ANTIBIOTIC RESISTANCE

TRANSPOSITION AND OTHER MECHANISMS

Editors
S. Mitsuhashi, L. Rosival, V. Krcmery

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Fourth International Symposium on
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Castle of Smolenice, Czechoslovakia, 1979

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S. MITSUHASHI, L. ROSIVAL, V. KRČMÉRY

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ANTIBIOTIC RESISTANCE:
TRANSPOSITION AND OTHER MECHANISMS

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PREFACE

Proceedings from the International Symposium on Antibiotic Resistance 1979, the fourth volume in the series of material edited from the Smolenice symposium, is a continuation in the tradition of communicating new scientific results on a currently important and interesting problem. It concerns not only human and veterinary medicine and practice, but also such important fields of contemporary biology as molecular biology and genetics.

Indeed, in both these disciplines, we have recently noted a strong and dynamic development of scientific knowledge directed toward such outgrowths of the study of plasmids as gene manipulations, transposons, and transposition, as well as the evolution of plasmids in general.

Because of a very real global development of resistance to the newest antibiotics, and the active participation of experts from medical science and practice, it was reasonable to separate the medical part of the program from the theoretical one. Consequently, priorities of the fourth symposium were transposons and the transposition of resistance of to antibiotics, with their serious impact on epidemiology, the resistance of *Pseudomonas aeruginosa*, that is becoming a prominent nosocomial pathogen, the ecology and epidemiology of R plasmids, and, last though not least, the computer-assisted surveillance of resistance to antibiotics.

Transposons which code for resistance to several antibiotics have been identified as a major mechanism by which this resistance is presently spread to new species and genera from its natural reservoir of bacteria. This has been found true for resistance to ampicillin, which appeared in *Neisseria* or *Hemophilus*, and additional transposons, e.g., for resistance to trimethoprim and even to gentamicin, have also been identified.

Gentamicin resistance is found to be an increasing problem in dealing with staphylococci, *Enterobacteriaceae*, and *Pseudomonas aeruginosa*. Enzymatic mechanisms for its inactivation are intensively studied so that we can now even address the question of the epidemiology of drug—inactivating enzymes in bacteria.

To guide antibiotic policy, computer-assisted systems for surveillance and monitoring of antibiotic resistance in selected species of "problem bacteria" have been developed in several countries and on an international basis. Comparison of the present and developing situation on this highest level, e.g., between selected hospitals and wards, seems to be highly productive and useful.

When setting forth this volume, many thanks of all participants should be expressed to AVICENUM, Czechoslovak Medical Press, and to SPRINGER-Verlag, for their efforts and help in editing these proceedings.

L. ROSIVAL

CONTENTS

I.D. Genetics and Molecular Biology

Chairwoman: P. Kontomichalou

I.E. Plasmids in the Nature

Chairman: W. Goebel

II. MEDICAL PART

II.A. New Drugs Against Resistant Bacteria

Chairman: S. M. Navashin

II.B. Natural Spread of Antibiotic Resistance

Cairman: R. Goméz-Lus

II.C. Enzymes Inactivating Antibiotics

Chairman: R. Goméz-Lus

II.D. Ecology and Epidemiology of Resistant Strains

Chairman: L. Rosival

I. THEORETICAL PART

A) INTRODUCTORY LECTURES

NONCONJUGATIVE DRUG RESISTANCE PLASMIDS

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It is a real pleasure for me to give the opening address at the 4th International Symposium on Antibiotic Resistance at the Castle of Smolenice in Czechoslovakia.

We are very glad once again to have met many representatives from all over the world who are studying plasmids and trying to solve the problems caused by new biohazards resulting from drug resistance plasmids. First of all, I want to express our sincere thanks to the Minister of Health of the Slovak Socialist Republic, the Minister of Agriculture of the Slovak Socialist Republic, and the Slovak Academy of Sciensces, and also to the Organizers of this symposium. Special thanks are due to the Secretary General, Organizing and Program Comittee, and to Publishers of the Proceedings.

TABLE I.

Isolation frequency of R plasmids from resistant strains

Drug	Isolation frequency of R plasmids from strains resistant to (%)
Tc	65.2
Cm	58.0
Sm	64.3
Su	55.8
Km	70.2
Ap	69.3
Gm	79.3

The results are based on surveys of 19,984 clinical isolates. Abbreviation of drugs: tetracycline (Tc), chloramphenicol (Cm), streptomycin (Sm), sulfanilamide (Su), kanamycin (Km), ampicillin (Ap), and gentamicin (Gm).

The introduction of antibacterial agents has contributed greatly to improved treatment of infectious diseases and to the progress of practical medicine. Only half a century after the real start of chemotherapy, however, we are now faced with the problems of bacterial resistance, and the prevalence of resistant bacteria has caused many problems in medicine, stock farming and fish breeding.

Transmissible drug resistance(R) plasmids were discovered in 1960, and since then the extensive studies of R plasmids have disclosed the epidemiology, genetics, and molecular biology of R plasmids. R plasmids are conjugally transferable and have a wide host range so that resistance spreads quickly from bacteria to bacteria, compounding the infectious spread by multiplying resistant organisms. It was further found that the drug resistance determinants on plasmids were easily transferred from plasmid to

bacterial chromosomes, bacteriophages and to other plasmids, or *vice versa*, the transferable unit is called "transposon" (Cohen, 1976, Mitsuhashi, 1977, Mitsuhashi et al., 1977). Easy transferability of resistance determinants has recently been explained by the presence of an insertion sequence(IS) (Starlinger and Saedler, 1976).

TABLE II.

Isolation frequency of R plasmids from strains resistant to Tc, Cm, Sm, and Su

Pattern of [a] resistance	Isolation frequency of R plasmids from strains resistant to (%)
Quadruple	67.9
Triple	55.2
Double	25.1
Single	4.3

The results are based on surveys of 14,530 strains.
[a] Resistance to Tc, Cm, Sm, and Su.

TABLE III.

Resistance patterns of R plasmids carrying Km or Ap resistance

R plasmids carrying	Resistance patterns	No. of R plasmids (%)
Ap resistance	Tc.Cm.Sm.Su.Km.Ap	87 (11.9)
	Quintuple resistance	252 (34.6)
	Quadruple resistance	219 (30.0)
	Triple resistance	75 (10.3)
	Double resistance	50 (6.9)
	Single Ap resistance	46 (6.3)
Total		729
Km resistance	Tc.Cm.Sm.Su.Ap.Km	87 (18.5)
	Quintuple resistance	78 (16.6)
	Quadruple resistance	91 (19.3)
	Triple resistance	137 (29.1)
	Double resistance	59 (12.5)
	Single Km resistance	19 (4.0)
Total		471

The R plasmids were isolated from 15,884 strains of gram-negative bacteria.

Surveys of clinical isolates disclosed that R plasmids were demonstrated at high frequencies, ranging from 50 to 80 percent, from drug-resistant bacteria (Table I.). Among drug-resistant strains to Tc, Cm, Sm and Su, R plasmids were most frequently seen in quadruply resistant strains, followed by triply, doubly and singly resistant strains, in that order (Table II.).

Resistance patterns of R plasmids carrying kanamycin (Km) and ampicillin (Ap) resistance are shown in Table III. R plasmids carrying Km- or Ap-resistance were isolated at a high frequency of 70% from Km- or Ap-resistant strains of gram-negative bacteria. Of those carrying Ap resistance, R plasmids with quintuple resistance were isolated most frequently, followed by those encoding quadruple, sexiduple, triple, double,

and single resistance, in that order, this distribution indicates the prevalence of Ap plasmids encoding multiple resistance. Of those with Km resistance, R plasmids carrying triple resistance were the most common, but the various patterns of multiple resistance were seen with very similar frequencies. These results are accountable for by the following factors:

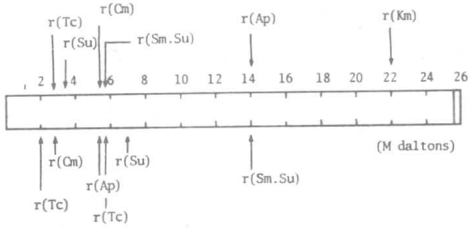

Fig. 1. The molecular size of nonconjugative resistance(r) plasmid DNAs.

(1) selection of multiple resistance plasmids by various drugs used,
(2) translocation of new resistance determinant on R plasmids, and
(3) spread of R plasmids by conjugal transmission.

Studies of multiple resistance in *Shigella* strains opened a way to the discovery of R plasmids (see Rev. Mitsuhashi, 1977, 1979). Strains of *S. aureus* are frequently isolated from clinical sources and play an important role in pathological lesions. This laboratory examined the reasons for the prevalence of drug-resistant strains and for the acquisition

TABLE IV.

Isolation of nontransferable (r) plasmids from bacteria carrying nontransmissible resistance

Bacteria	Isolation frequency of r plasmids (%)
S. aureus	85.0
S. pyogenes	95.0
H. influenzae	60.0
E. coli	96.0
Shigella	98.0
Salmonella	95.0
P. mirabilis	67.0
S. marcescens	63.0

The presence of r plasmids was examined using singly or doubly resistant strains.

of multiple resistance in staphylococci. The strains triple-and quadruple-resistant to tetracycline (Tc), streptomycin (Tc), penicillin (Pc) and sulfanilamide (Su) accounted for more than half of the strains showing multiple resistance. The isolation frequency of strains resistant to macrolide antibiotics (Mac), chloramphenicol (Cm), synthetic penicillins (Dmp and Mci), and kanamycin (Km) was highest among the triple- and quadruple-resistant strains. These results indicate that staphylococci easily develop multiple resistance when new drugs are introduced. Soon after the discovery of conjugative R plasmids in gram-negative bacteria, we examined the presence of plasmids

in staphylococci. In spite of the nontransmissibility of drug resistance in staphylococci, we found in 1963 that cross-resistance to Mac antibiotics was irreversibly eliminated by treatment with acriflavine (Mitsuhashi, Morimura, Kono, and Oshima, 1963). It was subsequently found that Pc resistance in staphylococcal strains was eliminated by treatment with acriflavine. Loss of the capacity to produce penicillinase explained the reversion from Pc resistance to Pc sensitivity (Hashimoto, Kono, and Mitsuhashi, 1964). These results indicate that drug resistance determinants in staphylococci are located on nonconjugative resistance (r) plasmids. We now have various methods to confirm

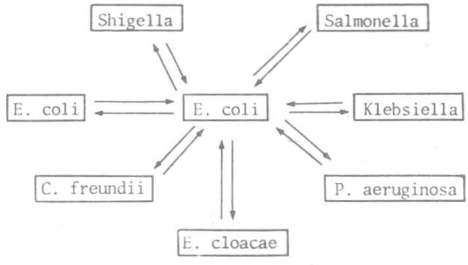

Fig. 2. Host range in the transformation of r plasmid DNAs.

the presence of nonconjugative r plasmids in bacteria: (1) artificial and spontaneous elimination of drug resistance, (2) demonstration of a satellite band of plasmid DNA by the density gradient centrifugation, (3) transformation of drug resistance by means of isolated plasmid DNA to a *rec*$^-$ recipient, and (4) transduction of drug ressistance to a *rec*$^-$ recipient. Using these techniques, we have isolated many r plasmids from staphylococcal strains, about 85% of the strains examined were found to carry r plasmids, indicating that drug resistance determinants in staphylococci are located primarily on these plasmids (Mitsuhashi et al., 1973, Mitsuhashi et al. 1976).

Further studies of drug resistance have disclosed that most single or double resistance in gram-positive or gram-negative bacteria is due to the presence of r plasmids that are present as multiple copies. Epidemiology of the r plasmid distribution in singly or double resistant strains is shown in Table IV. The r plasmids were demonstrated at a high frequency from singly and double resistant strains carrying nontransferable resistance. Drug resistance plasmids with molecular weight of less than 20×10^6 daltons are nonconjugative and lack the transfer region that is on the R plasmid (Fig. 1). The physical properties of r plasmid DNAs isolated from gram-positive and gram-negative bacteria are shown in Table V. Contour length of most r plasmids ranged from 1 to 3 μm. It is characteristic that r plasmids are present as multiple copies in a cell and most of them encode single resistance, except for r (Mac. Pc) and r (Sm.Su). Therefore, multiple resistance in staphylococci is due to the presence of various r plasmids in a cell, which are present as multiple copies.

The r plasmid DNAs in gram-negative bacteria were found to be easily transmitted to various species of bacteria through transformation, resulting in the stable existence and the expression of resistance in a new host cell. It is interesting to note that the r plasmid DNAs from *P. aeruginosa* strains are transmitted through transformation to *E. coli* strains, although most R plasmids from *P. aeruginosa* are transmissible only between *P. aeruginosa* strains and not to *E. coli*. One of the representative results is shown in Fig. 2 (Inoue and Mitsuhashi, unpublished observation).

It is well known that almost all of *S. aureus* strains are lysogenic and plasmid resistance in staphylococci is transduced with phage lysates obtained from multiply resistant strains. In the transduction of Tc resistance by means of phage lysates obtained from *S. aureus* strains, every strain was found to be competent when tested in various combinations of donor and recipient. The percentage of competent recipient strains in the various phage groups was: group I including 80/81 (89%), group II (22%), group III (0%),

TABLE V.

Summary of the physical properties of nonconjugative (r) plasmid DNAs isolated from gram-negative and gramm-positive bacteria

DNA sources	Resistance pattern	Contour length (μm)	No. of copies per chromosome
S. aureus	Tc	1.4	22–25
	Cm	1.4	5–7
S. pyogenes	Tc	1.0	NT
H. influenzae	Ap	12.5	NT
	Su	1.8	22–29
E. coli	Tc	3.1	3–6
	Sm.Su	2.5	15–19
Shigella	Tc	2.8	NT
	Su	1.8	22–23
	Sm.Su	2.5	17–19
Salmonella	Su	1.9	14–23
	Sm.Su	2.5	17–19
Proteus	Su	1.7	16–18
	Sm.Su	2.5	16–18
P. aeruginosa	Sm.Su	2.4	NT
A. hydrophilla	Tc. Su. Ap	1.0	NT
		2.8	NT

NT = not tested.

TABLE VI.

Mobilization of r plasmid with conjugative plasmid

Donor carrying	Selective drug	Resistance patterns (%) of transconjugants
RP4 (Km.Tc.CBPC) + rMS21 (Sm.Su)	Sm	rMS21 (3.3) rMS21 + RP4 (96.7)
	Km	RP4 (25.1) RP4 + rMS21 (74.9)
R9-5 (Cm) + rMS76 (Ap)	Ap	rMS76 (7.0) rMS76 + R9-5 (93.0)
	Cm	R-5 (27.3) R9-5 + rMS76 (72.7)

Donor, *E. coli* X2207 Nal^r; recipient, *E. coli* X1037 Rif^r. After 3 hr of incubation at 37C, the mixed culture of donor and recipient was spread on selective plates for either r plasmid or R plasmid marker.

and group IV (40%). The results coincide with the distributions of multiple resistance in staphylococcal phage groups (Mitsuhashi, Oshima, Kawaharada, and Hashimoto, 1965). Similarly, most *P. aeruginosa* strains are also lysogenic, and the r plasmids in these strains are transduced with phage lysates obtained from multiply resistant strains of *P. aeruginosa*.

The r plasmids were found to be transmitted by means of mobilization with conjugative plasmid. One of the results is shown in Table VI. indicating that more than 96 percent of the transconjugants carried both R and r plasmids when selected for r plasmid marker. Even when selected for R plasmid marker, about 70 percent of the transconjugants carried both R and r plasmid. In the epidemiologic studies of R plasmids in naturally

TABLE VII.

Types of resistance mediated by plasmid

Bacteria	Resistance mediated by
Gram-positive	r
	$r_1 + r_2 + r_3 + \ldots$
Gram-negative	R
	$R_1 + R_2$
	R + r
	$R + r_1 + r_2 + r_3 \ldots$

occurring resistant strains, we must be careful of the difference in resistance patterns of transconjugants after selection with various drugs, resulting from the mobilization of r plasmid with conjugative R plasmid.

According to the surveys of drug resistance in more than fifty thousands strains of bacteria isolated from clinical materials, livestock and cultured fish, we can conclude that most resistance in these strains is due to the presence of drug resistance plasmids. Gram-positive bacteria usually carry r plasmid and multiple resistance is due to the concomitant presence of various r plasmids in a cell. There are many types of resistance mediated by plasmids in gram-negative bacteria due to the presence of R, (R_1+R_2), (R+r), ($R+r_1+r_2+\ldots$), etc. (Table VII.). The studies of r plasmids have disclosed that the situations of resistance mediated by plasmid are rather complicated in naturally occurring resistant strains.

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