

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

A. Ribera,¹ F. Fernández-Cuenca,² A. Beceiro,³ G. Bou,³ L. Martínez-Martínez,²
A. Pascual,² J. M. Cisneros,⁴ J. Rodríguez-Baño,⁵ J. Pachón,⁴ J. Vila,^{1*}
and the Spanish Group for Nosocomial Infection (GEIH)[†]

Servei de Microbiologia, Institut Clínic Infeccions i Immunologia, IDIBAPS, Hospital Clínic, 08036 Barcelona,¹
Servicios de Microbiología² y de Enfermedades Infecciosas,⁵ Hospital Virgen de la Macarena, 41071 Seville,
Servicio de Enfermedades Infecciosas, Hospital Universitario Virgen del Rocío, 41013 Seville,⁴
and Servicio de Microbiología, Hospital Juan Canalejo, La Coruña,³ Spain

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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 (Gallosa, 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-

* Corresponding author. Mailing address: Department of Microbiology, Hospital Clínic, Facultat de Medicina, Universitat de Barcelona, Villarroel 170, 08036 Barcelona, Spain. Phone: 34.93.2275522. Fax: 34.93.2275454. E-mail: vila@medicina.ub.es.

[†] Contributing members of the Spanish Group for Nosocomial Infection are listed in Acknowledgments.

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

Antimicrobial agent	Range of MICs (µg/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 ≤ 1 µg/ml (susceptible) and MIC ≥ 4 µg/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'-ATGATGCCCTCACTTGCCAT-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.

Members of the Spanish Group for Nosocomial Infections (GEIH) of the Spanish Society of Infectious Diseases and Clinical Microbiology: Javier Ariza, M. Angeles Domínguez, Miquel Pujol, and Fe Tubau (Hospital Universitario de Bellvitge, Barcelona); Juan Pablo Horcajada, Anna Ribera, and Jordi Vila (Hospital Clinic, Barcelona); Jordi Cuquet, Carmina Martí, and Dolors Navarro (Hospital General de Granollers, Barcelona); Francisco Alvarez Lerma and Margarita Sal-

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 (µg/ml)		Level of affectation
	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.

vadó (Hospital del Mar, Barcelona); Fernando Chaves and Antonio Sánchez Porto (Hospital de la Línea de la Concepción, Cádiz); Fernando Rodríguez López and Elisa Vidal (Hospital Universitario Reina Sofía, Córdoba); Alejandro Beceiro and German Bou (Hospital Juan Canalejo, A Coruña); Manuel de la Rosa (Hospital Virgen de las Nieves, Granada); Fernando Chaves and Manuel Lisazoain (Hospital Doce de Octubre, Madrid); Paloma García Hierro and Josefa Gómez Castillo (Hospital de Getafe, Madrid); Belen Padilla (Hospital Gregorio Marañón, Madrid); Jesús Martínez Beltrán (Hospital Ramón y Cajal, Madrid); Manuel López Brea and Lucía Pérez (Hospital Universitario de la Princesa, Madrid); Manuel Causse and Pedro Manchado (Centro Hospitalario Carlos Haya, Málaga); Inés Dorronsoro and José Javier García Irure (Clínica de Navarra, Navarra); Almudena Tinajas (Complejo Hospitalario de Orense, Orense); Gloria Esteban and Begoña Fernández (Hospital Santa María de Nai, Orense); Nuria Borrell and Antonio Ramírez (Hospital Son Dureta, Palma de Mallorca); Isabel Alamo and Diana García Bardeci (Hospital del Pino, Las Palmas de Gran Canaria); José Angel García Rodríguez (Hospital Universitario, Salamanca); Carmen Fariñas and Carlos Fernández Mazarrasa (Hospital Marqués de Valdecilla, Santander); Eduardo Varela and Mercedes Treviño (Hospital Universitario, Santiago de Compostela); Luis Martínez, Alvaro Pascual, and Jesús Rodríguez Baño (Hospital Universitario Virgen Macarena, Seville); Ana Barreiros, José Miguel Cisneros, Jerónimo Pachón, and Trinidad Prados (Hospitales Universitarios Virgen del Rocío, Seville); Frederic Ballster (Hospital Universitario de Reus, Tarragona); María Eugenia García Leoni and Ana Leturia (Centro Nacional de Paraplégicos, Toledo); Susana Brea and Enriqueta Muñoz (Hospital Virgen de la Salud, Toledo); and Joaquina Sevillano and Irene Rodríguez Conde (Povisa, Vigo).

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