Antibiotic Susceptibilities of Parachlamydia acanthamoeba in Amoebae

M. Maurin, A. Bryskier, and D. Raoult

Unité des Rickettsies, CNRS UPRES A 6020, Faculté de Médecine, Université de la Méditerranée, 13385 Marseille Cedex 05, and Anti-Infective Research Department, Hoechst-Marion-Roussel, 93235 Romainville, France

Received 6 August 2001/Returned for modification 8 April 2002/Accepted 5 June 2002

Parachlamydia acanthamoeba are intracellular bacteria of amoebae and are considered potential etiological agents of human pneumonia. We have determined the in vitro antibiotic susceptibilities of two strains (strain Bn₁ and Hall’s coccus) in Acanthamoeba polyphaga. The two strains were susceptible to tetracyclines, macrolides, and rifampin, but resistant to fluoroquinolones.

Based on 16S ribosomal DNA sequence comparison, the taxonomic classification of species belonging to the order Chlamydiales has been recently reassessed, and a new family, Parachlamydiaceae, has been proposed (13). This family now comprises two genera: i.e., the genus Parachlamydia with Parachlamydia acanthamoeba as a type species (2), and the genus Neochlamydia with Neochlamydia hartmannellae as the type species (16). Strains belonging to the species P. acanthamoeba include the type strain Bn₁ (ATCC VR 1476), isolate Berg₁, Hall’s coccus (21), and unnamed isolates (2, R. J. Birtles, T. J. Rowbotham, C. Storey, T. J. Marrie, and D. Raoult, Letter, Lancet 349:925-926, 1997), which are all strictly intracellular bacteria with variable Gram staining properties and which display more than 99% 16S rRNA gene similarity (Birtles et al., Letter). Parachlamydia spp. naturally infect Acanthamoeba. Trophozoites of Acanthamoeba hosting chlamydia-like bacteria have been isolated in patients with fever associated with use of humidifiers in Vermont (i.e., Hall’s mydia-like bacteria have been isolated in patients with fever) (3, 6) apply to this group of pathogens.

Bacterial and amoebal strains. P. acanthamoeba strain Bn₁ was kindly provided by R. Amann (Lehrstuhl für Mikrobiologie, Technische Universität München, Munich, Germany), whereas Hall’s coccus was a gift from T. J. Robotham (Public Health Laboratory, Leeds, United Kingdom). Parachlamydia organisms were cultured in Acanthamoeba polyphaga, grown in 25-cm² culture flasks (Becton Dickinson, Le Pont de Clai, France) containing PYG medium (35) until almost complete lysis of amoebae (i.e., 4 days later). Cell supernatants were then recovered and centrifuged at 1,500 rpm (700 × g) for 10 min to remove cell debris. A Parachlamydia inoculum was prepared for each strain tested by diluting supernatants 1:100 in Page’s amoebal saline (28), which corresponded to approximately 10⁸ bacteria/ml. Titration of Parachlamydia was obtained by inoculating 10-fold serial dilutions of the primary inoculum to uninfected amoebal cultures and determining the highest dilution allowing lysis of amoebal monolayers after 4 days of incubation of cultures at 30°C.

Determination of MICs. Uninfected amoebae, cultured in PYG medium, were harvested by gentle shaking of monolayers and dispensed (160 µl per well of a 5.10⁵-organism/ml inoculum) in 96-well microtiter plates (D. Dutcher, Brumath, France). Each well received 20 µl of Parachlamydia inoculum (i.e., final inoculum of about 10⁶ bacteria/ml). After a 2-h incubation of infected amoebal cultures at 30°C, antibiotics were added (i.e., 20 µl of 10-fold the desired final concentrations). Controls were drug-free uninfected amoebae (as amoebal viability controls), uninfected amoebae with the various antibiotic concentrations tested (as amoebal antibiotic toxicity controls), and drug-free infected amoebae (as Parachlamydia growth controls). We also verified that the P. acanthamoeba strains tested did not grow in Page’s amoebal saline in the absence of amoebae. Drug-free controls received 20 µl of saline instead of the antibiotic solution. Cultures were incubated at 30°C and observed each day under an inverted microscope at a magnification of ×400 until complete lysis of amoebal monolayers in Parachlamydia-infected drug-free controls occurred. By this simple technique, MICs corresponded to the lowest antibiotic concentration that prevented Parachlamydia growth (i.e., destruction of amoebal cultures after 4 days of incubation). Escherichia coli C.I.P. 53.126 and Staphylococcus aureus C.I.P. 103811 were obtained from the Pasteur Institute (Institut Pasteur, Marnes La Coquette, France) and were used to control the antibiotic concentrations tested, with Mueller-
Hinton broth as the antibiotic assay medium according to the procedure recommended by the National Committee for Clinical Laboratory Standards (27).

No antibiotic concentration tested displayed a toxic effect against amoebae. MICs for E. coli C.I.P. 53.126 and Staphylococcus aureus C.I.P. 103811 were compatible with those determined by the Pasteur Institute. P. acanthamoeba strains grew well in A. polyphaga cells, with complete lysis of amoebal monolayers in drug-free cultures after 4 days of incubation at 30°C. Among the β-lactams tested, penicillin G, amoxicillin, ceftriaxone, and imipenem were ineffective at concentrations up to 32 μg/ml (Table 1). Both strains were susceptible to aminoglycosides, macrolides (including the newer ketolide, telithromycin), doxycycline, cotrimoxazole, and rifampin. In contrast, vancomycin, the activity of which is almost restricted to gram-positive bacteria, was ineffective. Thiophenicol and, more importantly, the fluoroquinolone compounds ofloxacin and ciprofloxacin were not bacteriostatic at the concentrations tested.

We have evaluated in vitro susceptibilities of two strains belonging to the species P. acanthamoeba (i.e., BN9 and Hall’s coccus), with A. polyphaga as an in vitro cell system to support growth of these strictly intracellular bacteria. An amoebal system was used because of the impossibility of growing these bacteria in the other cell systems we currently use in our laboratory, including McCoy cells, Vero cells, P388D1 macrophage-like cells, or human embryonic lung fibroblast cells. Our model was based upon inhibition of amoebal lysis due to bacterial multiplication when antibiotics were added to the culture supernatant compared to that of drug-free controls. Thus, it was critical to verify that amoebal lysis was not related to antibiotic toxicity. Despite these technical limitations, our model allowed us for the first time to define the antibiotic susceptibility pattern of P. acanthamoeba and to compare it with those previously reported for C. trachomatis, Chlamydothila pneumoniae, and Chlamydiwphila psittaci, species that also belong to the order Chlamydiales.

P. acanthamoeba strains BN9 and Hall’s coccus were found resistant to all β-lactams tested. The in vitro activity of β-lactams against C. trachomatis (4, 8, 25), C. pneumoniae (18), and C. psittaci (23, 40) has been demonstrated. Although these antibiotics are not considered first-line antibiotic therapy for Chlamydia-related pneumonia (19), amoxicillin has been shown to be bacteriostatic against C. trachomatis (9, 20, 39). In contrast, we found aminoglycosides to be bacteriostatic against P. acanthamoeba strains, whereas C. trachomatis has been shown to be highly resistant to gentamicin (17, 32, 41). Cotrimoxazole could inhibit the growth of P. acanthamoeba and is also effective against C. trachomatis (38). In contrast, C. pneumoniae and C. psittaci are resistant to this antibiotic combination. More surprisingly, P. acanthamoeba strains were found to be resistant to fluoroquinolones, whereas C. trachomatis, C. pneumoniae, and C. psittaci are highly susceptible to these drugs (24, 26, 31). DNA gyrase is usually the primary target of fluoroquinolones in gram-negative bacteria (11, 29), and resistance to fluoroquinolones due to mutation in gisA (the gene encoding the alpha subunit of DNA gyrase) has been reported in C. trachomatis (10). The possibility of gisA-mediated natural resistance to fluoroquinolones in P. acanthamoeba should be assessed.

Our results should be specifically examined considering the potential role of Parachlamydia spp., as etiological agents of human pneumonia (2, 21). β-Lactams are considered first-line antibiotic therapy of Streptococcus pneumoniae-related pneumonia, but are poorly effective against intracellular pathogens responsible for atypical pneumonia, such as Chlamydia spp., Legionella pneumophila, Mycoplasma pneumoniae, or Coxella burnetii (3, 6). This may also apply for P. acanthamoeba, a species resistant in vitro to these agents. In contrast, the susceptibility of P. acanthamoeba to macrolides and tetracycline suggests that the current practice of prescribing a macrolide or a tetracycline compound in patients with atypical pneumonia may well apply in case of Parachlamydia infection. The new ketolide compound telithromycin, which is active against eryth-

### Table 1. MICs for Parachlamydia sp., including the BN9 strain and Hall’s coccus, as determined in an A. polyphaga culture model

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>MIC (μg/ml) for:</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Parachlamydia</td>
<td></td>
</tr>
<tr>
<td></td>
<td>BN9</td>
<td>Hall’s coccus</td>
</tr>
<tr>
<td>Penicillin G</td>
<td>&gt;32</td>
<td>&gt;32</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>&gt;32</td>
<td>&gt;32</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>&gt;32</td>
<td>&gt;32</td>
</tr>
<tr>
<td>Imipenem</td>
<td>&gt;32</td>
<td>&gt;32</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>0.5</td>
<td>1</td>
</tr>
<tr>
<td>Amikacin</td>
<td>1</td>
<td>0.5</td>
</tr>
<tr>
<td>Thiophenicol</td>
<td>&gt;32</td>
<td>&gt;32</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>0.5</td>
<td>1</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Clarithromycin</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Telithromycin</td>
<td>0.25</td>
<td>0.5</td>
</tr>
<tr>
<td>TMP/SMX</td>
<td>0.5/2.5</td>
<td>2/10</td>
</tr>
<tr>
<td>Rifampin</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>&gt;16</td>
<td>&gt;16</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>&gt;16</td>
<td>&gt;16</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>&gt;16</td>
<td>&gt;16</td>
</tr>
</tbody>
</table>

*a MICs for C. trachomatis, C. pneumoniae, and C. psittaci reported in the literature with corresponding references have been incorporated for comparison.

*b TMP/SMX, trimethoprim-sulfamethoxazole.
romycin-resistant S. pneumoniae (5, 30), as well as against the intracellular pathogens C. pneumoniae (33), L. pneumophila (12, 36), M. pneumoniae (42), and C. burnetii (34), was found also active against the Parachlamydia strains. Fluoroquinolones, especially ofloxacin and ciprofloxacin, have been advocated as a possible alternative to macrolides in patients suffering atypical pneumonia, although their equivalence to erythromycin in case of legionellosis is still disputed (3, 6).

Interestingly, we found P. acanthamoeba to be highly resistant to these compounds in vitro.

REFERENCES


