Novel R-Plasmid-Mediated Beta-Lactamase from Klebsiella aerogenes

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A novel β-lactamase with a broad spectrum of activity against penicillins and cephalosporins has been detected in a strain of Klebsiella aerogenes. Its synthesis is mediated by an R-plasmid of molecular weight 64 × 10^6.

Recent serious hospital cross-infection in Bristol was caused by a strain of Klebsiella aerogenes which was phenotypically resistant to a wide range of penicillins and cephalosporins and to tetracycline, streptomycin, kanamycin, gentamicin, tobramycin, sulfonamides, nitrofurantoin, trimethoprim, and nalidixic acid (D. C. E. Speller, unpublished data). Examination of the basis of the resistance to β-lactam antibiotics showed that the strains produced a β-lactamase. Intact bacterial cultures expressed about 3.8 enzyme units/mg (dry weight) of bacteria with ampicillin as substrate, but after disruption in an ultrasonic disintegrator (4) the equivalent value was about 48 enzyme units/mg with the same substrate. The crypticity of the strains (6, 7) with respect to this enzyme and substrate was therefore about 13. Equivalent values obtained with benzyl penicillin and with cephaloridine were 15 and 1, respectively.

Standard overnight mating experiments (3) between the Klebsiella strain as donor and Escherichia coli UB1139 (a plasmidless E. coli that is resistant to rifampin to facilitate counterselection; see reference 9) gave transfer of penicillin resistance at a frequency of about 5 × 10^{-7}/donor when the cross was selected at 30°C on agar containing ampicillin (500 μg/ml) and rifampin (100 μg/ml). The penicillin resistance of the exconjugants obtained in this way was primarily due to β-lactamase production. The transfer of penicillin resistance to the recipient E. coli by conjugation was accompanied by the appearance in strain UB1139 of one plasmid of molecular weight 64 × 10^6. This plasmid has been designated RUB5451. The original Klebsiella strain, on the other hand, carried three plasmids, one of which also had a molecular weight about 64 × 10^6, the other two being smaller.

To determine the type of β-lactamase synthesized by E. coli UB1139 after receiving the penicillin resistance character from the Klebsiella strain, several E. coli lines (Table 1) were grown to a culture density of about 2 × 10^9 bacteria/ml in nutrient broth. The bacteria were then collected by centrifugation and resuspended in about one-fifth of their original volume of 0.1 M NaHPO_4/KH_2PO_4 buffer, pH 7.0. This suspension was then disrupted in an ultrasonic disintegrator as described by Jack and Richmond (4); the cell debris was removed by further centrifugation, and the supernatant fluid was used for enzyme studies.

The substrate profile of the enzyme was determined against a range of β-lactam antibiotics as described previously (7). Crude enzyme preparations of three other β-lactamases that are (also) mediated by bacterial plasmids (type IIA, Vα, and Vb enzymes; see references 7, 8) were also tested for comparison. The results are shown in Table 1. They have been calculated so that the rate of hydrolysis of benzyl penicillin is recorded as 100 in each case (7). The results obtained with the Klebsiella enzyme were different from those obtained with others in certain respects. Thus, type IIA enzyme was considerably more active than the Klebsiella enzyme against cephaloridine and cephalaxin, whereas the type Vα and Vb enzymes hydrolyzed cloxacillin, a penicillin barely affected by the new β-lactamase. As with the majority of β-lactamases from gram-negative species, cefuroxime and cefoxitin were stable to the new enzyme, having rates of hydrolysis less than 2% of those found with benzyl penicillin (Table 1).

Among the β-lactamases from gram-negative species, cefuroxime, cloxacillin, and clavulanic acid are commonly inhibitors of cephalexin hydrolysis. Crude preparations of the new enzyme were therefore examined to see whether these β-lactam compounds were inhibitors. In all cases they were (Table 2).

Isoelectric focusing of β-lactamases in poly-
acrylamide gels has proved an effective way of resolving molecular differences in these molecules (5). The novel β-lactamase was therefore subjected to isoelectric focusing under standard conditions (5), and the migration of crude preparations of type IIIa, Va, and Vb enzymes was included for comparison. The isoelectric point (pI) values obtained are shown in Table 3. The penicillin-susceptible E. coli UB1139, which was used as the recipient in the initial transfer experiment from the Klebsiella strain, itself expresses small amounts of type Ib β-lactamase, an enzyme commonly found in strains derived from E. coli K-12 (1). The pI of this enzyme is 8.1. After transfer of the penicillin resistance plasmid from the Klebsiella strain, E. coli UB1139 expresses two β-lactamases in the isoelectric focusing pattern. One is the type Ib enzyme (pI, 8.1), and the second has a pI value of 7.5.

Although the pI value for the new enzyme is similar to that shown by type Va enzyme (see reference 8), the substrate profile of that enzyme is quite different (see Table 1).

Antiserum capable of interacting with the type IIIa β-lactamase was available (2), and it was used to test crude preparations of the new β-lactamase for immunological cross-reaction. None was found.

All the enzyme studies reported up to this point have been carried out on enzyme expressed by E. coli UB1139. Studies on the enzyme as extracted from the original Klebsiella strain showed that it was indistinguishable from that expressed in E. coli after mating.

On the basis of the evidence summarized here, therefore, we conclude that a hitherto unrecognized β-lactamase was responsible for the penicillin and cephalosporin resistance of the K. aerogenes strains studied here. It is, as yet, uncertain whether this enzyme is widespread among clinical strains of gram-negative bacteria or whether it is still confined to a relatively small clone of K. aerogenes.

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LITERATURE CITED


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