Antimicrobial Susceptibilities and β-Lactamase Characterization of Capnocytophaga Species

DIANE L. ROSCOE,† S. JANET V. ZEMCOV, DAWN THORNBER, RICHARD WISE, and ALISON M. CLARKE

Division of Medical Microbiology, St. Paul's Hospital, Vancouver, British Columbia, Canada, and Department of Medical Microbiology, Dudley Road Hospital, Birmingham, England

Received 18 February 1992/Accepted 30 July 1992

Capnocytophaga species have been associated with a wide variety of infections in both immunocompetent and immunocompromised patients. On the basis of data from antimicrobial susceptibility studies, β-lactam antibiotics have been considered efficacious therapy. Six of 19 isolates from primarily clinical sources across Canada demonstrated β-lactamase production, and agar dilution susceptibility testing showed broad resistance to β-lactam antibiotics. For the β-lactamase producing isolates, clavulanate reduced the MIC of amoxicillin for 90% of the strains tested by 64-fold. Isolates were highly susceptible to clindamycin, imipenem, and ciprofloxacin. Characterization of the β-lactamases produced by two of these isolates (Van1 and Van2) was performed. Isoelectric focusing revealed an identical isoelectric point of 5.6 for both enzymes, but they had markedly different relative hydrolysis efficiencies, and different conditions were required to extract the enzymes. This study demonstrates the production of different types of β-lactamases by Capnocytophaga spp. and suggests the need to screen all clinical isolates of Capnocytophaga spp. for the presence of β-lactamases.

MATERIALS AND METHODS

Organisms. A total of 19 isolates of Capnocytophaga spp. were tested in this study, 16 of which were clinical isolates. Eight of the clinical isolates were from provinces other than British Columbia and were kindly provided by E. Pauline Ewan of the Laboratory Center for Disease Control, Ottawa, Canada, who also confirmed the identity of all the isolates in this study. The two β-lactamase-producing isolates recovered by our clinical laboratories within the period of a few months which prompted this investigation are subsequently identified as Van1 and Van2. All organisms were kept frozen in a glycerol-containing medium at −70°C until the time of the study.

Antimicrobial susceptibility studies. Testing for β-lactamase was done by using the chromogenic cephalosporin nitrocefin (BBL Microbiology Systems, Cockeysville, Md.) in accordance with the recommended procedure (31).

Susceptibility testing was performed by an agar plate dilution procedure with Wilkins-Chalgren agar. Antibiotics were obtained from their respective manufacturers and included penicillin, amoxicillin, clavulanate, cefazolin, cefoxitin, cefuroxime, cefotaxime, ceftazidime, imipenem, ciprofloxacin, gentamicin, vancomycin, clindamycin, and metronidazole. Antibiotic-containing plates were prepared on the day of testing for all beta-lactams and on the day prior to testing for the other antibiotics. Clavulanate was used in combination with amoxicillin at a concentration of 1 μg/ml and alone at 0.12, 1, and 4 μg/ml. The inoculum was prepared by making a turbid suspension in broth from 48-h cultures grown on Columbia agar base supplemented with 5% sheep blood. Direct inoculation to the antibiotic-containing plates was done with a Denley multiple inoculation device (Denley Instruments, Ltd., Billingshurst, United Kingdom). Viable surface colony counts verified that the inoculum contained 104 to 105 CFU. Incubation was in 10% CO2 at 35°C for 48 h. The MIC was defined as the lowest concentration of antibiotic that completely inhibited growth. The presence of a barely visible haze at the inoculum site was disregarded (3). Control organisms tested in parallel...
were *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), and *Bacteroides fragilis* (ATCC 25285).

**β-Lactamase characterization of Van1 and Van2.** (i) Preparation of β-lactamas. Colonies of each strain were removed from the surface of 100 blood agar plates and emulsified in 20 ml of sodium phosphate buffer, pH 7 (with the addition of 4% Triton-X for Van2). Cells were sonicated for a total of 3 min, with two 1-min cooling periods. The resultant preparations were ultracentrifuged for 30 min at 31,000 rpm at 4°C. The supernatant was used as crude β-lactamase.

(ii) Isoelectric focusing. Isoelectric focusing was carried out on broad-range acrylamide gels as described by Matthew et al. (20). As this method proved to be unsuitable for detection of Van2, both enzymes were also focused in a 4% Sephadex gel slurry, with the addition of 3% amphotline and 4% Triton-X. Electrophoresis was carried out on a water-cooled flat-bed apparatus, with a connected high-voltage power supply (Multiphorm System-LKB; Pharmacia, Uppsala, Sweden). Standard enzymes of known pI, as well as commercial isoelectric point markers (BDH, Darmstadt, Germany), were used.

(iii) Hydrolysis studies. Hydrolysis studies were carried out in a temperature-controlled spectrophotometer at 37°C as previously described (35). The reported kinetic constants were derived from half-time analysis of single-reaction progress curves (27).

**RESULTS**

All 19 isolates were tested for β-lactamase production by the nitrocefin test, and 6 gave positive results. Only one, Van1, gave a rapid reaction, turning dark pink within seconds. The other isolates developed a positive reaction more slowly, but all were positive within 5 min. The tests were observed for a total of 30 min before isolates were called negative.

Table 1 shows that the non-β-lactamase-producing strains were sensitive to all the beta-lactams tested, whereas the β-lactamase-producing strains were highly resistant to penicillin and amoxicillin. The addition of 1 µg of clavulanate per ml to amoxicillin resulted in a 64-fold lowering of the MIC for 90% of the β-lactamase-producing isolates (MIC90) and a lowering of the amoxicillin geometric mean MIC for all strains from 3.7 to 0.17 µg/ml. Results with the cephalosporins were more variable. The β-lactamase-producing isolates were highly resistant to cefazolin and were more resistant to cefoxitin, cefotaxime, and ceftazidime than non-β-lactamase-producing strains. β-Lactamase-producing and non-β-lactamase-producing strains were equally sensitive to imipenem. MIC90s of the non-beta-lactam antibiotics for all isolates indicated susceptibility to clindamycin and ciprofloxacin, resistance to gentamicin, and variable susceptibility to vancomycin and metronidazole.

Kinetic data for the enzymes from isolates Van1 and Van2 are shown in Table 2. Enzyme yields from Van2 were negligible without the addition of Triton-X. Although the MICs for the beta-lactam antibiotics, with the exception of cefoxitin and ceftazidime, were shown to be similar for Van1 and Van2 and although both produce an enzyme with an isoelectric point of 5.6, the substrate profiles of Van1 and Van2 are different. Compared with the activity against cephapridine, the enzyme from Van2 was shown to be inefficient at hydrolyzing the other cephalosporins and penicillins. However, Van1 was shown to be efficient at hydrolyzing a wide range of beta-lactams, including the extended-spectrum cephalosporins.

**Antimicrobial agent susceptibility of 19 isolates of *Capnocytophaga* spp.**

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>MIC (µg/ml)*</th>
<th>β-Lactam-negative isolates (n = 13)</th>
<th>β-Lactam-positive isolates (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
<td>50%</td>
<td>90%</td>
</tr>
<tr>
<td>Penicillin</td>
<td>≤0.008-0.25</td>
<td>0.015</td>
<td>0.25</td>
</tr>
<tr>
<td>Cefazolin</td>
<td>0.06-8</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>0.12-2</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Cefuroxime</td>
<td>0.03-2</td>
<td>0.06</td>
<td>2</td>
</tr>
<tr>
<td>Ceftazidine</td>
<td>≤0.008-2</td>
<td>0.06</td>
<td>0.25</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>≤0.008-1</td>
<td>0.03</td>
<td>0.25</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>0.008-0.5</td>
<td>0.03</td>
<td>0.25</td>
</tr>
<tr>
<td>Amox/Clav⁴</td>
<td>≤0.008-0.25</td>
<td>0.06</td>
<td>0.25</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>≤0.008-0.06</td>
<td>≤0.008</td>
<td>0.015</td>
</tr>
<tr>
<td>Metronidazole</td>
<td>0.12-64</td>
<td>2</td>
<td>16</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>≤2-64</td>
<td>8</td>
<td>16</td>
</tr>
<tr>
<td>Ciprofloxaix</td>
<td>≤0.008-0.5</td>
<td>0.06</td>
<td>0.25</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>4-64</td>
<td>32</td>
<td>&gt;64</td>
</tr>
</tbody>
</table>

* 50% and 90%, MICs for 50 and 90% of isolates tested, respectively.
*b* Amox/Clav, amoxicillin-clavulanate.

**Kinetics of beta-lactam hydrolysis by β-lactamas from Van1 and Van2**

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Van1</th>
<th>Van2</th>
<th>Kₚ (µM)</th>
<th>Rel Vₘₕ/Kₚ</th>
<th>Kₚ (µM)</th>
<th>Rel Vₘₕ/Kₚ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cephaloridine</td>
<td>760</td>
<td>100</td>
<td>108</td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>330</td>
<td>73</td>
<td>116</td>
<td>3.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cefaclor</td>
<td>351</td>
<td>60</td>
<td>216</td>
<td>2.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cefoxime</td>
<td>165</td>
<td>110</td>
<td>91</td>
<td>6.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>289</td>
<td>28</td>
<td>46</td>
<td>6.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>126</td>
<td>8</td>
<td>319</td>
<td>0.12</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Specific activity for cephaloridine of Van1 was 5.04 µmol/min/mg of protein and of Van2 was 3.41 µmol/min/mg of protein (after treatment with Triton-X). Rel Vₘₕ, relative maximum rate of metabolism.
DISCUSSION

The genus *Capnocytophaga* as described in 1979 incorporates the organisms formerly known as *Bacteroides ochronaceus* and CDC group DF-1 and includes three species (*C. ochronacea, C. sputigena,* and *C. gingivalis*) (19, 26). Distinguishing between the species is difficult, and the clinical significance of species differentiation is not known. In 1989, *C. canimorsus* (formerly CDC group DF-2) and *C. cynodegmi* (CDC group DF-2-like) were proposed as two new species in this genus (6). Previous antimicrobial susceptibility studies have included the first three named species and have shown predictable susceptibility to beta-lactam antibiotics, clindamycin, and the quinolone antibiotics; variable susceptibility to metronidazole; and fairly uniform resistance to aminoglycosides and vancomycin. There are very few reports on the susceptibility patterns of the newer additions to the genus, but the limited data available suggest similar susceptibility patterns (5, 16).

The results of this study support initial observations of others that beta-lactamase production is becoming more common in *Capnocytophaga* spp. (3, 4, 18, 30). Kinder et al. noted penicillin resistance in isolates from the subgingival flora of patients with periodontitis, usually in association with previous antibiotic therapy (18). Arlet and colleagues reported on the first case of a beta-lactamase-producing *Capnocytophaga* sp. recovered from a patient with sepsis (3). Rummens et al. included three beta-lactamase-producing isolates from immunocompromised patients in their series of 118 organisms (30), and the only beta-lactamase-producing isolate examined in detail was one of these. Foweraker et al. reported that this isolate from Rummens' collection hydrolyzed extended-spectrum cephalosporins and was thought from kinetic studies to belong to Richmond and Sykes beta-lactamase class 1c (11). The location of the gene coding for this beta-lactamase was not determined, and the enzyme was thought to be hydrophobic because isoelectric focusing could be achieved only if a nonionic detergent was added to the gel. Our study demonstrates that *Capnocytophaga* spp. may contain at least two different beta-lactamase enzymes. Van1 and Van2 produced enzymes which focused at the same isoelectric point, and the reduction in amoxicillin MICs in the presence of clavulanate suggests similar enzyme inhibition. However, the enzymes from Van1 and Van2 differed markedly in their substrate profile. The enzyme from Van2 appeared to be a membrane-associated low-efficiency cephalosporinase, properties which also appear to be shared by the enzyme described by Foweraker.

Considering the rarity of reported beta-lactamase-producing *Capnocytophaga* spp. in the literature and the fact that beta-lactamase-producing strains were not actively sought in this study, it was somewhat surprising to find that 6 of 19 (32%) *Capnocytophaga* spp. isolates, collected from primarily clinical sources across Canada, produced beta-lactamases. While the MIC<sub>90</sub>s for the beta-lactamase-negative isolates are consistent with previous published studies, the large number of beta-lactamase producers in our series resulted in a higher MIC<sub>90</sub> than previously reported for beta-lactam antibiotics. Beta-lactamase testing by the nitrocefin method predicted resistance to beta-lactams in our laboratory, and it seems appropriate to recommend that a beta-lactamase test be done on all clinical isolates of *Capnocytophaga* spp. Why Van1 was more rapidly positive than other beta-lactamase-producing isolates is not clear, although in the case of Van2 the slower nitrocefin reaction may be related to the fact that the enzyme appears to be membrane associated, and this may affect its catalytic activity in the cell.

Many infections with *Capnocytophaga* spp. occur in immunocompromised patients, which heightens the significance of identifying beta-lactamase production in this genus. As noted by others (4), empiric therapy in the febrile neutropenic host may not cover beta-lactamase-producing *Capnocytophaga* spp. Clinicians may need to consider this possibility, particularly in the immunocompromised patient with severe oral mucositis or periodontal disease or in the patient who is not responding to conventional therapy.

In summary, beta-lactamase production by *Capnocytophaga* spp. may be more common than previously realized. All clinical isolates should be tested for beta-lactamase production, and clinicians should be aware that standard empiric therapy for febrile neutropenic patients may not cover beta-lactamase-producing *Capnocytophaga* spp. Further studies are necessary to characterize the different beta-lactamases which are present.

ACKNOWLEDGMENTS

A special thanks to E. Pauline Ewan of the Special Bacteriology section of LCDC for providing eight isolates and for confirming all isolates as *Capnocytophaga* spp. and to E. Bryce and D. Henry (Vancouver General Hospital) and S. Damm (British Columbia Children’s Hospital) for providing isolates.

REFERENCES


