LETTERS TO NATURE

Transferable Carbenicillin Resistance in *Pseudomonas aeruginosa*

In 1969, after carbenicillin had been in use for three years in this unit, highly resistant strains of *Pseudomonas aeruginosa* were isolated for the first time[1]. Because these resistant strains included, from their first appearance, representatives of two unrelated types, it seemed likely that the resistance was transferable; this hypothesis was supported by experiments showing the transfer of carbenicillin resistance between *Ps. aeruginosa* and *Escherichia coli* K12 *in vitro* and *in vivo*[2-4]. The resistant *Ps. aeruginosa* produced a penicillinase (β lactamase) similar to that normally produced by some strains of Enterobacteria and different from that normally produced by *Ps. aeruginosa*[2,3], so it seemed likely that the *Ps. aeruginosa* had initially acquired resistance by the transfer of an R factor from a carbenicillin-resistant member of the Enterobacteriaceae colonizing the same burn. This hypothesis is now supported by a study on strains of Enterobacteria and *Ps. aeruginosa* isolated in a number of hospitals. We have also found evidence suggesting that *Ps. aeruginosa* which has acquired this R factor may not show resistance until it has been exposed repeatedly to carbenicillin.

Soon after the discovery of carbenicillin-resistant strains of *Ps. aeruginosa*, the therapeutic use of carbenicillin in the burns unit was stopped. Several months later, when resistant strains were no longer found in the unit, an extensively burned patient with pseudomonas infection was treated with carbenicillin. Three types of *Ps. aeruginosa* (serotypes 3, 5c and 10) were isolated, none of which had previously been found resistant to carbenicillin. Within a few days of starting treatment, however, highly resistant isolates of all three types were obtained from the burns; these resistant variants resembled the carbenicillin-resistant strains isolated previously in producing a carbenicillin-inactivating enzyme (carbenicillinase). A possible explanation of this rapid emergence of resistance is the transfer to the three types of *Ps. aeruginosa* of the R factor from a strain of *Klebsiella* or *Proteus* present in the same burns (such organisms were present), followed by the selection of the resistant *Ps. aeruginosa* on treating the patient with carbenicillin. The exceptionally high incidence of ampicillin-resistant strains of *Proteus mirabilis* in the unit between 1965 and 1967[5] suggests that antibiotic treatment had led to the selection of a range of organisms from which strains carrying transferable carbenicillin resistance emerged. A recent survey of *Klebsiella* spp. and *Proteus* spp. in the burns unit of this hospital has shown that a large proportion (90% and 72% respectively) of those tested carried an R factor determining linked resistance to tetracycline, carbenicillin, ampicillin, kanamycin and cephaloridine (resistance pattern TCAKCe). By contrast, a survey of ampicillin and/or carbenicillin-resistant *Klebsiella* spp. (ninety strains) and *Proteus* spp. (eighty-six strains) from eleven hospitals in England and Scotland showed only six strains, all of *Proteus* spp., which had the resistance pattern TCAKCe and transferred it to *E. coli* K12, from which it could be further transferred to a carbenicillin-sensitive *Ps. aeruginosa*; all but one of the six strains were isolated in a burns unit in Scotland, which was also the only source, apart from this unit, of carbenicillin-resistant, carbenicillinase-producing *Ps. aeruginosa* with a β lactamase substrate profile similar to that of some Enterobacteria (ref. 6 and M. H. Richmond and R. B. Sykes, personal communication). R factors determining other resistance patterns were common in *Klebsiella* and *Proteus* isolated in the Scottish burns unit, but though some of these strains transferred carbenicillin resistance to *E. coli* K12, the latter did not transfer carbenicillin resistance further to *Ps. aeruginosa*. These findings provide a possible explanation of the rarity of carbenicillin-resistant variants in other hospitals and of their delayed emergence in this unit.

Carbenicillin habituation tests were carried out in forty-five sensitive strains of *Ps. aeruginosa*, five on two or more occasions; a standard technique was used, involving twelve to eighteen subcultures from nutrient broth containing a sub-inhibitory concentration of carbenicillin to a range of doubling dilutions of carbenicillin in broth. In ten out of sixty-four tests (seven in strains of serotype 8 and one in each of types 3, 5c and 10, all previously found also as resistant variants in burns), there was a large increase in carbenicillin resistance (to MIC 4,000 μg ml. or more), associated with carbenicillinase production[1] and, in some strains tested, with an enterobacteria (R_{TEM}) type of β lactamase substrate profile[6]. Increased resistance also to tetracycline was found in a strain which had acquired carbenicillin resistance by habituation. These results, though variable even on replicate tests with the same strain, suggest that *Ps. aeruginosa* may sometimes have acquired the R factor from a resistant Gram negative bacillus of another species in a latent form, from which the resistant organisms emerged on subculture in the presence of carbenicillin. Carbenicillin resistance acquired on habituation was not transferable, but we found it impossible also to transfer resistance from some carbenicillinase-producing strains that occurred naturally in burns. Although newly isolated carbenicillin-resistant *Ps. aeruginosa* usually reverted to sensitivity, stable resistant variants sometimes emerged on repeated subculture of the resistant organisms.

Our findings raise some important questions on the interpretation of sensitivity tests as a guide to therapy. When mixed cultures are present, a sensitive pathogen may acquire resistance by transfer of an R factor from a resistant commensal organism which has not been tested. Even when the donor of an R factor is no longer present, resistance may still be selected in the pathogen by treatment with carbenicillin, if the infecting organism has a latent form of resistance.

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