Broad-spectrum antibiotic resistance of Planctomycetes organisms determined by Etest

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Objectives: The in vitro susceptibility of Planctomycetes organisms to antibiotics has seldom been studied and when it has, a variety of methods have been used. The objective of the study was to expand the knowledge of Planctomycetes antibiotic susceptibility patterns.

Methods: Planctomyces maris, Planctomyces brasiliensis, Blastopirellula marina, Planctomyces limnophilus, Gemmata obscuriglobus and Rhodopirellula baltica reference strains were tested for in vitro susceptibility to 18 antibiotics, representing 11 antibiotic families, using the Etest method.

Results: All Planctomycetes organisms were found to be resistant to β-lactams, with MICs of >32 mg/L for penicillin G and imipenem, and MICs of >256 mg/L for ampicillin, cefalotin and ceftriaxone. The organisms were resistant to nalidixic acid and vancomycin (MIC >32 mg/L), but susceptible to tetracycline (MICs < 0.016–0.05 mg/L) and doxycycline (MICs <0.016–1 mg/L). The MIC of gentamicin ranged from 1 mg/L (P. limnophilus) to >256 mg/L (B. marina and P. brasiliensis); the MIC of erythromycin ranged from 0.032 mg/L (P. limnophilus) to 2 mg/L (P. brasiliensis); the MIC for ciprofloxacin ranged from 0.008 mg/L (R. baltica) to >32 mg/L (P. brasiliensis); and the MIC for colistin ranged from 0.125 mg/L (P. limnophilus) to 96 mg/L (B. marina).

Conclusions: In addition to shedding new light on the biology of Planctomycetes organisms, these data could be used for the further phenotypic characterization of Planctomycetes organisms, and for the optimization of culture media for the primary isolation and growth of Planctomycetes organisms from contaminated specimens.

Keywords: antimicrobial agent, susceptibility testing, PVC phylum

Introduction

The phylum Planctomycetes is composed of environmental microorganisms characterized by budding reproduction, a peptidoglycan-less cell wall and cell compartmentation. It is phylogenetically closely related to Verrucomicrobia and Chlamydiae, which accommodate major human and veterinary pathogens, and is combined with them in the so-called PVC super-phylum. Knowledge of antibiotic resistance in Planctomycetes may therefore elucidate that in PVC organisms at large. The peptidoglycan-less characteristic of Planctomycetes bacteria explains a natural resistance to peptidoglycan synthesis inhibitors, including β-lactams, glycopeptides and D-cycloserine. The incorporation of the latter antibiotics in primary culture media has assisted in the isolation of Planctomycetes organisms from contaminated specimens. However, few studies have been published regarding the in vitro susceptibility of Planctomycetes organisms to antibiotics. The existing studies included relatively few antibiotics and Planctomycetes organisms. Additionally, testing methods were not standardized, being based on the antibiotic disc diffusion method, on the agar microdilution method or on macrodilution with direct observation of turbidity.

In an effort to increase our knowledge of Planctomycetes antibiotic susceptibility, we determined the susceptibility of representatives of the Planctomycetes phylum to 11 antibiotic families using the standardized Etest method.

Methods

Strains and culture

Seven reference isolates were studied, including Planctomyces maris ATCC 29201, Planctomyces brasiliensis ATCC 49424, P. brasiliensis ATCC 49425, Blastopirellula marina ATCC 49069 and Planctomyces limnophilus ATCC 65; 2119–2122
43296, all of which were purchased from the American Tissue Culture Collection (ATCC, Rockville, MA). Gemmata obscuriglobus DSM 58311 and Rhodopirellula baltica DSM 105277 were purchased from the German Collection of Microorganisms (DSMZ, Braunschweig, Germany). P. brasilensis, P. maris, R. baltica and B. marina were grown on half-strength marine agar (18.7 g of Difco marine broth 2216 per litre of sterile water; 15 g of agar sterilized by autoclaving at 121°C for 15 min) at 30°C for 7 days. P. limnophilus and G. obscuriglobus were grown on Caulobacter agar (2 g of peptone, 1 g of yeast extract, 0.2 g of MgSO4.7H2O and 10 g of agar per litre of sterile water, sterilized by autoclaving at 121°C for 15 min) at 30°C for 7 (G. obscuriglobus) or 14 days (P. limnophilus).

Antimicrobial susceptibility testing

A plate of appropriate medium (half-strength marine agar or Caulobacter agar) was flooded using 3 mL of a 2.5±0.2 MacFarland unit suspension of bacteria; the excess suspension was removed and the plate was dried at room temperature for 5 min under the air flow of a microbiological safety cabinet. An Etest strip (Biomerieux, Craponne, France) was deposited on the surface of the agar medium using sterile forceps. The plate was then incubated at 30°C and growth was observed after incubating for 7 days (except P. limnophilus, which was observed after incubating for 14 days). All tests were done in duplicate. A total of 18 antibiotics representing 11 antibiotic families were tested (Table 1). One clinical isolate of each of Staphylococcus aureus, Enterococcus faecalis and Escherichia coli with known antimicrobial susceptibility patterns were used as controls on plates of both media incubated for 1 day at 30°C to ensure the activity of Etest antibiotics. When S. aureus and E. faecalis strains were both susceptible to the same antibiotic (vancomycin and teicoplanin), we used the most susceptible strain as a control. (S. aureus was used to test vancomycin and E. faecalis to test teicoplanin). A Nikon TMS inverted phase-contrast microscope with objective E.10 0.25 160× (Nikon France S. A. S., Champigny Sur Marne, France) was used (100× magnification) to read the limit of growth inhibition. The presence of β-lactamase was tested twice with the cefinase test following the supplier’s instructions (Becton-Dickinson, Le Pont de Clai, France). A β-lactamase-producing Haemophilus influenzae isolate was used as a positive control.

Results and discussion

The limit of growth inhibition around the Etest strip was difficult to determine by the naked eye for B. marina and P. maris. Etest reading was therefore performed using an inverted microscope for these two strains. For antibiotic susceptibility controls, we observed no MIC variation for E. coli, E. faecalis or S. aureus on either set of medium.

We observed that Planctomycetes were resistant to β-lactams and glycopeptides, a feature previously linked to the absence of peptidoglycan in their cell wall (Table 1).1,5,7,9 Accordingly, we found that Planctomycetes lacked a β-lactamase in the presence of a positive control. These data agree with our observation that Pirellula staleyi and R. baltica genomes lack genes coding for essential peptidoglycan synthesis and lysis enzymes, i.e. glycosyltransferase (GT) 28 including murG, GT 51 including peptidoglycan glycosyltransferase, glycoside hydrolase (GH) 23 including lytic transglycosylases and GH 73. Candidatus Kueneiia and the Chloromya genomes encode only one enzyme of the GT 28 family (C. Cayrou, M. Drancourt and B. Henrissat, unpublished data).

Most Planctomycetes organisms were resistant to chloramphenicol and to the aminoglycoside gentamicin, targeting the 23S and the 16S rRNA of the 50S ribosomal subunit, respectively, and to rifampicin, a bacterial RNA polymerase inhibitor (Table 1).6 Rifampicin resistance has been primarily associated with mutations in the rpoB gene encoding the RNA polymerase β-subunit, reducing the affinity of the drug to the target.9 At least three other bacterial phyla include organisms naturally resistant to rifampicin, without phylogenetic relationships between these phyla (Figure 1). In Planctomycetes, the mechanism of resistance for these antibiotics is not elucidated. For chloramphenicol, we were unable to find any chloramphenicol-inactivating enzyme usually associated with resistance encoded by bacterial genomes. However, we observed that Planctomycetes genomes encode numerous efflux pumps: the P. staleyi genome (GenBank accession number NC_013720) has six such genes that encode proteins homologous with those involved in multidrug resistance or that belong to a gene family referenced as ‘resistance factor’; and the R. baltica genome (GenBank accession number NC_005027) has 20 efflux pump genes.

We observed a variable resistance to the sulfamethoxazole/trimethoprim association (Table 1). Sulfamethoxazole and trimethoprim inhibit DNA synthesis by inhibiting dihydropteroate synthase encoded by the folP gene and dihydrofolate reductase encoded by the folA gene. We observed that five Planctomycetes genomes (GenBank NC_005027, NZ_ABUK00000000, NZ_ABGO00000000, NZ_ABEO00000000 and NZ_AANZ00000000) encode the folP gene, but only the B. marina and R. baltica genomes encode folA. These two strains were susceptible to sulfamethoxazole/trimethoprim, whereas P. maris, G. obscuriglobus and P. limnophilus were resistant, suggesting that absence of the folA gene may explain the sulfamethoxazole/trimethoprim resistance in the latter organisms. We observed an important variability in the antibiotic susceptibility pattern between organisms belonging to the same genus (Table 1). For the genus Planctomyces, we observed that P. brasilensis was resistant to all antibiotics except for cyclo, whereas P. limnophilus was susceptible to eight antibiotics.

In this study, the Etest method was used for the first time to test the in vitro susceptibility of organisms in the phylum Planctomycetes to various antibiotics. We used the largest panel of antibiotic families and molecules ever tested on Planctomycetes organisms in a single study. The scope of the study allowed for the first in-depth depiction of the spectrum of antibiotic susceptibility, resistance and variation among Planctomycetes genera (Table 1). The Etest method was easy to use, because MIC values were directly observed without the need for further calculation and without the need for any apparatus other than an inverted microscope. Inverted microscopy allowed for the precise observation of growth limits. In addition, the Etest method required a minimum of manipulations.

Knowledge of the susceptibility pattern of Planctomycetes bacteria could help improve the rational composition of culture media. Indeed, some samples could be highly contaminated by bacteria resistant to commonly used antibiotics and susceptibility profiles easily allow the selection of new antibiotics to incorporate into media. Moreover, because we observed a variability of susceptibility in the strains tested in this study,
Table 1. Etest determination of the MIC (mg/L) of 18 antibiotics for 7 Planctomycetes representative isolates

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>P. maris ATCC 29201&lt;sup&gt;T&lt;/sup&gt;</th>
<th>B. marina ATCC 49069&lt;sup&gt;T&lt;/sup&gt;</th>
<th>G. obscuriglobus DSM 5831&lt;sup&gt;T&lt;/sup&gt;</th>
<th>P. brasiliensis ATCC 49424&lt;sup&gt;T&lt;/sup&gt;</th>
<th>P. brasiliensis ATCC 49425</th>
<th>R. baltica DSM 10527&lt;sup&gt;T&lt;/sup&gt;</th>
<th>P. limnophilus ATCC 43296&lt;sup&gt;T&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tetracycline (0.002–32 mg/L)</td>
<td>0.25</td>
<td>0.38</td>
<td>&lt;0.016</td>
<td>0.094</td>
<td>0.094</td>
<td>0.5</td>
<td>&lt;0.016</td>
</tr>
<tr>
<td>Doxycline (0.002–32 mg/L)</td>
<td>0.25</td>
<td>3</td>
<td>&lt;0.016</td>
<td>0.047</td>
<td>0.032</td>
<td>1</td>
<td>&lt;0.016</td>
</tr>
<tr>
<td>Minocycline (0.002–32 mg/L)</td>
<td>0.094</td>
<td>1</td>
<td>&lt;0.016</td>
<td>0.094</td>
<td>0.064</td>
<td>2</td>
<td>&lt;0.016</td>
</tr>
<tr>
<td>Erythromycin (0.016–256 mg/L)</td>
<td>0.032</td>
<td>0.19</td>
<td>0.5</td>
<td>2</td>
<td>1</td>
<td>0.5</td>
<td>0.032</td>
</tr>
<tr>
<td>Colistin (0.064–1024 mg/L)</td>
<td>0.38</td>
<td>96</td>
<td>24</td>
<td>16</td>
<td>16</td>
<td>0.5</td>
<td>0.125</td>
</tr>
<tr>
<td>Chloramphenicol (0.016–256 mg/L)</td>
<td>&gt;256</td>
<td>6</td>
<td>12</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>16</td>
<td>0.25</td>
</tr>
<tr>
<td>Trimethoprim/sulfamethoxazole (0.002–32 mg/L)</td>
<td>&gt;32</td>
<td>0.5</td>
<td>&gt;32</td>
<td>&gt;32</td>
<td>&gt;32</td>
<td>1</td>
<td>&gt;32</td>
</tr>
<tr>
<td>Gentamicin (0.016–256 mg/L)</td>
<td>24</td>
<td>&gt;256</td>
<td>6</td>
<td>192</td>
<td>&gt;256</td>
<td>128</td>
<td>1</td>
</tr>
<tr>
<td>Rifampicin (0.002–32 mg/L)</td>
<td>&gt;32</td>
<td>&gt;256</td>
<td>&gt;32</td>
<td>&gt;32</td>
<td>&gt;32</td>
<td>&gt;32</td>
<td>&gt;32</td>
</tr>
<tr>
<td>Nalidixic acid (0.016–256 mg/L)</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>&gt;256</td>
</tr>
<tr>
<td>Ciprofloxacin (0.002–32 mg/L)</td>
<td>0.38</td>
<td>0.25</td>
<td>0.094</td>
<td>&gt;32</td>
<td>24</td>
<td>0.008</td>
<td>0.125</td>
</tr>
<tr>
<td>Penicillin G (0.002–32 mg/L)</td>
<td>&gt;32</td>
<td>&gt;32</td>
<td>&gt;32</td>
<td>&gt;32</td>
<td>&gt;32</td>
<td>&gt;32</td>
<td>&gt;32</td>
</tr>
<tr>
<td>Ampicillin (0.016–256 mg/L)</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>&gt;256</td>
</tr>
<tr>
<td>Imipenem (0.002–32 mg/L)</td>
<td>&gt;32</td>
<td>&gt;32</td>
<td>&gt;32</td>
<td>&gt;32</td>
<td>&gt;32</td>
<td>&gt;32</td>
<td>&gt;32</td>
</tr>
<tr>
<td>Cefalotin (0.016–256 mg/L)</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>&gt;256</td>
</tr>
<tr>
<td>Ceftriaxone (0.016–256 mg/L)</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>&gt;256</td>
</tr>
<tr>
<td>Teicoplanin (0.016–256 mg/L)</td>
<td>&gt;12</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>&gt;256</td>
</tr>
<tr>
<td>Vancomycin (0.016–256 mg/L)</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>&gt;256</td>
</tr>
</tbody>
</table>

Dark shading indicates susceptibility to the antibiotic, light shading indicates that the strain had intermediate susceptibility and no shading indicates that the strain was resistant. *The MIC range of the Etest strip is indicated in parentheses.*
In conclusion, the data presented indicate that the phylum Planctomycetes is composed of broad-spectrum antibiotic-resistant organisms. While the Planctomycetes organisms are naturally resistant to some antibiotic families, we observed large differences in the resistance profiles among genera and species, indicating that the systematic study of antibiotic families performed in the present study was warranted. We recommend the Etest for the accurate determination of antibiotic MICs for Planctomycetes organisms. The precise determination of the antibiotic susceptibility/resistance profile sheds new light on the biology of these organisms, and may help in the rational design of media for the primary isolation and culture of Planctomycetes organisms. Planctomycetes, being harmless organisms, may be used as model organisms to decrypt the molecular bases of antibiotic resistance in PVC organisms at large.

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**Transparency declarations**
None to declare.

**References**


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**Figure 1.** Schematic diagram adapted from Rappe and Giovannoni, featuring bacterial phyla comprising genera naturally resistant to rifampicin (black arrows) and those phyla that have either no natural resistance or for which no representatives were tested for rifampicin resistance (grey arrows). This variability could be used to select for one genus or species during isolation protocols.