In Vitro Susceptibilities of Capnocytophaga Isolates to β-Lactam Antibiotics and β-Lactamase Inhibitors

ANNE JOLIVET-GOUGEON,1* ANNE BUFFET,1 CÉCILE DUPUY,1 JEAN-LOUIS SIXOU,2 MARTINE BONNAURE-MALLET,1 SANDRINE DAVID,1 AND MICHEL CORMIER1

Laboratoire de Microbiologie Pharmaceutique, UPRES-EA 1254, and Equipe de Biologie Buccale, UPRES-EA 1256, UFR Odontologie, Université de Rennes I, 35000 Rennes, France

Received 21 December 1999/Returned for modification 2 May 2000/Accepted 11 August 2000

The susceptibilities of 43 pharyngeal isolates of Capnocytophaga to beta-lactam antibiotics, alone or in combination with beta-lactamase inhibitors, were tested by an agar dilution method. The 34 beta-lactamase-positive strains were highly resistant to beta-lactams, but the intrinsic activities of clavulanate, tazobactam, and sulbactam against Capnocytophaga, even beta-lactamase producers, indicate that these beta-lactamase inhibitors could be used for empirical treatment of neutropenic patients with oral sources of infection.

The genus Capnocytophaga is composed of a group of cappnophilic, gram-negative fusiform bacteria that are part of the normal oral flora in humans and animals. Capnocytophaga species have been identified as the cause of a variety of infections in immunocompetent hosts (18). In immunocompromised and neutropenic patients, Capnocytophaga spp. have been isolated more frequently from patients with bloodstream infections, including bacteremia (2, 5, 9), and patients with endocarditis (4) with severe chemotherapy-induced ulcers (8). Variations in the prevalence, number, and proportion of Capnocytophaga spp. have been shown to occur in the dental plaque of pediatric cancer patients undergoing a course of immunosuppressive chemotherapy (22). In general, many antibiotics, including penicillins, clindamycin, macrolides, and quinolones, are effective in treating Capnocytophaga infections (6, 10, 11, 21). However, strains that produce beta-lactamases and that cause septicemia have recently been described (1, 7, 9, 19). These beta-lactamase-producing strains increase the risk of infection in neutropenic patients, especially during chemotherapy. The aim of this study was to determine the susceptibilities of 43 Capnocytophaga strains isolated from neutropenic pediatric patients to beta-lactams and beta-lactamase inhibitors.

Forty-three Capnocytophaga strains were isolated by swabbing the throats of pediatric cancer patients undergoing a course of chemotherapy in the Department of Pediatric Oncology at Centre Hospitalier Université Sud (Rennes, France). Two reference strains (Escherichia coli CIP 7624 and Staphylococcus aureus CIP 7625) were also included in the study. Throat samples were collected with sterile swabs, which were immediately taken to the Department of Microbiology, dispersed in sterile distilled water, and inoculated onto TBBP agar (4%). Trypticase soy agar supplemented with 5% sheep blood, 0.1% yeast extract [AES Laboratory, Combourg, France], 100 μg of polymyxin per ml, 50 μg of bacitracin [Sigma] per ml (15). The agar plates were incubated in a 10% CO₂ atmosphere for 5 days at 37°C. The isolates were identified on the basis of colony morphology, Gram staining, negative catalase and oxidase reactions, and API ZYM profiles (BioMérieux, Marcy l’Etoile, France) (13, 23).

All isolates were tested for beta-lactamase production by a chromogenic cephalosporin nitrocefin method (Cefinase; BBL Microbiology Systems, Cockeysville, Md.) by the recommended procedure (Becton Dickinson). The test results were recorded after 20 min.

One susceptibility testing method, not standardized for Capnocytophaga spp., was performed to determine the resistance phenotypes of the strains. An agar dilution procedure with Columbia agar base (44 g/lt; AES Laboratory) supplemented with 1% Polytite (BioMérieux), and 1% hemoglobin (Difco) and with antibiotics at various concentrations (21) was used to study the effectiveness of several beta-lactam antibiotics against the 43 pharyngeal Capnocytophaga colonizers. Pure preparations of the following antimicrobial agents were kindly supplied by the manufacturers: amoxicillin, amoxicillin-clavulanate, ticarcillin, ticarcillin-clavulanate, and clavulanate (SmithKline Beecham Laboratories, Philadelphia, Pa.), sulbactam (Plizer Inc, New York, N.Y.), piperacillin, piperacillin-tazobactam and tazobactam (Lederle Laboratories, Pearl River, N.Y.), mecillinam (Leo Pharmaceutical Products, Ballerup, Denmark), imipenem and ceftoxitin (Merck Sharp & Dohme, West Point, Pa.), aztreonam (Squibb Manufacturing, Humacao, P.R.), cefotaxime (Roussel UCLAF, Romainville, France), ceftazidime (Glaxo Wellcome, Stevenage, United Kingdom), and moxalactam (Eli Lilly & Co., Indianapolis, Ind.). Clavulanate was also combined with amoxicillin at a ratio of 1:2 and with ticarcillin at a ratio of 1:1.5; tazobactam-piperacillin was tested at a ratio of 1:8. Stock solutions of 6,400 μg/ml were prepared as recommended by the manufacturer, and serial twofold dilutions were prepared to give final concentrations in the agar medium ranging from 0.03 to 128 μg/ml. Fresh Capnocytophaga cultures grown for 48 h in brucella broth (AES Laboratory) were diluted in Mueller-Hinton broth (Difco) and inoculated onto the test medium with an automatic multipoint inoculator (Denley, Billingshurst, United Kingdom) to give an inoculation spot of 10⁷ CFU. Control plates containing no antibiotics before each set of antibiotic-containing plates were inoculated. Reference organisms were included on each plate to assess the reproducibility of the assay. The plates were incubated for from 48 h to 5 days at 37°C in 10% CO₂.

All 43 strains belonged to the Capnocytophaga ochracea-C. sputigena group. Thirty-four of the isolates produced beta-lactamase according to the nitrocefin test. The MIC breakpoints used are those recommended for anaerobes by the National Committee for Clinical Laboratory Standards (NCCLS) (16). The susceptibilities of the 43 Capnocytophaga strains to...
three beta-lactam antibiotics and three beta-lactamase inhibitors, alone or in combination, expressed as the range of MICs and the MICs at which 50% (MIC50) and 90% (MIC90) of the strains are inhibited, are shown in Table 1. No significant differences were noted when the incubation was prolonged up to 5 days or when the results of the susceptibility tests were recorded after a 48-h incubation period (data not shown). The 9 beta-lactamase-negative strains were sensitive to the beta-lactams tested, whereas the 34 beta-lactamase-positive strains were highly resistant, especially to amoxicillin, ticarcillin, and mezlocillin. The results for the cephalosporins were more variable, but all were more active against the beta-lactamase-negative strains. Cefoxitin remained active, as did imipenem and aztreonam, although most beta-lactamase-positive strains were resistant to cefazidime. The addition of clavulanate to amoxicillin or ticarcillin or the addition of tazobactam to piperacillin resulted in low MICs for all isolates. The MIC measurements (Table 1) indicated that the three beta-lactamase inhibitors (clavulanate, sulbactam, and tazobactam) all had nearly the same intrinsic activity against the Capnocytophaga strains. The reproducibility of the MIC determinations for the reference strains was good for all the antimicrobial agents tested and varied only by a twofold dilution in separate measurements, as expected (1).

The bacterial flora of the throat is a complex ecosystem. In pediatric cancer patients, it is influenced by the disease itself as well as numerous drugs, including antimicrotics, corticosteroids, and antibiotics, which may lead to the selection of multidrug-resistant bacteria. Resistance to antibiotics acts as a virulence factor, favoring the spread of localized and systemic infections. Periodic sampling of the pharyngeal flora of neutropenic children using appropriate media has been used to detect the presence of Capnocytophaga. However, susceptibility testing by routine culture techniques is difficult because Capnocytophaga is a slowly growing, fastidious organism. Many different media have been used to determine MICs. In this study, beta-lactam MIC reproducibility was better on hemoglobin-supplemented medium with a 48-h incubation, which is in agreement with the work of Rummens et al. (21). The agar dilution technique is recognized as the best method for evaluation of the susceptibility to antimicrobial agents of Capnocytophaga and other fastidious anaerobic bacteria (19, 21).

The results of this study support initial observations that beta-lactamase production is becoming increasingly common in Capnocytophaga spp., as in other oral bacteria (3, 12, 17), but the location of the gene(s) coding for these beta-lactamases is not yet determined. Most investigators report that Capnocytophaga remains susceptible to imipenem and beta-lactamase inhibitor combinations. However, susceptibilities to other beta-lactams have been found to vary. Rummens et al. (21) investigated the in vitro activities of antimicrobial agents against Capnocytophaga strains, while Fowlerak et al. (7) studied a strain that was resistant to cephalosporins (MICs, >16 μg/ml). Roscoe et al. (20) characterized a membrane-associated low-efficiency cephalosporinase that appears to be similar to the enzyme described by Fowlerak et al. (7). Bilgrami et al. (2) reported a case of Capnocytophaga bacteremia caused by a resistant strain that was susceptible to a number of beta-lactam antibiotics but resistant to cefazidime (MIC, 32 μg/ml). Gomez-Garces et al. (9) described a multidrug-resistant strain for which the MICs were similar to those reported here. Although imipenem and cefoxitin are always very active against Capnocytophaga (7, 9, 20), it is interesting that aztreonam and moxalactam are not. Variable susceptibilities have generally been reported for these beta-lactam antibiotics (10, 14, 21).

The intrinsic activities of beta-lactamase inhibitors, already described for clinical isolates of Acinetobacter species, with sulbactam being the most effective (24), have led to their combination with beta-lactam antibiotics for empirical antibiotic treatment of suspected bacteremia of oral origin in neutropenic patients, especially when Capnocytophaga is involved. Their use should be encouraged, as they are effective against both beta-lactamase producers and cephalosporin-resistant strains.

REFERENCES


