

Antimicrobial Susceptibility and Mechanisms of Resistance to Quinolones and β -Lactams in *Acinetobacter* Genospecies 3

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Antimicrobial susceptibility was determined in 15 epidemiologically unrelated clinical isolates of *Acinetobacter* genospecies 3. Moreover, the mechanisms of resistance to some β -lactam antibiotics may be associated with the presence of a chromosomal cephalosporinase, AmpC, and the resistance to quinolones related to mutations in the *gyrA* and *parC* genes.

The genus *Acinetobacter* has a complex history, and it has long been difficult to find phenotypic criteria for speciation (R. E. Weaver and L. A. Actis, Letter, J. Clin. Microbiol. **32**: 1833, 1994). Since 1986, this genus has been shown to consist of at least 23 genospecies which can be identified by DNA-DNA hybridization (4, 6, 18).

Genospecies 1, 2, 3, and 13 (18) have been shown to be closely genetically related and difficult to separate phenotypically. Therefore, they are known as the *A. calcoaceticus*-*A. baumannii* complex (6). Except for *Acinetobacter* genospecies 1, which plays little or no role as a human pathogen, the remaining genospecies in this complex are all important nosocomial pathogens able to cause infections and spread in hospitals (1, 6, 8).

Although some reports have been published about the antimicrobial susceptibility of these genospecies, mainly *A. baumannii* (7, 9, 16, 17, 19, 21), there are no reports concerning the mechanisms of resistance to antimicrobial agents in *Acinetobacter* genospecies 3. Thus, we herein analyze the antimicrobial susceptibility and mechanisms of resistance to β -lactam antibiotics and quinolones in 15 epidemiologically unrelated clinical isolates of *Acinetobacter* genospecies 3.

In November 2000, all the isolates of *A. baumannii* from clinical samples were collected in 28 hospitals around Spain. A total of 244 strains of *Acinetobacter* spp. were collected: 226 *A. baumannii*, 15 *Acinetobacter* genospecies 3, and 3 unidentified *Acinetobacter* strains. These species were identified by amplified ribosomal DNA restriction analysis. *Acinetobacter* genospecies 3 was also identified by sequencing the 16S rRNA gene (20). To our knowledge, no commercial identification system can completely discriminate within the *A. calcoaceticus*-*A. baumannii* complex (2). Thus, no reactions besides growth at 44°C would discriminate between *A. baumannii* and genospecies 3.

However, while *Acinetobacter* genospecies 3 strains are reported not to grow at 44°C, exceptions to this rule do occur (6). In fact, we found that 46.6% (7 of 15 isolates) of the studied strains of genospecies 3 grew at 44°C.

A microdilution assay following the guidelines established by the NCCLS (12) was used to determine the MICs of the following antimicrobial agents: ampicillin, piperacillin, cephalothin, cefoxitin, gentamicin, amikacin, tobramycin, tetracycline, minocycline, doxycycline, rifampin, colistin (Sigma, Madrid, Spain), ceftazidime (GlaxoSmithKline, Uxbridge, United Kingdom), cefepime, (Bristol-Myers Squibb, Madrid, Spain), sulbactam and azithromycin (Pfizer, Sandwich, United Kingdom), imipenem (Merck, Hoddesdon, United Kingdom), meropenem (AstraZeneca, Macclesfield, United Kingdom), ciprofloxacin (Bayer, Leverkusen, Germany), and cotrimoxazole (Gallosol, Madrid, Spain). The breakpoints used were those recommended by the NCCLS for nonfermentative bacteria (12).

Table 1 shows the MICs of the different antimicrobial agents. The antibiotics with the best activity against this species of *Acinetobacter* were ceftazidime, ampicillin-sulbactam, meropenem, imipenem, amikacin, tetracycline, doxycycline, and minocycline. Cephalothin, cefoxitin, ampicillin, rifampin, and azithromycin showed the least activity. Our results agree with those of other studies reporting the high level of susceptibility of this species to most antimicrobial agents (1, 7, 9, 16, 19, 24, 25).

Interestingly, Houang et al. (9) described some cases of resistance of *Acinetobacter* genospecies 3 to imipenem, amikacin, gentamicin, ceftazidime, rifampin, sulbactam, and cotrimoxazole. Antibiotics with poor activity against *A. baumannii* (21), such as tetracycline, ciprofloxacin, ceftazidime, and gentamicin, showed good activity against *Acinetobacter* genospecies 3. Therefore, when a highly antibiotic-susceptible clinical isolate is identified as *A. baumannii*, it may be suspected to be another species of *Acinetobacter*; thus, further genetic identification should be performed, since a correct identification is necessary for surveillance and epidemiological studies.

To study the mechanisms of resistance to β -lactam antibiot-

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TABLE 1. Distribution of antibiotic susceptibilities for isolates of *Acinetobacter* genospecies 3

Antimicrobial agent	Range of MICs ($\mu\text{g/ml}$)	MIC ₅₀ ^d	MIC ₉₀	%R ^e
Ampicillin ^a	8–128	32	128	66.7
Piperacillin	32–512	32	64	6.7
Cephalothin ^a	256	256	256	100
Cefoxitin ^a	128–256	128	256	100
Ceftazidime	1–8	8	8	0
Cefepime	1–32	4	16	6.7
Sulbactam ^{a,b}	1–8	1	2	0
Ampicillin-sulbactam	1–4	2	2	0
Imipenem	0.12–1	0.12	0.5	0
Meropenem	<0.5–2	<0.5	1	0
Nalidixic acid ^a	4–1,024	8	16	6.7
Ciprofloxacin	<0.5–32	<0.5	1	6.7
Gentamicin	<1–16	<1	4	6.7
Tobramycin	0.25–8	0.5	8	0
Amikacin	0.5–16	1	4	0
Tetracycline	<1–4	<1	4	0
Doxycycline	<0.5	<0.5	<0.5	0
Minocycline	<1	<1	<1	0
Azithromycin ^a	0.5–64	4	64	20
Rifampin ^c	2–16	4	8	66.7
Cotrimoxazole	<0.5–2	<0.5	1–2	0
Colistin ^b	0.25–2	1	2	0

^a The breakpoints used for ampicillin, cephalothin, cefoxitin, sulbactam, nalidixic acid, and azithromycin were those recommended by the NCCLS for *Enterobacteriaceae* (12).

^b The breakpoints used for colistin and sulbactam were those described by MENSURA (11). For colistin, ≥ 4 (resistant); for sulbactam, ≥ 32 (resistant).

^c *Neisseria meningitidis* and *Haemophilus* spp. are the only gram-negative bacteria for which the NCCLS gives interpretative criteria for rifampin, these being MIC $\leq 1\mu\text{g/ml}$ (susceptible) and MIC $\geq 4\mu\text{g/ml}$ (resistant) (12).

^d MIC₅₀, MIC at which 50% of the isolates were inhibited.

^e %R, percent resistant isolates.

ics, the presence of TEM, OXA 1-4-like, OXA 2-3-like, OXA 5-7-like (5'-TATATTCCAGCATCAACATT-3' and 5'-ATG ATGCCCTCACTTGCCAT-3'), and OXA 20-37-like β -lactamases, AmpC chromosomal cephalosporinase, and integrons by PCR with primers and conditions previously described (3, 5, 13). The determination of the β -lactamase isoelectric point was performed as described by Mathew et al. (10). The results of the isoelectric focusing assay showed the production of a β -lactamase with a pI of >8 in the 15 strains. Neither OXA-type or TEM-type β -lactamases nor integrons of type 1 were amplified by PCR in any strain. However, when specific primers were used for the *AmpC* gene of *A. baumannii*, a PCR product was obtained for all the studied strains (data not shown), suggesting that the expression of this AmpC cephalosporinase, probably of chromosomal origin, may play a role in the resistance to some β -lactam antibiotics, although other concomitant mechanisms of resistance cannot be discarded.

The MICs of ampicillin and ceftazidime were also determined in either the absence or the presence of 4 μg of Syn2190/ml (5), known to inhibit chromosomal AmpC β -lactamases, including that of *A. baumannii* (5, 14). This inhibitor did not affect the MICs of ampicillin and ceftazidime in *Acinetobacter* genospecies 3. These results may suggest either the presence of possible genetic differences between the AmpC β -lactamases of *A. baumannii* and *Acinetobacter* genospecies 3 or the presence of marked differences between the membrane permeability of both species of the complex.

Regarding quinolone resistance, PCR amplification of the

QRDR sequence produced by the *gyrA* and *parC* genes was undertaken by using the primers and following the conditions previously described (22, 23). The EMBL accession numbers for the *gyrA* and *parC* genes of *Acinetobacter* genospecies 3 are AY204699 and AY204702, respectively. Only one strain (AC060) was resistant to quinolones and presented a substitution of Ser for Leu in positions 83 of GyrA and 80 of ParC. These results agree with previous reports for *A. baumannii*, in which mutations in both the *gyrA* and *parC* genes are required to obtain high levels of fluoroquinolone resistance (22, 23).

The MICs of nalidixic acid (Sigma) were also determined in either the absence or the presence of 20 μg of Phe-Arg- β -naphthylamide (MC207,110; Sigma) per ml (15), an efflux pump inhibitor. The MIC of nalidixic acid decreased at least fourfold in 60% (9 of 15) of the isolates (Table 2). According to these results, it may be suggested that, similar to *A. baumannii* (15), genospecies 3 possesses an efflux pump inhibited by MC207,110 that is able to pump nalidixic acid out of the cell. This decrease in nalidixic acid accumulation may provide a basal level of resistance to this antimicrobial agent in this *Acinetobacter* species.

In summary, we have described the antimicrobial susceptibility and the mechanisms of resistance to β -lactam antibiotics and quinolones in 15 clinical isolates of *Acinetobacter* genospecies 3. Our results suggest that, in spite of the high level of susceptibility to most antimicrobial agents, the percentage of isolates resistant to ampicillin, cephalothin, and cefoxitin is high. On the other hand, the resistance to quinolones is associated with mutations in both the *gyrA* and *parC* genes and, in the case of nalidixic acid, with the concomitant expression of an efflux system.

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TABLE 2. MICs of nalidixic acid in the absence and in the presence of 20 μg of Phe-Arg- β -naphthylamide (MC207,110) per ml

No. of strains	MICs of nalidixic acid ($\mu\text{g/ml}$)		Level of affection
	Without I ^a	With I	
14	8	4	2×
20	16	2	8×
21	16	2	8×
52	8	2	4×
56	4	2	2×
60	1,024	128	8×
65	8	4	2×
67	16	2	8×
69	4	2	2×
90	16	8	2×
103	4	2	2×
109	8	2	4×
128	16	2	8×
195	8	2	4×
243	8	2	4×

^a I, Phe-Arg- β -naphthylamide.

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REFERENCES

- Bergogne-Berezin, E., and K. J. Towner. 1996. *Acinetobacter* spp. as nosocomial pathogens: microbiological, clinical, and epidemiological features. *Clin. Microbiol. Rev.* **9**:148–165.
- Bernards, A. T., J. Van der Toorn, C. P. A. Van Boven, and L. Dijkshoorn. 1996. Evaluation of the ability of the API 20NE system to identify *Acinetobacter* genomic species. *Eur. J. Clin. Microbiol. Infect. Dis.* **15**:303–308.
- Bou, G., and J. Martínez-Beltrán. 2000. Cloning, nucleotide sequencing, and analysis of the gene encoding an AmpC β -lactamase in *Acinetobacter baumannii*. *Antimicrob. Agents Chemother.* **44**:428–432.
- Bouvet, P. J. M., and P. A. D. Grimont. 1986. Taxonomy of the genus *Acinetobacter* with the recognition of *Acinetobacter baumannii* sp. nov., *Acinetobacter haemolyticus* sp. nov., *Acinetobacter johnsonii* sp. nov., and *Acinetobacter junii* sp. nov. and emended descriptions of *Acinetobacter calcoaceticus* and *Acinetobacter iwoffii*. *Int. J. Syst. Bacteriol.* **36**:228–240.
- Danés, C., M. M. Navia, J. Ruiz, F. Marco, A. Jurado, M. T. Jiménez de Anta, and J. Vila. 2002. Distribution of β -lactamases in *Acinetobacter baumannii* clinical isolates and the effect of Syn2190 (AmpC inhibitor) in their MICs to different β -lactam antibiotics. *J. Antimicrob. Chemother.* **50**:261–264.
- Gerner-Smidt, P., and I. Tjernberg. 1993. *Acinetobacter* in Denmark. II. Molecular studies of the *Acinetobacter calcoaceticus*-*Acinetobacter baumannii* complex. *APMIS* **101**:826–832.
- Henwood, C. J., T. Gatward, M. Warner, D. James, M. W. Stockdale, R. P. Spence, K. J. Towner, D. M. Livermore, and N. Woodford. 2002. Antibiotic resistance among clinical isolates of *Acinetobacter* in the UK, and *in vitro* evaluation of tigecycline (GAR-936). *J. Antimicrob. Chemother.* **49**:479–487.
- Horrevorts, A., K. Bergman, L. Kollée, I. Breuker, I. Tjernberg, and L. Kijkshoorn. 1995. Clinical and epidemiological investigations of *Acinetobacter* genomospecies 3 in a neonatal intensive care unit. *J. Clin. Microbiol.* **33**:1567–1572.
- Houang, E. T. S., Y. W. Chu, K. Y. Chu, K. C. Ng, C. M. Leung, and A. G. B. Cheng. 2003. Significance of genomic DNA group delineation in comparative studies of antimicrobial susceptibility of *Acinetobacter* spp. *Antimicrob. Agents Chemother.* **47**:1472–1475.
- Mathew, M., A. M. Harris, M. J. Marshall, and G. W. Ross. 1975. The use of analytical isoelectric focusing for detection and identification of β -lactamases. *J. Gen. Microbiol.* **88**:169–178.
- Mesa Española de Normalización de la Sensibilidad y Resistencia a los Antimicrobianos. 2000. Recomendaciones del grupo MENSURA para la selección de antimicrobianos en el estudio de la sensibilidad y criterios para la interpretación del antibiograma. *Rev. Esp. Quimioter.* **13**:73–86.
- National Committee for Clinical Laboratory Standards. 2001. Performance standards for antimicrobial susceptibility testing: approved standard M100-S11. NCCLS, Wayne, Pa.
- Navia, M. M., L. Capitano, J. Ruiz, M. Vargas, H. Urassa, D. Schelleberg, J. Gascon, and J. Vila. 1999. Typing and characterization of mechanisms of resistance of *Shigella* spp. isolated from feces of children under 5 years of age from Ifakara, Tanzania. *J. Clin. Microbiol.* **37**:3113–3117.
- Nishida, K., C. Kunugita, T. Uji, F. Higashitani, A. Hyodo, N. Unemi, S. N. Maiti, O. A. Phillips, P. Spevak, K. O. Atchison, S. M. Salama, H. Atwal, and R. G. Micetic. 1999. *In vitro* and *in vivo* activities of Syn2190, a novel β -lactamase inhibitor. *Antimicrob. Agents Chemother.* **43**:1895–1900.
- Ribera, A., J. Ruiz, M. T. Jiménez de Anta, and J. Vila. 2001. Effect of an efflux pump inhibitor on the MIC of nalidixic acid for *Acinetobacter baumannii* and *Stenotrophomonas maltophilia* clinical isolates. *J. Antimicrob. Chemother.* **49**:697–698.
- Seifert, H., R. Baginski, A. Schulze, G. Pulverer. 1993. Antimicrobial susceptibility of *Acinetobacter* species. *Antimicrob. Agents Chemother.* **37**:750–753.
- Shi, Z. Y., P. Y. Liu, Y. Lau, Y. Lin, B. S. Hu, and J. M. Shir. 1996. Antimicrobial susceptibility of clinical isolates of *Acinetobacter baumannii*. *Diagn. Microbiol. Infect. Dis.* **24**:81–85.
- Tjernberg, I., and J. Ursing. 1989. Clinical strains of *Acinetobacter* classified by DNA-DNA hybridization. *APMIS* **79**:595–605.
- Traub, W. H., and M. Spohr. 1989. Antimicrobial drug susceptibility of clinical isolates of *Acinetobacter* species (*A. baumannii*, *A. haemolyticus*, genomospecies 3, and genomospecies 6). *Antimicrob. Agents Chemother.* **33**:1617–1619.
- Vanechoutte, M., L. Dijkshoorn, I. Tjernberg, A. Elaichouni, P. de Vos, G. Claeys, and G. Verschraegen. 1995. Identification of *Acinetobacter* genomic species by amplified ribosomal DNA restriction analysis. *J. Clin. Microbiol.* **33**:11–15.
- Vila, J., A. Marcos, F. Marco, S. Abdalla, Y. Vergara, R. Reig, R. Gomez-Lus, and 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.
- Vila, J., J. Ruiz, P. Goñi, and M. T. Jimenez de Anta. 1997. Quinolone-resistance mutations in the topoisomerase IV *parC* gene of *Acinetobacter baumannii*. *J. Antimicrob. Chemother.* **39**:757–762.
- Vila, J., J. Ruiz, P. Goñi, A. Marcos, and M. T. Jimenez de Anta. 1995. Mutation in the *gyrA* gene of quinolone-resistant clinical isolates of *Acinetobacter baumannii*. *Antimicrob. Agents Chemother.* **39**:1201–1203.
- Visalli, M. A., M. R. Jacobs, T. D. Moore, F. A. Renzi, and P. C. Appelbaum. 1997. Activities of β -lactams against *Acinetobacter* genomospecies as determined by agar dilution and E-test MIC methods. *Antimicrob. Agents Chemother.* **41**:767–770.
- Wisplinghoff, H., M. B. Edmond, M. A. Pfaller, R. N. Jones, R. P. Wenzel, and H. Seifert. 2000. Nosocomial bloodstream infections caused by *Acinetobacter* species in United States hospitals: clinical features, molecular epidemiology, and antimicrobial susceptibility. *Clin. Infect. Dis.* **31**:690–697.