Transferable Resistance to Carbenicillin and Gentamicin in *Pseudomonas aeruginosa*

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SUMMARY

Antibiograms on 482 routine isolates of *Pseudomonas aeruginosa* yielded 64 (13,3%) carbenicillin-resistant strains. In 40 of these strains resistance could be ascribed to R factors. R factors accounted for resistance in 3 out of 11 gentamicin-resistant strains. The significance of resistance in *Ps. aeruginosa* is discussed.

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Gentamicin[1] and carbenicillin[2] are the drugs of choice in the treatment of infection with *Pseudomonas aeruginosa*. However, this regimen of treatment has been accompanied by the emergence of drug resistance, particularly to carbenicillin.[3-7] Since the establishment of intensive care units at the Bloemfontein National and Pelonomi Hospitals we have noted an exceptional increase in the number of *Ps. aeruginosa* strains isolated, as well as in its incidence of resistance to gentamicin and carbenicillin.

This article reports on the occurrence of transmissible drug resistance in *Ps. aeruginosa* strains, isolated in our laboratory.

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MATERIALS AND METHODS

Antibiotic Sensitivity Tests

Mast sensitivity discs were used to screen isolates of *Pseudomonas aeruginosa* for resistance to carbenicillin and gentamicin. Resistance was confirmed by plating on MacConkey agar containing the antibiotics at a concentration of 1 000 μg/ml for carbenicillin or 25 μg/ml for gentamicin.

Minimum inhibitory concentrations (MIC) of the antibiotics were determined by spotting small drops (0,1 ml) of diluted cultures (10^3 bacteria/ml) on Welco sensitivity test agar plates containing doubling dilutions of carbenicillin or gentamicin. The MIC was taken as the lowest concentration of antibiotic that prevented growth.

Transfer of Carbenicillin and Gentamicin Resistance

Transfer of R factors was done as previously described.[8,9] The recipient was *Escherichia coli* K12 strain J53.[8]

RESULTS

Carbenicillin Resistance in Pseudomonas aeruginosa

Out of 482 routine isolates of *Ps. aeruginosa* 64 (13,3%) showed high levels of resistance to carbenicillin (>2 500

μg/ml). In 40 strains (62,5%) this resistance was transferred to the *E. coli* recipient. Different resistant markers were transferred simultaneously with carbenicillin resistance (Table I). In 17 isolates markers for ampicillin, carbenicillin, kanamycin and tetracycline resistance were located on the same R factor, while 11 strains showed grouping of resistance markers to carbenicillin, ampicillin, kanamycin and tetracycline. One of these R factors (RP-638) was examined in further detail.[9]

TABLE I. ANTIBIOTIC RESISTANCE DETERMINED BY R FACTORS

Antibiotic resistance	No. of R factors
Am, Ca, Sm	**11**
Su, Cm, Ca, Am, Sm, Km, Tc... ...	**8**
Am, Ca, Su, Sm, Cm	**6**
Am, Ca, Sm, Cm, Tc	**5**
Am, Ca, Km, Tc	**17**
Gm, Km	**3**

Am = ampicillin, Ca = carbenicillin, Cm = chloramphenicol, Gm = gentamicin, Km = kanamycin, Su = sulphonamide, Sm = streptomycin, Tc = tetracycline.

Gentamicin Resistance in Pseudomonas aeruginosa

Eleven (2,3%) strains were resistant to gentamicin at a MIC of 25 μg/ml. Three of these strains possessed R factors which determined resistance to gentamicin and kanamycin.

DISCUSSION

Activity of carbenicillin against *Pseudomonas aeruginosa* is low, MIC: 32 - 64 μg/ml, compared with values of 2 - 4 μg/ml for *Escherichia coli* and *Proteus mirabilis*.[10] However, toxicity and poor *in vivo* activity of other drugs make carbenicillin an important antibiotic in the treatment of infections by *Ps. aeruginosa*. Marked increases in levels of carbenicillin resistance in *Ps. aeruginosa* were described by Lowbury *et al.*[3] We now report even higher levels of resistance in our isolates, with minimum inhibitory concentrations of 2 500 μg/ml or more.

Inadequate dosage of carbenicillin is a potent method for the selection of resistant mutants. These strains, however, have been isolated from the intensive care units where carbenicillin is only used when indicated by microbiological reports. This can thus be excluded as a cause for the emergence of resistant strains. It is known that treatment with penicillins may result in the appearance of carbenicillin resistance and the common use of ampicillin probably contributes to this problem. A further possibility is the transmission of R factors from resistant *E. coli* and *Proteus* strains to *Pseudomonas aeruginosa*. That such transfer does occur *in vivo* has been adequately demonstrated.[4] We have, however, shown that the *Pseudomonas* R factors isolated by us are unique for this species,[9] which argues against this hypothesis.

Problems are encountered in achieving high enough concentrations of carbenicillin in lesions such as burns and respiratory tract infections. These areas provide ideal circumstances for the selection of resistant mutants and the sole use of carbenicillin in the treatment of such infections by *Ps. aeruginosa* is contra-indicated. In our study only 2,3% of the *Ps. aeruginosa* strains were resistant to gentamicin (MIC 25 μg/ml). In view of the synergy reported for combined gentamicin and carbenicillin therapy,[12] a combination of these two drugs may be recommended in the treatment of all *Pseudomonas* infections.

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