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Molecular and Biochemical Analysis of AST-1, a Class A β-Lactamase from *Nocardia asteroides* Sensu Stricto

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A β-lactamase gene was cloned from a *Nocardia asteroides* sensu stricto clinical isolate. A recombinant plasmid, pAST-1, expressed the β-lactamase AST-1 in *Escherichia coli* JM109. Its pI was 4.8, and its relative molecular mass was 31 kDa. *E. coli* JM109(pAST-1) was resistant to penicillins and narrow-spectrum cephalosporins. The β-lactamase AST-1 had a restricted hydrolytic activity spectrum. Its activity was partially inhibited by clavulanic acid but not by sulbactam and tazobactam. AST-1 is an Ambler class A β-lactamase sharing 65% amino acid identity with β-lactamase FAR-1, the most closely related enzyme.

The *Nocardia* genus includes several species that are opportunistic pathogens in immunocompromised patients (3, 13). Species of *Nocardia asteroides* sensu stricto are the predominant human pathogens and are involved in pulmonary and brain abscesses (13). Since nocardiosis requires a long treatment (6 to 12 months or longer) and may cause a high mortality rate, the choice of the optimal antibiotic treatement is crucial (7).

β-Lactams have been used as first-line treatment with little concern for the β-lactam susceptibility of *Nocardia* sp. isolates (13). Knowledge of the mechanisms of β-lactam resistance profiles of *Nocardia* isolates may be critical for assessing the potential clinical efficacy of β-lactams. A study of the antimicrobial susceptibility patterns of 78 clinical isolates belonging to the *N. asteroides* complex found that 95% of the isolates exhibit one of the four major antibiotic resistance patterns (24). Type I (20% of the isolates) is susceptible to ampicillin and carbenicillin but intermediate in susceptibility to imipenem; type III (18%) is susceptible to ampicillin and erythromycin; type V (17%) is resistant to broad-spectrum cephalosporins; and type VI, the most prevalent group (35%), is resistant to ampicillin but susceptible to extended-spectrum cephalosporins and imipenem. Type II and type IV are extremely rare and not well characterized. Wallace et al. show that drug resistance patterns of type III and type V correlate with taxonomic groups and have been reclassified as *Nocardia nova* and *Nocardia farcinica*, respectively (21, 22). Isolates belonging to types I, IV, and VI are grouped into the same subspecies, named *N. asteroides* sensu stricto.

Although some nocardial β-lactamases have been characterized biochemically in *N. asteroides* (9, 17), *Nocardia brasiliensis* (19, 23), and *N. farcinica* (11, 20), the accurate role of β-lactamase in the β-lactam resistance pattern has scarcely been explored. Sequences of β-lactamase genes are available only for *N. farcinica* and the nonhuman pathogen *Nocardia lactamdurans* (5, 11).

We report on the molecular and biochemical characterization of a class A β-lactamase named AST-1 from a clinical isolate belonging to the most prevalent group of *N. asteroides* sensu stricto species. Hydrolytic activity of β-lactamase AST-1 was compared to that of β-lactamase FAR-1.

MATERIALS AND METHODS

Bacterial strains and plasmids. The *N. asteroides* sensu stricto isolate JPL was from a pulmonary abscess of a 30-year-old human immunodeficiency virus-infected man. It was identified by molecular methods based on the restriction analysis of PCR fragments corresponding to the heat shock protein gene, as described previously (12, 18). The recipient strain *Escherichia coli* JM109 for cloning experiments and phagemid cloning vector pBK-CMV have been reported previously (11).

Antimicrobial agents and MIC determinations. Antibiotic powders and their sources have been described previously (11). Antibiogram disks were used for routine antibiograms (Sanofi-Diagnostics Pasteur, Marnes-La-Coquette, France). MICs were determined by an agar dilution technique on Mueller-Hinton agar (Sanofi-Diagnostics Pasteur) with an inoculum of 10^4 CFU per spot as reported previously (11). All plates were incubated at 35°C for 18 h for *E. coli* and for 72 h for *N. asteroides* according to NCCLS guidelines (15). MICs of β-lactams were determined alone or in combination with a fixed concentration of clavulanic acid (2 μg/ml), sulbactam (8 μg/ml), and tazobactam (4 μg/ml).

Cloning experiments and genetic analysis. Genomic DNA from *N. asteroides* sensu stricto JPL was extracted as previously described (13). Partially digested *Sau*3AI fragments of genomic DNA of *N. asteroides* JPL were ligated into *Bam*HI-restricted phagemid pBK-CMV (Stratagene, La Jolla, Calif.). Ligation was performed at a 1:2 vector/insert ratio at a final concentration of 200 ng of DNA in a ligation mixture containing 1 U of T4 DNA ligase at 4°C for 18 h. Recombinant plasmids were transformed by electroporation (Gene Pulser II; Bio-Rad, Ivry-sur-Seine, France) into electrocompetent *E. coli* JM109 cells. Antibiotic-resistant colonies were selected onto Trypticase soy (TS) agar plates containing amoxicillin (50 μg/ml) and kanamycin (30 μg/ml) that were analyzed as described previously (13). Plasmid DNAs of recombinant strains were obtained using Qiagen columns (Qiagen, Courtaboeuf, France). Plasmid mapping was performed after double restriction analysis. DNA of one recombinant plasmid with the shortest insert was sequenced on both strands by using an ABI 377 sequencer (Applied Biosystems, Foster City, Calif.). The nucleotide and the deduced protein sequences were analyzed with software available over the

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internet at the National Center of Biotechnology Information webwite (http://www.ncbi.nlm.nih.gov) and at Pedro's Biomolecular Research Tools website (http://www.fmi.chbiology/research_tools.html).

β-Lactamase preparation. Cultures of *E. coli* JM109 harboring recombinant plasmid pAST-1 were grown overnight at 37°C in 4 liters of TS broth with amoxicillin (50 μg/ml). The bacteria were harvested for 10 min at 6,000 × *g*, and the bacterial pellet was resuspended in 30 ml of 20 mM bis-Tris (pH 5.5) [bis (2-hydroxyethylimino)tris(hydroxymethyl)methane] at 4°C. The bacterial cells were disrupted by sonication (two times for 20 s at 20 Hz) (Vibra Cell 75022 Phospholyser; Bioblock, Illkirch, France) and were centrifuged (30 min, 10,000 × *g*, 4°C). The supernatant containing the enzyme extract was purified by ion-exchange chromatography with AGMP-1 exchanger (Bio-Rad). The exchanger in the chloride form was treated with 0.1 M ammonia in water and was then washed extensively with water. After adsorption of the extracts, elution was performed with 0.1 M NaCl. The active fractions were pooled, dialyzed extensively, and lyophilized.

Kinetic measurements. Kinetic measurements were performed with the semi-purified β-lactamase preparation extracted from *E. coli* JM109(pAST-1). The kinetic constants were determined by the online computerized microacidimetric method at pH 7.0 and 37°C as described previously (10). V_{max} and V_{max}/K_m were expressed relative to that of benzylpenicillin (V_{max} = 100). The 50% inhibitory concentrations (IC_{50}s) were determined for clavulanate, sulbactam, and tazobactam as the concentration that reduced the hydrolysis rate of 100 μM benzylpenicillin by 50% under conditions in which the enzyme was preincubated with various concentrations of inhibitor for 5 min at 30°C before addition of the benzylpenicillin (10). The specific activity of the semipurified enzyme from *E. coli* JM109 harboring pAST-1 (AST-1) was obtained as described previously (16). One unit of the enzyme was defined as the activity which hydrolyzed 100 μmol of cephalothin per min per mg of protein. The total protein content was determined with bovin serum albumin as the standard (Bio-Rad DC protein assay kit).

IEF and determination of relative molecular mass. Cultures of *N. asteroides* JPL were grown in TS broth at 35°C for 72 h in an aerobic atmosphere. β-Lactamase extracts from these cultures were obtained as described previously (11) and were submitted with the β-lactamase preparation from cultures of *E. coli* JM109 harboring recombinant plasmid pAST-1 to isoelectric focusing (IEF) analysis on an ampholine polyacrylamide gel, as described previously (11). The relative molecular mass of the β-lactamase from *E. coli* JM109(pAST-1) culture was estimated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis, as reported previously (16).

Nucleotide sequence accession number. The nucleotide sequence data reported in this paper have been assigned to the GenBank nucleotide database under accession no. AF 279904.

RESULTS AND DISCUSSION

Identification and susceptibility testing of *N. asteroides* sensu stricto isolate. The *N. asteroides* isolate JPL was assigned to the type VI group of Steingrube et al. (18), which includes most of the *N. asteroides* sensu stricto isolates. MICs of β-lactams showed that this isolate was resistant to amino- and ureidopenicillins, narrow-spectrum cephalosporins, ceftazidime, and aztreonam (Table 1). Addition of clavulanate partially decreased the MICs of amino- and ureidopenicillins, while tazobactam and sulbactam did not have any significant effect (Table 1). These results were consistent with those obtained for other *N. asteroides* isolates (9), for *N. farcinica* (11, 20), and for *Mycobacterium fortuitum* (2, 6). Disk susceptibility testing showed that the *N. asteroides* isolate JPL was also susceptible to aminoglycosides (except kanamycin), tetracycline, and sulfonamides and resistant to fluoroquinolones, macrolides, fosfomycin, and chloramphenicol.

Characterization of the bla_{AST-1} gene and its expression in *E. coli*. Two recombinant plasmids were obtained harboring the same 1.7-kb insert as a result of cloning experiments. One of them, pAST-1, was further characterized, and its insert was sequenced. It contained a 933-bp open reading frame (ORF), bla_{AST-1}, encoding a 310-amino-acid protein named AST-1 (Fig. 1). The G+C content of bla_{AST-1} was 71.3%, which lies within the G+C ratios for other chromosomally encoded *Nocardia* sp. genes as recorded in the EMBL and GenBank sequence databases (64 to 72%). Moreover, 18 bp upstream of this ORF, part of another ORF was identified, the deduced protein of which shared 42% identity with a 561-amino-acid protein of unknown function from *Streptomyces coelicolor* (GenBank accession no. T35845). Additionally, 244 bp from bla_{AST-1}, another ORF was identified, the protein of which shared 59% amino acid identity (within 89 amino acids) with a probable phosphorylating protein, UreD, from *Mycobacterium leprae* (GenBank accession no. S72992). These results are consistent with the *Actinomycetales* origin of bla_{AST-1}. Since no ATG initiation codon was found for bla_{AST-1}, a putative GTG

TABLE 1. MICs of β-lactams for *N. asteroides* sensu stricto JPL, *E. coli* JM109 harboring recombinant plasmid pAST-1, and reference strain *E. coli* JM109

β-Lactam[a]	MIC (μg/ml)		
	N. asteroides JPL	*E. coli* JM109(pAST-1)	*E. coli* JM109
Amoxicillin	64	>512	2
Amoxicillin + CLA	4	32	2
Amoxicillin + TZB	64	>512	2
Amoxicillin + SUL	64	>512	2
Ticarcillin	256	256	2
Ticarcillin + CLA	8	8	1
Ticarcillin + TZB	128	256	1
Ticarcillin + SUL	256	256	2
Piperacillin	512	128	1
Piperacillin + CLA	32	4	0.5
Piperacillin + TZB	128	128	0.5
Piperacillin + SUL	256	128	0.5
Cephalothin	128	8	4
Cephalothin + CLA	64	4	2
Cephalothin + TZB	128	8	2
Cephalothin + SUL	128	8	2
Cefoxitin	128	4	4
Cefoxitin + CLA	64	4	4
Cefoxitin + TZB	64	4	4
Cefoxitin + SUL	64	4	4
Ceftazidime	>512	0.25	0.25
Ceftazidime + CLA	>512	0.25	0.25
Ceftazidime + TZB	>512	0.25	0.25
Ceftazidime + SUL	>512	0.25	0.25
Cefotaxime	<0.06	0.06	0.06
Cefotaxime + CLA	<0.06	0.06	0.06
Cefotaxime + TZB	<0.06	0.06	0.06
Cefotaxime + SUL	<0.06	0.06	0.06
Aztreonam	>512	0.12	0.12
Aztreonam + CLA	>512	0.12	0.06
Aztreonam + TZB	>512	0.12	0.06
Aztreonam + SUL	>512	0.12	0.06
Cefepime	0.25	0.12	0.06
Cefepime + CLA	0.25	0.06	0.06
Cefepime + TZB	0.25	0.06	0.06
Cefepime + SUL	0.25	0.06	0.06
Imipenem	0.5	0.06	0.06
Imipenem + CLA	0.5	0.06	0.06
Imipenem + TZB	0.5	0.06	0.06
Imipenem + SUL	0.5	0.06	0.06
Meropenem	0.25	<0.06	<0.06
Meropenem + CLA	0.25	<0.06	<0.06
Meropenem + TZB	0.25	<0.06	<0.06
Meropenem + SUL	0.25	<0.06	<0.06

[a] CLA, clavulanic acid at a fixed concentration of 2 μg/ml; TZB, tazobactam at a fixed concentration of 4 μg/ml; SUL, sulbactam at a fixed concentration of 8 μg/ml.

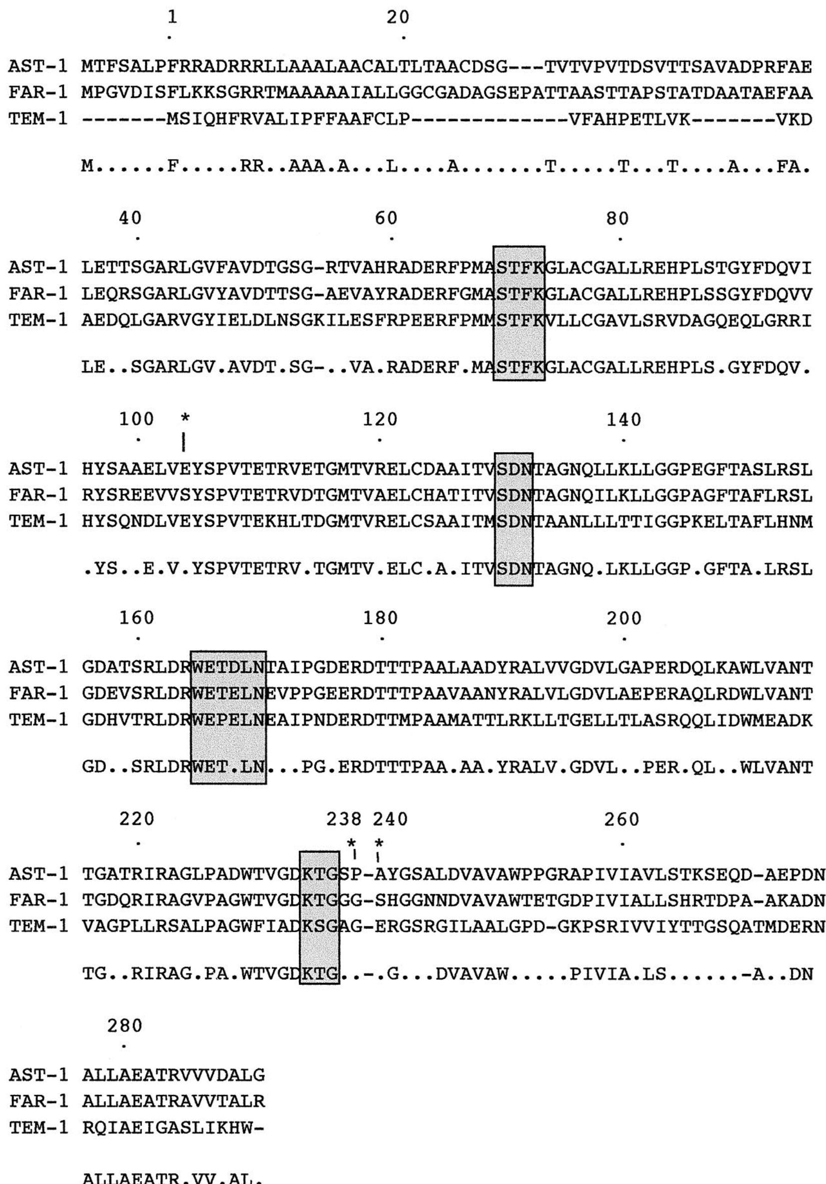

FIG. 1. Alignment of the amino acid sequence of AST-1 with those of TEM-1 and FAR-1 β-lactamases. Numbering is performed according to the method described by Ambler et al. (1). The conserved residues among nocardial β-lactamases are reported below the alignment, with dots indicating conserved residues. Four structural elements characteristic of class A β-lactamases are boxed in grey. Some relevant amino acid positions that may correspond to amino acid changes in extended-spectrum TEM derivatives are indicated by asterisks. Dashes indicate gaps within the alignment.

was retained as its initiation codon (data not shown), as in several *Streptomyces* and *Mycobacterium* sp. genes (11). Within the deduced protein, structural elements characteristic of serine and Ambler class A β-lactamases were identified (1, 8) (Fig. 1). The comparison of the AST-1 sequence with those of other class A β-lactamases showed that it was distantly related to class A β-lactamases, including those of *Streptomyces* and *Mycobacterium* spp. (35 to 50% of amino acid identity). It was related mostly to FAR-1 β-lactamase from *N. farcinica* VIC, sharing 65% amino acid identity (11).

MICs of β-lactams for *E. coli* JM109(pAST-1) showed mostly resistance to penicillins that was partially antagonized by addition of clavulanate (Table 1). These MICs mirrored those obtained for *N. asteroides* isolate JPL and for *E. coli* JM109(pFAR-1) expressing β-lactamase FAR-1, except that in this latter case, a slight increase of the MIC of aztreonam was observed (11).

Biochemical properties of the β-lactamase AST-1. IEF analysis showed that cultures of *N. asteroides* isolate JPL and of *E. coli* JM109(pAST-1) produced an identical β-lactamase with a pI of 4.8 (data not shown). This pI was similar to those observed for β-lactamases of other *N. asteroides* isolates (4.2 to 4.6) (9) and was different from the pI of 5.8 of an *N. asteroides* isolate, as reported recently (17). Thus, *N. asteroides* isolates may possess different β-lactamases of acidic pI values. However, valid comparison of β-lactamase content based on pI val-

TABLE 2. Compared kinetic parameters for AST-1 from *N. asteroides* sensu stricto and FAR-1 from *N. farcinica*

Substrate	Parameter for: AST-1			Parameter for: FAR-1		
	V_{max}[a]	K_m (μM)	V_{max}/K_m	V_{max}	K_m (μM)	V_{max}/K_m
Benzylpenicillin	100	30 ± 2	100	100	30 ± 2	100
Amoxicillin	53 ± 4	50 ± 4	32	115 ± 12	50 ± 3	69
Ticarcillin	8 ± 0.7	7 ± 0.5	33	30 ± 1	31 ± 1	29
Piperacillin	90 ± 6	330 ± 27	8	250 ± 26	45 ± 2	166
Cephalothin	40 ± 4	20 ± 1	60	85 ± 6	104 ± 9	2.5
Cephaloridine	57 ± 3	>500	<3.5	80 ± 5	>500	<5
Cefoperazone	12 ± 0.7	>500	<0.7	NS[b]	NS	NS
Ceftazidime	<1	>500	$<6 \times 10^{-2}$	<1	>500	$<6 \times 10^{-2}$
Cefotaxime	<1	>500	$<6 \times 10^{-2}$	3 ± 0.2	>500	<0.2
Aztreonam	<1	>500	$<6 \times 10^{-2}$	8 ± 0.5	400 ± 36	0.6

[a] V_{max} and V_{max}/K_m relative to that of benzylpenicillin, which was set at 100. Data are the means and standard deviations from three independent experiments.
[b] NS, not studied.

ues is difficult, since in the previous studies (9, 17), *N. asteroides* sensu stricto isolates were not differentiated from other *N. asteroides* spp. by molecular techniques.

The relative molecular mass of the β-lactamase AST-1 expressed in *E. coli* JM109(pAST-1) was estimated to be 31 kDa (data not shown), close to the value of 32 kDa for the β-lactamase FAR-1 (11).

The β-lactamase AST-1 was very poorly expressed from *E. coli* JM109(pAST-1) and *N. asteroides* JPL cultures (data not shown). The specific activity of the semipurified extract of *E. coli* JM109(pAST-1) was 0.11 mU · mg of protein^{-1} with 100 μM cephalothin as the substrate. Its purification factor was between 10- and 15-fold. Kinetic parameters of β-lactamase AST-1 revealed its strong activity against penicillins and narrow-spectrum cephalosporins (Table 2). As opposed to β-lactamase FAR-1, hydrolysis of aztreonam was not detected. As assessed by IC_{50}s, the activity of inhibitors was weak, especially for sulbactam and tazobactam (Table 3). Similar results were obtained for β-lactamases extracted from *N. asteroides*, *M. fortuitum*, and *N. farcinica* (FAR-1) isolates (2, 6, 9, 11). Thus, it may be hypothesized that β-lactamases of *Nocardia* spp. are not susceptible to the β-lactamase inhibitors sulbactam and tazobactam. Susceptibility of *N. brasiliensis* β-lactamases to clavulanate is, however, greater than that of AST-1 (22). In one report, hydrolytic activity toward cefotaxime was noted for *N. asteroides* isolates (17). However, comparison with the activity of AST-1 is difficult, again since these *N. asteroides* isolates have not been grouped by molecular techniques (17).

AST-1, like FAR-1, is tazobactam resistant, like the inhibitor-resistant TEM derivatives occurring as acquired resistance mechanisms. Thus, AST-1 is another example of naturally occurring inhibitor-resistant β-lactamases that mimic molecular mechanisms involved in acquired β-lactam resistance. It would be interesting to investigate whether the *N. asteroides* isolate JPL produces clavulanate derivatives in a manner similar to that of the β-lactamase-producing *N. lactamdurans* isolate, which produces cephamycin derivatives (5). Since AST-1 activity is partially or totally resistant to inhibitors, antibiotic combinations containing amoxicillin-clavulanate, piperacillin-tazobactam, and ampicillin-sulbactam should be avoided in treatment of nocardiosis due to *N. asteroides* sensu stricto.

TABLE 3. Inhibition profiles of AST-1, FAR-1, and TEM-1 β-lactamases

β-Lactamase	IC_{50} (μM) for: Clavulanic acid	Sulbactam	Tazobactam
AST-1	0.7	960	67
FAR-1	0.3	600	20
TEM-1	0.08	6.1	0.1

AST-1, as opposed to β-lactamase FAR-1, did not hydrolyze aztreonam. A few substitutions in TEM-derivative β-lactamases, such as Glu104Lys, Gly238Ser, and Glu240Lys, increase hydrolytic activity toward aztreonam (4, 14). FAR-1 possessed a serine residue in positions 104 and 240, as not found in the AST-1 sequence (Fig. 1). Thus, sequence differences between the two nocardial β-lactamases may account for the observed difference in the hydrolytic activity toward the monobactam aztreonam. Moreover, proline in position 238 in AST-1 sequence may modify the β-3 sheet structure, thus explaining the weak catalytic properties of AST-1.

Conclusion. β-Lactamase AST-1 is the second class A β-lactamase characterized in a *Nocardia* sp. clinical isolate. As already mentioned for β-lactamase FAR-1 from *N. farcinica*, AST-1 expression cannot explain the entire β-lactam resistance profile of the *N. asteroides* sensu stricto isolate, especially concerning its resistance to aztreonam and ceftazidime. Additionally, other undetected β-lactamases and/or penicillin-binding affinities may account for this naturally occurring β-lactam resistance profile. Since AST-1 and FAR-1 β-lactamases shared significant amino acid identity and similar biochemical properties, they may derive from a common ancestor.

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L.P. and F.L. contributed equally to this work.

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REFERENCES

1. **Ambler, R. P., A. F. W. Coulson, J.-M. Frère, J. M. Guysen, B. Joris, M. Forsman, R. C. Lévesque, G. Tiraby, and S. G. Waley.** 1991. A standard numbering scheme for the class A β-lactamases. Biochem. J. **276:**269–272.
2. **Amicosante, G., N. Franceschini, B. Segatore, A. Oratore, L. Fattorini, G. Orefici, J. Van Beeumen, and J.-M. Frère.** 1990. Characterization of a beta-lactamase produced in *Mycobacterium fortuitum* D316. Biochem. J. **271:** 729–734.
3. **Beaman, B. L., P. Boiron, L. Beaman, G. H. Brownell, K. Schaal, and M. E. Gombert.** 1992. Nocardia and nocardiosis. J. Med. Vet. Mycol. **30:**317–331.
4. **Cantu, C., III, W. Huang, and T. Palzkill.** 1996. Selection and characterization of amino acid substitutions at residues 237–240 of TEM-1 beta-lactamase with altered substrate specificity for aztreonam and ceftazidime. J. Biol. Chem. **271:**22538–22545.
5. **Coque, J. J., P. Liras, and J. F. Martin.** 1993. Genes for a β-lactamase, a penicillin-binding protein and a transmembrane protein are clustered with the cephamycin biosynthetic genes in *Nocardia lactamdurans*. EMBO J. **12:** 631–639.
6. **Fattorini, L., G. Amicosante, D. Fiorentino, N. Franceschini, L. Di Marzio, A. Oratore, and G. Orefici.** 1989. Inhibitors and inactivators of beta-lactamase from *Mycobacterium fortuitum*. J. Chemother. **1:**293–297.
7. **Gombert, M. E., L. B. Berkowitz, T. M. Aulicino, and L. Dubouchet.** 1990. Therapy of pulmonary nocardiosis in immunocompromised mice. Antimicrob. Agents Chemother. **34:**1766–1768.
8. **Joris, B., P. Ledent, O. Dideberg, E. Fonze, J. Lamotte-Brasseur, J. A. Kelly, J.-M. Ghuysen, and J.-M. Frère.** 1991. Comparison of the sequences of class A β-lactamases and of the secondary structure elements of penicillin-recognizing proteins. Antimicrob. Agents Chemother. **35:**2294–2301.
9. **Kitzis, M. D., L. Gutmann, and J. F. Acar.** 1985. In-vitro susceptibility of

Nocardia asteroides to 21 beta-lactam antibiotics, in combination with three beta-lactamase inhibitors, and its relationship to the beta-lactamase content. J. Antimicrob. Chemother. **15:**23–30.
10. **Labia, R., J. Andrillon, and F. Legoffic.** 1973. Computerized microacidimetric determination of β-lactamase Michaelis-Menten constants. FEBS Lett. **33:**42–44.
11. **Laurent, F., L. Poirel, T. Naas, E. B. Chaibi, R. Labia, P. Boiron, and P. Nordmann.** 1999. Biochemical-genetic analysis and distribution of FAR-1, a class A β-lactamase from *Nocardia farcinica*. Antimicrob. Agents Chemother. **43:**1644–1650.
12. **Laurent, F. J., F. Provost, and P. Boiron.** 1998. Rapid identification of clinically relevant *Nocardia* species to genus level by 16S rRNA gene PCR. J. Clin. Microbiol. **37:**99–102.
13. **Lerner, P. I.** 1996. Nocardiosis. Clin. Infect. Dis. **22:**891–903.
14. **Matagne, A., J. Lamotte-Brasseur, and J.-M. Frère.** 1998. Catalytic properties of class A beta-lactamases: efficiency and diversity. Biochem. J. **330:** 581–598.
15. **National Committee for Clinical Laboratory Standards.** 1993. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard M7–A3. National Committee for Clinical Laboratory Standards, Villanova, Pa.
16. **Poirel, L., T. Naas, M. Guibert, E. B. Chaibi, R. Labia, and P. Nordmann.** 1999. Molecular and biochemical characterization of VEB-1, a novel class A extended-spectrum β-lactamase encoded by an *Escherichia coli* integron gene. Antimicrob. Agents Chemother. **43:**573–581.
17. **Scopetti, F., L. Fattorini, N. Franceschini, G. Amicosante, and G. Orefici.** 1997. Non-inducible, mainly cell-associated β-lactamase from *Nocardia asteroides* strain 108. J. Antimicrob. Chemother. **40:**5–11.
18. **Steingrube, V. A., B. A. Brown, J. L. Gibson, R. W. Wilson, J. Brown, Z. Blacklock, K. Jost, S. Locke, R. F. Ulrich, and R. J. Wallace, Jr.** 1995. DNA amplification and restriction endonuclease analysis for differentiation of 12 species and taxa of *Nocardia*, including recognition of four new taxa within the *Nocardia asteroides* complex. J. Clin. Microbiol. **33:**3096–3101.
19. **Steingrube, V. A., R. J. Wallace, Jr., B. A. Brown, Y. Pang, B. Zeluff, L. C. Steele, and Y. Zhang.** 1991. Acquired resistance of *Nocardia brasiliensis* to clavulanic acid related to a change in β-lactamase following therapy with amoxicillin-clavulanic acid. Antimicrob. Agents Chemother. **35:**524–528.
20. **Steingrube, V. A., R. J. Wallace, Jr., B. A. Brown, Y. Zhang, L. C. Steele, G. Young, and D. R. Nash.** 1993. Partial characterization of *Nocardia farcinica* β-lactamases. Antimicrob. Agents Chemother. **37:**1850–1855.
21. **Wallace, R. J., Jr., B. A. Brown, M. Tsukamura, J. M. Brown, and G. O. Onyi.** 1991. Clinical and laboratory features of *Nocardia nova*. J. Clin. Microbiol. **29:**2407–2411.
22. **Wallace, R. J., Jr., D. R. Nash, W. K. Johnson, L. C. Steele, and V. A. Steingrube.** 1987. Beta-lactam resistance in *Nocardia brasiliensis* is mediated by beta-lactamase and reversed in the presence of clavulanic acid. J. Infect. Dis. **156:**959–966.
23. **Wallace, R. J., Jr., L. C. Steele, G. Sumter, and J. M. Smith.** 1988. Antimicrobial susceptibility patterns of *Nocardia asteroides*. Antimicrob. Agents Chemother. **32:**1776–1779.
24. **Wallace, R. J., Jr., M. Tsukamura, B. A. Brown, J. Brown, V. A. Steingrube, Y. S. Zhang, and D. R. Nash.** 1990. Cefotaxime-resistant *Nocardia asteroides* strains are isolates of the controversial species *Nocardia farcinica*. J. Clin. Microbiol. **28:**2726–2732.