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Role of a Cefoxitin-Inducible Beta-Lactamase in a Case of Breakthrough Bacteremia

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Development of resistance during therapy with cefamandole contributes to treatment failure. A simple cefoxitin disk test was recently described which detects a cefamandole-active inducible beta-lactamase not otherwise detectable with cefamandole as the inducer. A case of breakthrough *Enterobacter* bacteremia due to selection of a resistant subpopulation is reported in an immunocompromised patient. The use of this simple disk test in selected clinical cases is advocated.

Cefamandole nafate, a semisynthetic cephalosporin, is effective against a wide variety of microorganisms and has been used successfully to treat both systemic and localized infections (11). Although active against *Enterobacter* species where older cephalosporins and cefoxitin are not, the rapid appearance of resistant strains was noted in the in vitro evaluation of the antibiotic (4). The significance of these resistant subpopulations to the clinical outcome of human infections by *Enterobacteriaceae* is not well documented. Sanders and Sanders (16) developed a simple disk test to detect bacterial populations with inducible beta-lactamases capable of inactivating cefamandole and noted the need for data relating the presence of inducible enzymes and the outcome of therapy in humans. We include an example of breakthrough bacteremia with *Enterobacter aerogenes* in an oncology patient treated with cefamandole. This case is illustrative of the necessity to predict the existence of inducible beta-lactamase activity if treatment of infections with cefamandole is to be successful in this selected patient population.

MATERIALS AND METHODS

A 37-year-old Caucasian male with known macroglobulinemia was admitted to our oncology service complaining of epistaxis. Therapy in the past year included melphalan, vincristine, prednisone, and multiple plasmaphereses. One month before the present admission the patient had received a 5-day course of continuous intravenous therapy with bleomycin, vincristine, and cyclophosphamide.

Physical examination disclosed a temperature of 38.7°C and evidence of recent bleeding from the right nostril but no obvious infection site.

A complete blood count showed the following values: hemoglobin, 8.9 g/dl; hematocrit, 26%; and leukocyte count, 1,300/mm^3 with 37% polymorphonuclear leukocytes, 7% bands, 39% lymphocytes, and 17% monocytes. The platelet count was 5,000/mm^3. Therapy with tobramycin and ticarcillin was initiated after cultures were obtained. Although cultures remained negative, antibiotic therapy was continued. The patient experienced intermittent low-grade fever most probably due to his underlying disease. On hospital day 13 these antibiotics were discontinued because of ototoxicity and problems with hemostasis.

On hospital day 15 the patient spiked a fever, and two blood cultures drawn at that time were radiometrically detected as positive 12 h later. *E. aerogenes* (BC494) was identified from the blood cultures and was also isolated from a bulbous lesion of the tongue and from the throat. This initial isolate was susceptible to tobramycin, gentamicin, cefamandole, carbenicillin, and chloramphenicol but resistant to ampicillin, cephalothin, and cefoxitin. Cefamandole therapy was instituted.

After 6 days, the patient spiked a fever, and at this time blood cultures were positive for *E. aerogenes* (BC564) additionally resistant to cefamandole and ticarcillin. Cefamandole was discontinued, and chloramphenicol therapy was begun. In the next 4 days the patient's condition continued to deteriorate. Two additional blood cultures drawn at this time were again positive for *E. aerogenes*. Two populations were clearly discernible on the sensitivity plates, one susceptible (BC618-1) and the other resistant (BC618-2) to cefamandole. The patient expired 2 days later.

Blood cultures. Blood cultures were performed radiometrically using a BACTEC 225 to detect positive cultures.

Species identification and biotyping were performed using the API 20E strip and the API ZYM strip for aminopeptidase activity to determine 47 biochemical and growth characteristics of the isolate. The API 20E inoculum was standardized using a no. 1 MacFarland standard (12).

Susceptibility testing. Bauer-Kirby disk diffusion (13) and agar dilution (18) techniques were performed using standard techniques.

Beta-lactamase detection. The following assays were performed as previously described: a rapid aci-

dometric beta-lactamase procedure (17), induction experiments using the chromogenic cephalosporin substrate 87/312, cephaloridine, and cefamandole (these assays were performed at Eli Lilly & Co. [9, 14]), and the Sanders and Sanders cefoxitin disk test (16). Determination of the "spontaneous mutation rate to resistance" was performed as described by Ott et al. (14). For determining the incidence of cefoxitin-inducible enzymes in our geographic area, isolates were obtained from clinical specimens at St. Luke's Hospital, Bethlehem, Pa. and kindly provided by J. F. Salventi (Allentown-Sacred Heart Hospital Center, Allentown, Pa.) and Diane Halstead-McFarland (Allentown Hospital, Allentown, Pa.). Persistence of beta-lactamase activity after exposure to cefoxitin was assayed by selecting colonies from the truncated zone area where cefamandole had been inactivated (see Fig. 1) and retesting by disk diffusion methods.

RESULTS

The four strains of *E. aerogenes* isolated were considered identical biotypes as determined by the API 20E and API ZYM systems. The API 20E code number 5305773 is an excellent identification for *E. aerogenes*. In addition, each isolate possessed alkaline phosphatase, leucine aminopeptidase, acid phosphatase, phosphoamidase, alpha-glucosidase, and beta-glucosidase. The only discrepancy was that our first isolate (BC494) also possessed weak (2+) alpha-galactosidase activity, whereas the others did not. However, the four isolates differed in their susceptibility to cefamandole (Table 1).

The detection of beta-lactamase activity with the acidometric test and the detection of inducible beta-lactamase activity using the cephalosporin substrates 87/312 (Glaxo), cephaloridine, cefamandole, and cefoxitin are summarized in Table 1.

Figure 1 illustrates the cefoxitin disk induction test with the initial cefamandole-susceptible strain of *E. aerogenes* (BC494) from our patient.

The spontaneous rate of mutation to resistance in the two susceptible strains isolated from this patient was 2×10^{-5} for BC494 and 3×10^{-5} for BC618-1 at 10 times the minimal inhibitory concentration for cefamandole.

At this institution, the percentage of cefamandole-susceptible *Enterobacter cloacae* clinical isolates for a 14-month period was 68%, and the percentage of susceptible *E. aerogenes* was 81% as determined by Bauer-Kirby disk diffusion tests. Of 51 strains of *Enterobacter* consisting of *E. cloacae* (24) and *E. aerogenes* (27) resistant to cefoxitin but susceptible to cefamandole by Bauer-Kirby criteria, 90% possessed inducible beta-lactamases with cefoxitin as the inducer. The criterion employed was a 4-mm reduction in the radius of the cefamandole zone of inhibition in the area between the cefamandole and cefoxitin disks. Five isolates demonstrated a truncated zone of less than 4 mm.

Inoculum removed from the truncated cefamandole zone in the cefoxitin disk test and retested using Bauer-Kirby disk diffusion methods did not exhibit smaller or negligible zones of inhibition. A smaller zone might be expected if the cefoxitin had selected a subpopulation of *Enterobacter* expressing a beta-lactamase active without induction.

DISCUSSION

Recognition that resistant subpopulations of *Enterobacter* and indole-positive *Proteus* species were selected in the presence of cefamandole first occurred when discrepancies were noted between broth dilution and agar dilution minimal inhibitory concentration endpoints. Findell and Sherris (4) examined the parameters of broth and inoculum inactivation and felt these two mechanisms were not contributory to the discrepancies between the broth and agar methods. They did, however, isolate a subpopulation of *E. aerogenes* by both direct and indirect selection with 16-fold greater resistance to cefamandole than the parent strain. They concluded that the discrepancies observed were explicable by a high mutation rate to resistance and determined that these variants occurred at a frequency of 10^{-6} or 10^{-7}. Ott et al. (14) were unable

TABLE 1. *Minimal inhibitory concentration (MIC) and beta-lactamase assays*

Strain	Agar dilution MIC for cefamandole (μg/ml)	Acidometric test	Inducible beta-lactamase on substrate			
			87/312	Cephaloridine	Cefamandole	Cefoxitin
BC494	4	−	−	−	−	+
BC564	128	−	+	+	−	NA[a]
BC618-1	8	−	−	−	−	+
BC618-2[b]	128	+	+	+	+	NA

[a] NA, Not applicable. Only isolates with a measurable zone of inhibition around cefamandole can be employed in the cefoxitin disk test.

[b] Isolate 618-2 possessed 30 to 200 times the specific activity of the inducible beta-lactamases in the other strains.

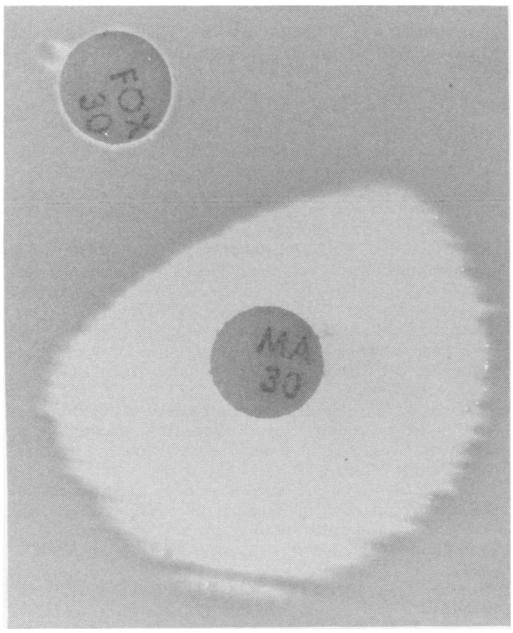

FIG. 1. *The cefoxitin disk test with E. aerogenes (BC494). Note the truncated zone of inhibition indicating an inducible beta-lactamase.*

to correlate resistance to cefamandole and cefoxitin in spontaneous mutants of *Enterobacteriaceae* with constitutive or inducible beta-lactamase production. They did, however, confirm the heterogeneity of population susceptibility in the bacteria studied to all the antibiotics tested. Resistance to cefamandole by a study strain of *E. aerogenes* occurred at a frequency of 3×10^{-6}. Although no inducible beta-lactamase activity had been detected using the chromogenic cephalosporin substrate 87/312, cephaloridine, or cefamandole, Sanders and Sanders (16) reported that cefoxitin was an effective inducer of a cefamandole-active beta-lactamase.

With the API 20E and the API ZYM systems, no significant strain variation could be detected by biotyping of 47 characteristics of the *E. aerogenes* strains isolated from our patient concomitant with the emergence of resistance to cefamandole. The fact that organisms removed from the truncated cefamandole zone of inhibition in which cefamandole had been inactivated were not resistant to cefamandole on repeat Bauer-Kirby testing supports the presence of an inducible enzyme. Coupled with low incidence of detectable beta-lactamase activity in cefamandole-susceptible strains at 10 times the minimal inhibitory concentration, enzyme activity appears to be expressed only in a minor subpopulation without cefoxitin induction. It is not a trait detectable in *Enterobacter* isolates except under the conditions of selective in vivo cefamandole therapy or with the in vitro use of cefoxitin as an inducer. Routine detection of minor resistant subpopulations by Bauer-Kirby disk diffusion techniques is dependent on chance selection of organisms or colonies expressing resistance (2). Since the incidence of enzyme expression is low without cefoxitin as an inducer, the probability of detecting resistant colonies within the cefamandole zone of inhibition is likewise low. In the case presented there was no in vitro indication of a resistant subpopulation until after cefamandole therapy, when the predominant antibiotype became cefamandole resistant (BC564). After cefamandole was discontinued, the population began to revert to the susceptible antibiotype, and a mixed population was evident on the Bauer-Kirby plates (BC618-1 and BC618-2) in the last cultures obtained.

Recurrent bacteremia in spite of appropriate therapy may be related to several factors. McHenry et al. (10) studied 29 cases of bacteremia of long duration and documented the importance of intravascular devices and abscesses in perpetuation of the infection. No development of resistance during antimicrobial therapy was noted. Harris and Cobbs (7) corroborated these observations in an additional 20 patients and again did not cite development of resistance as contributory to persistence. Anderson et al. (1) divided "breakthrough" bacteremia into "early" and "late" categories, with early breakthrough (24 to 72 h after therapy) occurring because of suboptimal antibiotic levels and late breakthrough (more than 72 h after therapy) occurring in the presence of undrained abscesses or impaired host defenses. In these cases bacteria isolated after breakthrough were still susceptible to the drug employed. Breakthrough bacteremia due to selection of a resistant subpopulation in spite of "appropriate" therapy is not mentioned in these studies as contributing to recurrence. Our case of late breakthrough, occurring more than 72 h after cefamandole therapy, was caused by our patient's inability to combat the resistant subpopulation selected by therapy.

Cefamandole has been recommended for the treatment of infections despite recognition that selection of variants resistant to the antibiotic occurs during treatment with the drug. The selection of cefamandole resistance has been important in treatment failures in animal models (6). In humans, treatment failure of enterobacterial infections due to development of resistance has been reported in 2 out of 45 patients with urinary tract obstruction (8), and in 3 out of 52 patients with skin and soft tissue infections (15).

Beta-lactamase activity was not determined in these cases.

Our case represents failure of cefamandole therapy in treatment of an *Enterobacter* bacteremia mediated by a cefoxitin-inducible beta-lactamase. Resistant subpopulations may be successfully treated with an antibiotic if the host is immunocompetent and his defenses are able to overcome small numbers of selected variants. Patients who are immunocompromised by therapy or disease, on the other hand, may fail to handle an assault by even small populations of resistant bacteria. It would be useful to the clinician treating oncology or other immunocompromised patients to know whether significant isolates of *Enterobacter* or other organisms known to have a high spontaneous mutation rate to cefamandole resistance, e.g., indole-positive *Proteus* or *Serratia* species, possess a detectable cefoxitin-inducible beta-lactamase. Possession of this capability would contraindicate use of cefamandole as the sole agent in treating the infection. This is particularly pertinent when hemodynamic or toxic considerations discourage use of recommended paired antibiotic regimens, e.g., cefamandole and either carbenicillin or an aminoglycoside (3, 5).

The cefoxitin disk test is useful in that it detects beta-lactamase activity and inactivation of cefamandole in bacterial populations when it would not be detected using other assays. Inducible beta-lactamase activity is common in *E. cloacae* and *E. aerogenes*. Sanders and Sanders (16) reported activity in 88% of *Enterobacter* species tested. In clinical isolates from the Lehigh Valley it occurred 90% of the time. Therefore, with these *Enterobacter* strains, single-drug therapy with cefamandole may be predictably contraindicated in immunocompromised patients regardless of apparent susceptibility when tested by disk diffusion. However, the test may still be necessary in species in which the incidence of inducible beta-lactamase is not as predictably high as it is with *Enterobacter*, e.g., *Providencia* sp. and *Proteus rettgeri* (16).

The cefoxitin disk test is a simple procedure easily performed in any laboratory with Bauer-Kirby susceptibility testing capability. Microbiologists should be familiar with tests which, although not routine, are applicable to special clinical problems. Communication between the clinician and the microbiologist will permit selective employment of the cefoxitin disk test as indicated by therapeutic considerations and the etiology and nature of the infection.

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