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 A. baumannii and genospecies 3. Therefore, 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 (Gallos, 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-
the presence of TEM, OXA 1-4-like, OXA 2-3-like, OXA 37-like (5’-TATATTCCAGCATCAAATT-3’ and 5’-ATG ATGCCTCACTTGGCAT-3’), and OXA 20-37-like β-lactama-
es, 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 nor 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 gyrA 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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