2$ solution.
[e] Enzyme activity in the presence of 1 mM EDTA solution after 10 min of preincubation with extract at 30°C.

concern due to the large spectrum of these enzymes. Therefore, it is crucial to rationalize the use of this class of compounds in an attempt to minimize the selection of these new β-lactamases.

REFERENCES

1. **Bush, K.** 1998. Metallo-β-lactamases: a class apart. Clin. Infect. Dis. **27** (Suppl. 1)**:**S48–S53.
2. **Bush, K., G. A. Jacoby, and A. A. Medeiros.** 1995. A functional classification scheme for β-lactamases and its correlation with molecular structure. Antimicrob. Agents Chemother. **39:**1211–1233.
3. **Clark, R. B.** 1996. Imipenem resistance among *Acinetobacter baumannii*: association with reduced expression of 33–36 KDa outer-membrane protein. J. Antimicrob. Chemother. **38:**245–251.
4. **Forster, D. H., and F. D. Daschner.** 1998. *Acinetobacter* species as nosocomial pathogens. Eur. J. Clin. Microbiol. Infect. Dis. **17:**73–77.
5. **Gehrlein, M., H. Leying, W. Cullman, S. Wendt, and W. Opferkuch.** 1991. Imipenem resistance in *Acinetobacter baumannii* is due to altered penicillin-binding proteins. Chemotherapy **37:**405–412.
6. **National Committee for Clinical Laboratory Standards.** 1998. Performance standards for antimicrobial susceptibility testing: eighth informational supplement. NCCLS document M100-S8. National Committee for Clinical Laboratory Standards, Wayne, Pa.
7. **Paton, R., R. S. Miles, J. Hood, and S. G. B. Amyes.** 1993. ARI-I: β-lactamase-mediated imipenem resistance in *Acinetobacter baumannii*. Int. J. Antimicrob. Agents **2:**81–88.
8. **Seifert, H., R. Baginski, A. Schulze, and G. Pulverer.** 1993. Antimicrobial susceptibility of *Acinetobacter* species. Antimicrob. Agents Chemother. **37:**750–753.
9. **Vila, J., A. Marcos, F. Marco, S. Abdalla, Y. Vergara, R. Reig, R. Gomez-Lus, and M. T. Jimenez de Anta.** 1993. In vitro antimicrobial production of β-lactamases, aminoglycoside-modifying enzymes, and chloramphenicol acetyltransferase by and susceptibility of clinical isolates of *Acinetobacter baumannii*. Antimicrob. Agents Chemother. **37:**138–141.
10. **Weinbren, M. J., A. P. Johnson, M. E. Kaufmann, and D. M. Livermore.** 1998. *Acinetobacter* spp. isolates with reduced susceptibilities to carbapenems in a UK burns unit. J. Antimicrob. Chemother. **41:**574–576.

Gabriela J. Da Silva*
Rui Leitão
Laboratório de Microbiologia
Faculdade de Farmácia
Universidade de Coimbra
Couraça dos Apóstolos, 51, r/c E
3030 Coimbra, Portugal

*Phone: (351) 39 852567
Fax: (351) 39 852569
E-mail: gjsilva@cygnus.ci.uc.pt

Luísa Peixe
Laboratório de Microbiologia
Faculdade de Farmácia
Universidade do Porto
R. Aníbal da Cunha, 164
4050 Porto, Portugal