PLASMID-MEDIATED GENTAMICIN RESISTANCE NOT ASSOCIATED WITH KANAMYCIN RESISTANCE IN ENTEROBACTERIACEAE

Sir:

R-Factors mediating resistance to aminoglycoside antibiotics have been shown to govern the synthesis of various drug-inactivating enzymes: a kanamycin-acetyl-transferase, a kanamycin-phospho-transferase, a streptomycin-adenylylate-synthetase, a streptomycin-phospho-synthetase and a gentamicin-adenylylate-synthetase[1,14]. Recently, we obtained, from several hospitals, strains of *Enterobacter*, *Serratia*, *Klebsiella* and *Escherichia* sp. which were able to transfer into *E. coli* K12 a gentamicin resistance character different from the Gk character previously described in strain LA290 R55[15,16].

Naturally occurring gentamicin resistant strains were conjugated to mutants of *E. coli* K12 resistant to sodium azothydrate (LA 290) or to rifampicin (LA 534). Original strains and their R-factors are described in Table 1. Sensitivity to aminosidic antibiotics of *E. coli* recipients was determined by the agar dilution method[17] using a STEER's apparatus (10^4~10^5 bacteria per spot). The MICs of gentamicin C, kanamycin A, tobramycin, paromomycin and lividomycin for *E. coli* J5 carrying R-factors listed in Table 1, are shown in Table 2. These strains were compared to reference strains: *E. coli* R5 kindly supplied by Dr. H. UMEZAWA which acetylates kanamycin and adenylates streptomycin; *E. coli* R11-2 which phosphorylates kanamycin; *E. coli* R55 which adenylates gentamicin (J. DAVIES, personal communication).

Table 1. Origin of R factors studies

Wild type strain	Origin	R factor	Transferred resistance characters*
Enterobacter 705	Foch hosp. Suresnes	R135	T Su G S
Klebsiella 452	Foch hosp. Suresnes	R136	A C T Su G
Klebsiella 12	C.H.U. Caen	R138	A C T Su G S
Serratia 006	C.H.U. Angers	R140	A C T Su G
E. coli 2423-1	C.H.U. Angers	R141	A C T Su G S
Klebsiella 535	Claude Bernard hosp. Paris	R137	A C T Su G S
Enterobacter 536	Claude Bernard hosp. Paris	R146	A C T Su G S

* Resistance characters (disc method): T: tetracycline; Su: sulfonamides; A: ampicillin; C: chloramphenicol; G: gentamicin; S: streptomycin.

Table 2. MIC in μg/ml of deoxystreptamine antibiotics for *E. coli* J5 R$^+$

R factor	Gen*	Tob.	Kan.	Par.	Liv.
R135	32	0.25	1	2	4
R136	64	0.25	1	4	4
R137	32	0.25	1	2	4
R138	32	0.25	1	2	4
R140	64	0.25	1	2	4
R141	128	0.25	1	4	8
R146	32	0.25	1	4	4
Control					
R55	64	32	64	4	4
R11-2	0.25	0.25	512	>512	>512
R5	0.5	8	16	4	8

Gen: gentamicin C: Unilabo 3804, Tob: tobramycine; Lilly XU 3740. Kan: kanamycin A; Bristol 595. Paromomycin; Parke Davis 10.254. Liv: lividomycin; Roger Bellon.
* Levels for each fraction of gentamicin C: C1 C1a C2 kindly supplied by Dr. M. WEINSTEIN are similar.

E. coli harbouring R-factors R135, R136, R137, R138, R140, R141, R146 are resistant to gentamicin (32~128 μg/ml) but remain sensitive to kanamycin (1 μg/ml), tobramycin (0.25 μg/ml), paromomycin (2~4 μg/ml) and lividomycin (4~8 μg/ml). This type of resistance to deoxystreptamine antibiotics is clearly distinct from the one previously described in *E. coli* R55 inactivating gentamicin C, kanamycin A and tobramycin. Levels of resistance for streptomycin and spectinomycin when present are similar to those observed with strains adenylating streptomycin as *E. coli* R5.

R-Factor R135 (the first isolated), was transduced into *E. coli* K12 by phage P1 Kc; the procedure has been described in a previous article[15]. Resistance determinants for tetracycline, sulfonamides, streptomycin and gentamicin were transduced simultaneously and are likely to belong to one single plasmid. Precise determination of inhibitory concentrations for deoxystreptamine antibiotics was done for *E. coli* transductant. Inhibitory concentrations (IC) 50 % values were calculated by a probit procedure[18]. Comparative results for sensitive strain *E. coli* J5, *E. coli* J5 R55 and

E. coli J5 R135 are summarized in Fig. 1. Differences in drug resistance specificity mediated by R55 adenylating gentamicin, and R135 are significant. There is actually a good correlation between drug resistance (MICs) and the inactivation of aminosidic drugs by bacterial enzymes[19] although it should be noted that N-acetylation due to R-factor R5 of gentamicin Cla, for instance, does not lead to real inactivation[20].

The R-factor studied here mediates resistance to gentamicin C but not to kanamycin A and tobramycin (G character). These results suggest that the enzyme involved, if any, could be different from the one previously described and responsible for transferable gentamicin-kanamycin (Gk) resistance character.

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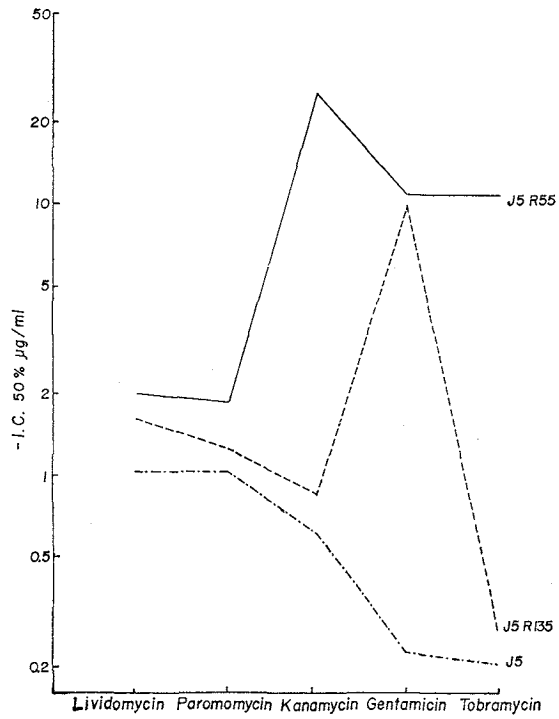
Fig. 1. Inhibitory concentrations 50% in μg/ml for *E. coli* J5 and *E. coli* J5 carrying R factors R135 and R55.



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