PREVALENCE OF *PSEUDOMONAS AERUGINOSA* STRAINS POSSESSING R FACTOR IN A HOSPITAL

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Pseudomonas aeruginosa strains possessing R factor, or transferable drug-resistant factor, have been described[1~9]. We have made an attempt to test some of the *P. aeruginosa* strains recently isolated in our hospital for the transferability of resistance to various antibiotics.

The 51 *P. aeruginosa* strains studied had been isolated from various pathological specimens submitted to the Central Clinical Laboratories, Shinshu University Hospital, Matsumoto, for bacteriological examination. Those specimens had been collected from 40 in-patients and 11 out-patients. The criteria used for the identification of *P. aeruginosa* were as follows: Gram-negative motile rods that were oxidase-positive, utilized glucose oxidatively in Hugh and Leifson's medium, and grew on Simmons' citrate agar. Hydrogen sulfide was not produced in a butt of TSI agar. The strains produced a diffusible green pigment on King's A medium. They hydrolyzed arginine but did not decarboxylate lysine when tested by Møller's method.

Auxotrophic mutants were propagated from cultures treated with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine by a combination of penicillin screening and replica plating methods, and were characterized as recommended by Glover[10]. Tryptosoy agar, a product of the Eiken Chemical Co., Tokyo, was used for antibiotic susceptibility tests. It was also employed as a basal medium for the test of pyocinogenic properties. The minimal medium used contained the following substances per liter: K_2HPO_4, 10.5 g; KH_2PO_4, 4.5 g; $(NH_4)_2SO_4$, 1.0 g; $MgSO_4 \cdot 7H_2O$, 0.05 g; NaCl, 0.5 g; glucose, 3.5 g; and agar, 15 g. This medium was enriched, when necessary, by adding amino acid(s) at a concentration of 20 μg/ml.

Susceptibility to antibiotics was examined by streaking a loopful of saline suspension (about 5×10^7 cells/ml) of a fresh agar culture onto agar plates containing varying concentrations of the antibiotics listed below: streptomycin (SM), kanamycin (KM), gentamicin (GM), tetracycline (TC), chloramphenicol (CM), colistin (CL), benzylpenicillin (PC-G), ampicillin (AB-PC), and carbenicillin (CB-PC). Results were read after incubation at 37°C for 20 hours.

Tests of transferability of antibiotic resistances and chromosomal markers were performed as follows: Broth cultures of donor (auxotrophic resistant strain) and recipient (doubly auxotrophic susceptible strain) in the logarithmic phase of growth (about 5×10^8 cells/ml) were mixed at the ratio 1:5. The mixture was incubated at 37°C for an hour with gentle agitation. After the incubation the mating culture was centrifuged, resuspended in saline at a concentration of about 5×10^8 cells/ml, and 0.1 ml of sample was spread onto each selecting plate. All the plates were incubated at 37°C for 48 hours. Colonies appearing on them were picked, purified by streaking on nutrient agar plates, and then tested for transfer of antibiotic resistance(s) and for transfer of chromosomal markers. Frequency of transfer of antibiotic resistance or chromosomal marker was estimated from the number of colonies per input donor.

Pyocinogenic properties were tested in such manner as described by Darrell and Wahba[11]. Serotypes (O antigens) were determined by using autoclaved and/or living cultures, as described previously[12].

The susceptibilities of the 51 *P. aeruginosa* strains to the 9 antibiotics tested are shown in Table 1. On the basis of susceptibilities to SM, KM, TC, CM, and CB-PC, these strains were classified into 11 susceptibility types, as listed in Table 1. None of them

Table 1. Susceptibilities of *P. aeruginosa* strains to antibiotics

Susceptibility types	No. of strains	SM			KM			GM	CB-PC		PC-G	AB-PC	TC		CM		CL
		<100*	>100~<500	>1,000	<100	>100~<500	>1,000	<100	<100	1,000	>200	>200	<100	>100~<300	<100	>100~<300	<50
1	31	●**			●			●	●		●	●	●		●		●
2	2		●		●			●	●		●	●	●		●		●
3	6			●			●	●	●		●	●	●		●		●
4	1	●				●		●	●		●	●	●		●		●
5	3			●	●			●	●		●	●	●		●		●
6	2	●					●	●	●		●	●	●		●		●
7	2			●			●	●	●		●	●	●		●		●
8	1			●			●	●		●	●	●	●		●		●
9	1			●	●			●	●		●	●		●		●	●
10	1			●	●			●	●		●	●		●	●		●
11	1			●		●		●	●		●	●		●		●	●

* Concentration of antibiotic (μg/ml)
** • Minimum inhibitory concentration of the antibiotic indicated above over the strains of the given susceptibility type.

Table 2. Transfer of antibiotic resistances in *P. aeruginosa* strains Ps 2-72, Ps 38-72, Ps 39-72 and Ps 33-72.

Donor	Recipient	Selected marker	Frequency of transfer	No. of colonies tested for genotype	Genotype
Ps 2-72, *arg*$^-$*	Ps 33-72**, *his*$^-$*, *lys*$^-$*	CB-PC/400 μg^r ***	3×10^{-4}	50	CB-PC^r, SM^r, KM^r, *his*$^-$, *lys*$^-$
		SM/500 μg^r	4×10^{-4}	50	CB-PC^r, SM^r, KM^r, *his*$^-$, *lys*$^-$
		KM/300 μg^r	3×10^{-4}	50	CB-PC^r, SM^r, KM^r, *his*$^-$, *lys*$^-$
		his$^+$	$<10^{-8}$	0	
		lys$^+$	$<10^{-8}$	0	
Ps 38-72, *met*$^-$*	Ps 33-72, *leu*$^-$*, *trp*$^-$*	TC/200 μg^r	4×10^{-5}	50	TC^r, SM^r, *leu*$^-$, *trp*$^-$
		SM/500 μg^r	1×10^{-5}	50	TC^r, SM^r, *leu*$^-$, *trp*$^-$
		KM/300 μg^r	$<10^{-8}$	0	
		CM/150 μg^r	$<10^{-8}$	0	
		leu$^+$	$<10^{-8}$	0	
		trp$^+$	$<10^{-8}$	0	
Ps 39-72, *arg*$^-$	Ps 33-72, *his*$^-$, *lys*$^-$	TC/200 μg^r	5×10^{-4}	50	TC^r, SM^r, *his*$^-$, *lys*$^-$
		SM/500 μg^r	3×10^{-4}	50	TC^r, SM^r, *his*$^-$, *lys*$^-$
		CM/150 μg^r	$<10^{-8}$	0	
		his$^+$	$<10^{-8}$	0	
		lys$^+$	$<10^{-8}$	0	

* *arg*, arginine; *his*, histidine; *lys*, lysine; *met*, methionine; *leu*, leucine; *trp*, tryptophan. The minus and plus signs indicate requirement and independence of the given substance, respectively.
** Ps 33-72 is a susceptible strain belonging to susceptibility type 1 in Table 1.
*** Letter r indicates the resistance to the given concentration of the given antibiotic.

was resistant to GM or CL. PC-G and AB-PC allowed all the strains to grow at the concentrations indicated. Twenty strains that were classified into susceptibility types 2~11, as shown in Table 1, were tested for the occurrence of transfer of antibiotic resistance(s).

Nearly all the *P. aeruginosa* strains have been reported to be pyocinogenic[18]. Therefore, for an optimal transfer of R factor, it is required to choose a suitable recipient; that is, no strain chosen as recipient should be a producer of pyocin(s) that kills the donor or one susceptible to pyocin(s) secreted by the donor. For these reasons, so far as the test of pyocinogenic properties is concerned, the 51 strains were divided into two groups; *i. e.*, one group consisting of resistant strains (susceptibility types 2~11, inclusive) and the other of susceptible strains (susceptibility type 1 alone). Each strain of one group was tested for pyocinogenic properties by using all the strains of the other group as indicators. The recipients of a donor were selected. Furthermore, in order to differentiate exactly the transfer of an R factor

from a sex factor-mediated or from phage-mediated transfer of chromosomal gene(s) associated with the antibiotic resistance, selections were made both for resistant marker(s) and for two different auxotrophic markers. For these selections, it was assumed that if acquisition of the resistance(s) resulted from the transfer of chromosomal gene(s), the transfer of the other chromosomal markers would take place at a similar frequency.

Three strains, Ps 2-72, Ps 38-72 and Ps 39-72, were found to be able to transfer their antibiotic resistances. Detailed results are given in Table 2.

Ps 2-72 transferred its resistances to CB-PC, SM and KM. It had been isolated from the urine of a patient with a hypertrophic prostate. Heated cultures of this strain became autoagglutinable, and hence its serotype could not be determined. Both Ps 38-72 and Ps 39-72 transferred resistant markers to SM and TC, but the resistances of these strains to other antibiotics were non-transferable. The 2 strains belonged to serotype 06 (HABS' type). Of them, Ps 38-72 had been obtained from the urine of a patient with a urinary tract infection, and Ps 39-72 from that of a patient with a hypertrophic prostate.

Attempts were made repeatedly to transfer the resistance of the other 17 strains to antibiotics by employing three different recipients for each donor, but in vain. No reasons for the failure are yet known.

Strains Ps 2-72, Ps 38-72 and Ps 39-72 were able to transfer their resistance alone, and no selections by the auxotrophic markers were fertile. Therefore, there is no doubt that they possess R factor. R factor of Ps 2-72 conferred a high resistance to CB-PC upon its host cells. No study of enzymes or compatibility groups has been done. The results of the present study apparently indicate that the occurrence of *P. aeruginosa* with R factor in Japan is not rare.

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References

1) AYLIFFE, G. A.; E.J.L. LOWBURY & E. ROW: Transferable carbenicillin resistance in *Pseudomonas aeruginosa*. Nature 235: 141, 1972
2) BRYAN, L. E.; H. M. VAN DEN ELZED & J. T. TSENG: Transferable drug resistance in *Pseudomonas aeruginosa*. Antimicr. Agents & Chemoth. 1: 22~29, 1972
3) DATTA, N.; R. W. HEDGES, E. J. SHAW, R. B. SYKES & M.H. RICHMOND: Properties of an R factor from *Pseudomonas aeruginosa*. J. Bact. 108: 1244~1249, 1970
4) FULLBROOK, P. D.; S. W. ELSON & B. SLOCOMBE: R factor mediated β-lactamase in *Pseudomonas aeruginosa*. Nature 226: 1054~1056, 1970
5) LOWBURY, E.J.L.; A. KIDSON, H. A. LILLY, G. A. AYLIFFE & R. J. JONES: Sensitivity of *Pseudomonas aeruginosa* to antibiotics: Emergence of strains highly resistant to carbenicillin. Lancet 1969-2: 448~452, 1969
6) ROE, E.; B. J. JONES & E.J.L. LOWBURY: Transfer of antibiotic resistance between *Pseudomonas aeruginosa*, *Escherichia coli*, and other gram-negative bacilli in burns. Lancet 1971-1: 149~152, 1971
7) STANISICH, V. A. & B. W. HOLLOWAY: Chromosome transfer in *Pseudomonas aeruginosa* mediated by R factors. Genet. Res. 17: 169~172, 1971
8) SYKES, R.B. & M.H. RICHMOND: Intergeneric transfer of a β-lactamase gene between *P. aeruginosa* and *E. coli*. Nature 226: 952~954, 1970
9) WITCHITZ, J.L. & Y.A. CHABBERT: Résistance transférable à la gentamicine. II. Transmmision et liaisons du caractère de résistance. Ann. Inst. Past. 122: 367~378, 1972
10) GLOVER, S. W.: The induction, isolation and analysis of auxotrophic mutants. *In* Experiments in Microbial Genetics. (CLOWES, R. C. & W. HAYES, Eds.) Blackwell, Oxford, England. 1968
11) DARRELL, J. H. & A. H. WAHBA: Pyocin-typing of hospital strains of *Pseudomonas pyocyanea*. J. Clin. Path. 17: 236~242, 1964
12) MATSUMOTO, H. & T. TAZAKI: Relationship of O antigens of *Pseudomonas aeruginosa* between Hungarian types of LÁNYI and HABS' type VERDER and EVANS' types. Japan. J. Microbial. 13: 209~211, 1969
13) HOLLOWAY, B. W.; V. KRISHNAPILLAI & V. STANISICH: *Pseudomonas* genetics. Ann. Rev. Genetics 5: 425~446, 1971