Spontaneous Mutant with Loss of β -Lactamase in *Aerobacter cloacae*

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The β -lactamase hydrolysis of penicillin and cephalosporin has been consistently observed in certain species of *Enterobacteriaceae* (1, 3, 5). This activity can be demonstrated in single colonies by the rapid screening test of Knox and Smith (6), which is based on the detection of acid formed by the action of β -lactamase on penicillin or cephalosporin. From a highly active β -lactamase strain of *Aerobacter cloacae* (3, 4), a mutant was isolated which did not produce β -lactamase and was sensitive to cephalosporin C.

Serial log dilutions of a 10-ml broth culture inoculated from a single colony of HSC 18410 were plated in nutrient agar by use of the agar-overlay technique. After incubation at 37 C, isolated colonies were used to inoculate the surface of membrane filters (Millipore Corp., Bedford, Mass.) according to the Knox and Smith test (6) with cephalosporin C as substrate (3). Among the population of β -lactamase-producing colonies, one was observed which reacted negatively. Repeated tests on this isolate with cephalosporin C and penicillin G as substrates failed to reveal β -lactamase activity. Back-mutation to β -lactamase production was not demonstrated by testing colonies from 20 successive subcultures by the Knox and Smith method, nor after testing the more resistant colonies obtained by inoculation of gradient plates containing cephalosporin C according to the method of Szybalski (9). The mutant strain was subjected to a complete set of characterization tests for bacterial identification (2) and was found to be identical to that of the *A. cloacae* parent strain.

The minimal inhibitory concentration, using a tube dilution method and an inoculum of 10^6 cells in Penassay broth incubated overnight at 37 C, was found to be 30 μ g of cephalosporin C per ml for the mutant strain as compared with 6,000 μ g/ml for the parent strain.

Aerobacter β -lactamase preparations of increasing potency derived from the HSC 18410 strain retain their relative β -lactamase activities on cephalosporin C and penicillin G, indicating that both these activities are probably due to a single β -lactamase. For example, an increase of 50-fold after partial purification (M. A. Mos-

carello and P. C. Fleming, unpublished data) did not affect the ratio of activities on cephalosporin C and penicillin G, which remained at about 100 to 1 (molar basis). This would explain the loss of the β -lactamase activity on both cephalosporin C and penicillin G in the mutant strain.

Penicillin resistance has been attributed completely to β -lactamase only among the gram-positive species (7, 8). In the present work, a 200-fold increase in cephalosporin sensitivity was conferred by a spontaneous mutation involving loss of β -lactamase in a gram-negative organism. This increase in sensitivity to cephalosporin C was accompanied by a 400-fold and a 125-fold increase in sensitivity to cephaloridine and penicillin G, respectively. As lack of β -lactamase in the mutant strain was associated with sensitivity to cephalosporin and penicillin, it appears that β -lactamase production in at least one strain of *A. cloacae* is mainly responsible for the bacterial resistance to β -lactam antibiotics. This suggests the need for further assessment of the relative importance of intrinsic factor(s) and β -lactamase production in the resistance of gram-negative organisms to the β -lactam antibiotics.

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