Communications to the editor

HIGH LEVEL TRANSFERABLE RESISTANCE TO GENTAMICIN

Sir:

Resistance to aminoglycoside antibiotics is frequently observed among enteric bacteria harbouring transferable drug-resistance factors (R factor). Streptomycin or kanamycin can be inactivated by various enzymes[1~11] but the activity of commercially available gentamicin is generally unaffected. One exception has been reported by SMITH,[12,13] who observed a low level (<1~2 μg/ml) of resistance to gentamicin in *Enterobacteria* carrying R factors.

Recently we isolated from urinary tract infections multi-resistant *Enterobacteria* (three *Klebsiella pneumoniae*, one *Escherichia coli* and one *Enterobacter aerogenes*). These strains were resistant to high concentrations of gentamicin (32~128 μg/ml), and the relevant resistance determinants were transferred with the other drug-resistance determinants in conjugation experiments. The complete drug resistance patterns A=ampicillin, C=chloramphenicol, T = tetracycline, Su = sulfonamides, S = streptomycin, K=kanamycin, P=paromomyicn, Ge=gentamicin), and the minimal inhibitory concentrations (M.I.C.) of gentamicin for the newly isolated strains, *Klebsiella pneumoniae* LCCB 433, LCCB 182, and LCCB 184, *Enterobacter aerogenes* LCCB 183 and *Escherichia coli* LCCB 3 are shown in Table 1. These strains were conjugated with *E. coli* K12 LA 115 lac$^+$ or *E. coli* K12 LA 116 lac$^-$, (sodium azide-resistant mutants of *E. coli* K12 54117 lac$^+$ and *E. coli* K12 C600 lac$^-$, kindly supplied by Drs. LE MINOR and WOLLMAN). The frequency of transfers after 16 hours of contact was between 1.10^{-3} and 1.10^{-5} per donor bacteria. The transferred characters, and the nomen-

Table 1.

	Donor Original strain resistance pattern	MIC of gentamicin (μg/ml)	Recipient *E. coli* K12	Selecting antibiotic	Transferred characters	Nomenclature of resistant recipient strains
	LCCB 433	128	LA 115	A	ACSu K Ge	
	ACSu SKPGe			C	ACSu K Ge	
				K	ACSu K Ge	
				G	ACSu K Ge	LA 115 R 55
Transfer from			LA 116	C	ACSu K Ge	
Klebsiella				K	ACSu K Ge	
pneumonie				G	ACSu K Ge	LA 116 R 55
	LCCB 182	32	LA 115	G	ACSu K Ge	
	ACTSu SKPGe				ACSuT K Ge	LA 116 R 58
	LCCB 184	32	LA 115	G	ACSu K Ge	LA 115 R 57a
	ACTSu SKPGe			K	ACSu K Ge	LA 115 R 57b
Transfer from *Enterobacter aerogenes*	LCCB 183 ACTSu SKPGe	32	LA 115	G	ACSu K Ge	LA 115 R 59
	LCCB 3	64	LA 115	G	ACSu K Ge	LA 115 R 56
	ACTSu SKPGe				ACSuT K Ge	
				G	ACSuT K Ge	
					ACSuT KP Ge	
Transfer from				A	ACSuT KP Ge	
Escherichia coli				K	T KP	
				T	T KP	
			LA 116	T	T KP	
					ACSuT K Ge	
				G	ACSuT KP Ge	

Ampicillin (Delagrange) 20 μg/ml, chloramphenicol, (Roussel) 10 μg/ml, kanamycin sulfate (Bristol) 12.5 μg/ml, tetracycline hydrochloride (Pfizer-Clin) 12.5 μg/ml, gentamicin sulfate (Uni-labo) 4 μg/ml. Sodium azothydrate (Rhône-Poulenc) 300 μg/ml, was added to inhibit donor bacteria on selecting medium.

clature for resistant recipients can also be seen in Table 1. Some variations in the drug resistance pattern of the recipients were observed according to the selecting drug, but gentamicin-resistant recipients were always obtained when this antibiotic was used as the selector (see Table 1). Transfer of gentamicin resistance was obtained 19 times in various conjugations, ampicillin, chloramphenicol, kanamycin, tetracycline or gentamicin being used as a selector. Transfer of kanamycin and paromomycin resistance was sometimes obtained independently. Subsequent transfers of R factor R 55 and R 57b were successful between *E. coli* LA 115 lac^+ R^+ and LA 116 lac^- and *E. coli* LA 116 lac^- R^+ and *E. coli* K12 LA 106 lac^+ (mutant resistant to rifampicin of *E. coli* K12 Hfr H IP 6533).

Transduction of gentamicin and kanamycin resistance, associated with resistance to ampicillin, chloramphenicol and sulfonamides, was obtained with phage P1-b (a mutant of phage P1-Kc, kindly supplied by Dr. F. JACOB). *E. coli* K12 LA 115 R 57b (A, C, Su, K, Ge) was lysed by phage P1-b. The sterile lysate, containing 1.10^9 p.f.u./ml was mixed with *E. coli* K12 LA 116 (m.o.i. =1) and gentamicin was used to select transductants. The frequency of transduction was 5.10^{-8}/p.f.u. The transductants were able to transfer *"en bloc"* their resistance characters into sensitive *E. coli* K12 LA 115 or *E. coli* K12 LA 106 (Hfr H). Stability, co-transfer and co-transduction of the five resistance determinants A, C, Su, K, Ge support the hypothesis that they belong to a single factor.

E. coli K12 LA 106 (Hfr H): R 57b (A, C, Su, K, Ge) was found to be sensitive to the male specific phage MS2 and f2, so the R 57b factor does not repress the synthesis of F pili and can be considered as an "fi^-" factor.

The MICs of gentamicin, kanamycin, paromomycin, neomycin, framycetin and monomycin for *E. coli* K12 LA 115 and LA 116 carrying R factors R 55, R 56, R 57a, R 57b, R 58 and R 59 are shown in Table 2. These strains were compared with *E. coli* LA 116 R 5, which carries an R-factor transferred from *E. coli* K12 CS2 R 5 (NIHJ B 265), kindly supplied by Dr. UMEZAWA, and with our strain LA 115 R 11(2).

Table 2. MIC in μg/ml of aminoglycosidic antibiotics (agar plate method)

E. coli K12	R factor	Gentamicin	Kanamycin	Paromomycin	Neomycin	Framycetin	Monomycin
LA 115	no	0.5	1	2	1	2	4
LA 115	R 55	128	64	2	1	2	4
LA 115	R 56	32	32	2	1	2	4
LA 115	R 57a	32	32	8	2	2	4
LA 115	R 57b	256	128	8	8	8	4
LA 115	R 58	32	32	4	1	2	4
LA 115	R 59	32	32	4	2	2	4
LA 116	no	0.25	0.5	2	1	1	4
LA 116	R 55	32	32	1	1	1	4
LA 116	R 57b	64	32	2	1	1	4
LA 106	no	2	4	8	4	8	8
LA 106	R 55	128	128	8	4	8	8
Transducted strains							
LA 116	R 57b	32	32	2	1	1	4
LA 116	R 57b	64	64	2	1	1	4
LA 115	R 57b	32	32	2	1	2	4
Control							
LA 116	R 5	0.25	32	2	2	2	4
LA 115	R 11(2)	0.5	512	512	512	512	512

Three types of resistant recipients were observed:

a) *E. coli* K12 LA 116 R 5 carrying the R factor transferred from *E. coli* K12 CS2 R 5 was found resistant to kanamycin but sensitive to the other aminoglycoside antibiotics including gentamicin. This strain harbouring this R factor has been shown to acetylate kanamycin.[6,7,8]

b) *E. coli* K12 LA 115 R 11(2), was found sensitive to gentamicin (M.I.C. : 0.5 μg/ml), but resistant to kanamycin and the other antibiotics of the deoxystreptamine group (>512 μg/ml). A similar resistance pattern has been shown in strains phosphorylating kanamycin.[9~11]

c) *E. coli* strains K12 LA 115 and LA 116 harbouring the factors R 55, R 56, R 57a, R 57b, R 58 and R 59 were found resistant to kanamycin (32~128 μg/ml) and gentamicin (32~256 μg/ml) but sensitive to paromomycin and other antibiotics of the deoxystreptamine group.

The strains of *K. pneumoniae*, *E. coli* and *E. aerogenes* recently isolated in a hospital in Paris harbour transferable R factors which are able to confer on them a high level of resistance to commercial preparations of gentamicin, that is constantly associated with kanamycin resistance, but they are still sensitive to other aminoglycosidic antibiotics. This type of transferable resistance clearly differs from the two types previously described to inactivate kanamycin, since the latter were not associated with resistance to gentamicin.

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