

SPONTANEOUS SEGREGATION OF R PLASMIDS PRESENT IN GRAM NEGATIVE BACTERIA OF POULTRY ORIGIN

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ABSTRACT

R plasmids of Gram negative bacteria isolated from poultry in Karachi were studied for their spontaneous segregation in *E. coli* AB 712. Of 32 R plasmids studied, 16 were lost by spontaneous segregation.

INTRODUCTION

R plasmids are extra chromosomal elements that confer resistance to one or many antibiotics (Khatoon & Mohammed, 1986).

R plasmids mediated antibiotic resistance is quite dangerous from view point of chemotherapy as R plasmids may spread in bacterial population by cell contact or conjugation (Cohen & Miller, 1970). Non pathogenic bacteria bearing R plasmids are equally dangerous because they can transfer R plasmids to pathogenic bacteria.

R plasmids like other extra chromosomal elements, can be lost from the host cells spontaneously due to some errors in replication or segregation (Derylo & Lorkiewicz, 1970; Novick, 1969). Plasmid loss is used as a criterion for establishing the extra chromosomal nature of certain genes. Such losses are increased when the cells are treated with certain chemical agents such as acridine orange or ethidium bromide (Iyer & Iyer, 1969; Mitsuhashi et al., 1961; Watanabe & Fukasawa, 1961). However, the extent of spontaneous loss or segregation is a property of a particular plasmid. The losses are also more frequent when *Shigella* or *Salmonella* host strains are used (Mitsuhashi et al., 1961; Watanabe & Fukasawa, 1961). Susceptibility to curing agents also varies among plasmids. Some plasmids are not curable by treatment with acridine orange or ethidium bromide (Amir & Khatoon 1976, Khatoon 1987).

Thirty two R plasmids of Gram negative bacteria isolated from poultry in Karachi (Ansari & Khatoon, 1994) were studied for their spontaneous segregation in *E. coli* AB712. Sixteen of these R plasmids were lost spontaneously (Table 1).

All these R plasmids were also studied for their curing by acridine orange (Ansari & Khatoon, 1996) and ethidium bromide (unreported data).

MATERIALS AND METHODS

Bacterial strain

All the R plasmids were studied for their curing by ethidium bromide in *E. coli* AB712 provided by E. Adelberg of U.S.A.

Media

MacConkey's agar (M.A.) was from E. Merck, Germany. Nutrient agar (N.A.) had the following composition: Beef extract 3 gms, peptone 10 gms, NaCl 5 gms, agar 20 gms, distilled water 1000 ml, pH 7. Nutrient broth (N.B.) had the same composition as N.A. except that agar was not added.

Antibiotics and other chemicals

The antibiotics used were: ampicillin trihydrate, chloramphenicol levo (Opal Laboratories Ltd., Karachi), furazolidone (Risma Laboratories, Karachi), kanamycin sulphate, neomycin sulphate (Glaxo Laboratories, Karachi), polymyxin B (Pfizer Laboratories, Karachi) and tetracycline HCl (Lederle Laboratories, Karachi).

All the antibiotics except chloramphenicol and furazolidone were dissolved in distilled water to give a concentration of 10 mg/ml. Chloramphenicol and furazolidone were dissolved in ethyl alcohol and dimethyl formamide respectively.

Ethyl alcohol was from E. Merck, Germany and dimethyl formamide came as a gift from Prof. Salim-uz-Zaman Siddiqui FRS of H.E.J. Research Institute of Chemistry, University of Karachi.

Antibiotics were sterilized by millipore filtration and kept frozen when not in use. The concentration of working solution of antibiotics was 50 µg/ml.

Spontaneous segregation of R plasmids

Techniques were similar as described by Khatoon (1971). The R plasmids were studied for curability in *E. coli* AB712. The bacterium bearing R plasmid was grown in antibiotic free nutrient broth, through 30-45 consecutive transfers. Each time the culture was grown for 24 hours and transferred to the fresh broth to give a 20 fold dilution. At the end of 30-45 transfers, the culture was diluted and plated on M.A., to obtain isolated colonies. Some 100 colonies were grided onto M.A. plates. After overnight incubation at 37°C, these were replicated on antibiotic containing plates to check for the loss (or its absence) of antibiotic resistance determinants.

RESULTS AND DISCUSSION

Table-1 lists the R plasmids of Gram negative bacteria isolated from poultry in Karachi which were cured by spontaneous segregation. Of the 32 R plasmids studied, 16 were lost by spontaneous segregation. 50% R plasmids were stable in *E. coli* host even after spontaneous segregation. The stability of R plasmids even after repeated subculturing reflects that these are quite dangerous from epidemiologic point of view as infections caused by organisms bearing these R plasmids will not be easy to control. Some of these R plasmids were stable even after treatment with ethidium bromide (Ansari & Khatoon, in press) and acridine orange (Ansari & Khatoon, 1996).

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Table 1
Spontaneous segregation of R plasmids in *E. coli* AB712

R-plasmids	Antibiotic* Resistance Determinants	% of colonies with lost marker(s) @	Markers lost
pFK-1	KNP	0.0	-
pFK-2	KN	0.0	-
pFK-4	KN	0.0	-
pFK-5a	A	0.0	-
pFK-5b	KN	0.0	-
pFK-6	KT	0.0	-
pFK-7	KNT	0.0	-
pFK-8a	AT	0.0	-
pFK-9	PT	1.0	PT
pFK-10a	KN	0.0	-
pFK-10b	T	0.0	-
pFK-13	T	0.0	-
pFK-15	CT	88.0	CT
pFK-16	ACFKNT	98.0	ACFKNT
pFK-17	T	0.0	-
contd...			

R-plasmids	Antibiotic* Resistance Determinants	% of colonies with lost marker(s) @	Markers lost
pFK-18	KNT	0.0	-
pFK-19	T	1.0	T
pFK-20	AKN	50.0	AKN
pFK-22a	A	1.0	A
pFK-22b	T	7.0	T
pFK-22c	KN	2.0	KN
pFK-23	KNT	3.0	KNT
pFK-24	T	0.0	-
pFK-25	T	1.0	T
pFK-26	KN	65.0	KN
pFK-27	T	40.0	T
pFK-28	T	1.0	T
pFK-29	T	0.0	-
pFK-30	FKN	0.0	-
pFK-31	T	2.0	T
pFK-32a	C	1.0	C
pFK-32b	KN	1.0	KN

*A = ampicillin, C = chloramphenicol, F = furazolidone, K = kanamycin,
N = neomycin, P = polymyxin B and T = tetracycline.

@ 100 colonies were tested.

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