

Drug-Resistance and Enterotoxigenic Plasmids in Enteropathogenic Bacteria With Special Reference to Clinical Isolates of San Lazaro Hospital*

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The Genetic background of drug-resistant bacteria is multiple in origin (Table 1). The simplest mechanism may be the simple selection of preexisting resistant strains by drugs. This is the case of emergence of penicillin G-resistant staphylococci in clinical fields. (Figure 1):

Table 1. Genetic Background of Bacterial Drug Resistance

1. Direct Relation of Bacteria with Drugs	
a. Spontaneous Mutation and Selection	SM-R, PAS-R M. tuberculosis MCIPC-R Staphylococcus aureus PC-R Streptococcus pneumoniae
2. Gene Transfer Mechanisms	
a. Plasmid Transfer	Multiple-R Gram-Negative Rods
b. Transduction	Multiple-R Staphylococcus, Streptococcus
c. Transformation	
d. Phage Conversion	
e. Chromosomal Recombination	

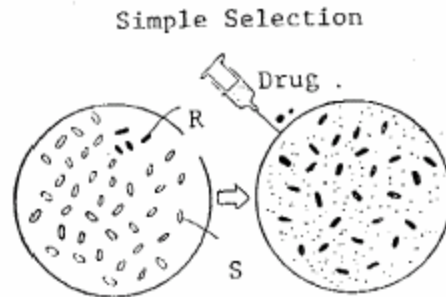


Figure 1. Mechanism of bacterial drug resistance

In 1948, Demerec in USA proposed the famous "Spontaneous Mutation and Selection" theory suggesting that the origin of drug-resistant bacteria randomly occurred as spontaneous mutants during cell division and drugs play a role only as the selective agents. This concept had been adopted until 1959 as the genetic background for drug-resistant bacteria not only in vitro but also in vivo (Figure 2).

Although bacteria had been considered as an unisexual and bifissionally dividing primitive creature, various mechanisms of gene transfer were discovered since 1946. There were chromosomal recombinations like sexual reproduction, Transduction by bacteriophages, carrier of genes by virus-like parasite in bacterial cells, and transformation by purified DNA. With these discoveries, it became logical to assume that drug-resistance of bacteria can be transmitted to

other species by these phenomena. In 1959, Akiba et al and Ochitai et al in Japan discovered a phenomenon of transfer of bacterial resistance from clinical isolates of *Shigella flexneri* and *Escherichia coli* to sensitive bacteria. It was the first discovery of R factors.

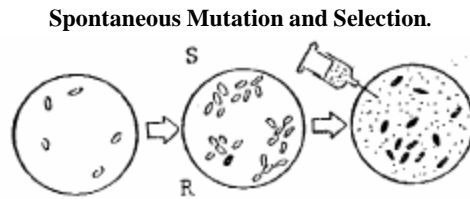


Figure 2. Mechanism of bacterial drug resistance

Today, it has been understood that the origin of streptomycin (SM) and p-aminosalicylic acid-resistant *Mycobacterium tuberculosis*, cloxacillin resistant *Staphylococcus aureus* and penicillin-resistant *Streptococcus pneumoniae* is the result of spontaneous mutation and selection. Whereas those of multiple drug-resistant gram-negative bacilli and gram-positive cocci are based upon the plasmid transfer, i.e., R factors and transduction by bacteriophages, respectively.

R plasmids and drug resistance in enteropathogenic bacteria

Plasmids are extra-chromosomal and cytoplasmic genetic elements Consisting of small circular DNA, the size of which is 1 to 3% of the bacterial chromosome.

Plasmids carrying drug-resistance genes were originally called R factors but are now called R plasmids by international agreement. Most R plasmids obtained from gram-negative bacteria are self-transferable by cellular conjugation, while those from gram-positive cocci are non-transferable and consist of a smaller circular DNA which can be carried from one coccus to another by bacteriophage transduction.

Common resistance patterns carried by transferable R plasmids are 4 drugs, 5 drugs or 6 drugs, and single drug resistance is rather rarely borne by them. On the other hand, the number of drug resistant genes borne by non-transferable miniplasmids is usually smaller, and they are contranduced by bacteriophages (Table 2).

As the result of bacteriophage transduction of miniplasmids, a large proportion of clinical isolates of *Staphylococcus aureus* and *Streptococcus pyogenes* have become multiple drug-resistant in Japan (Figure 3).

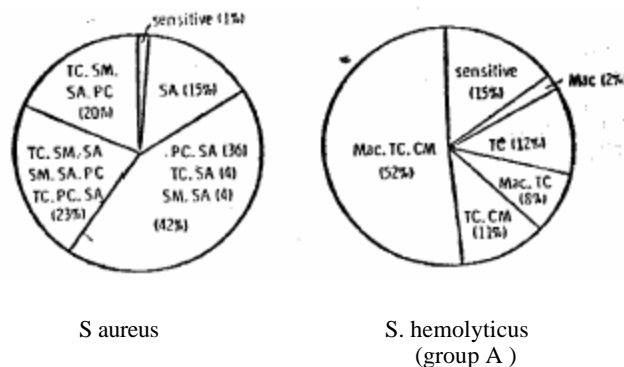


Figure 3. Resistance pattern of gram-positive cocci (By Mitsuhashi et al., 1975)

R plasmids of gram-negative bacilli can be transferred by themselves rather freely among almost all species of gram-negative rods. The host ranges, however, vary from one plasmid to

another. Some can be transmitted over bacterial families but others are restricted to a species (Figure 4). Pairing of bacterial cells for conjugation is usually mediated by a filamentous appendage named sex pill.

Table 2. Drug Resistance Patterns Carried by Plasmids

Resistance patterns		Natural distribution
Transferable Plasmids	CM.TC.SM.SA (Hg)	Very Common
	CM.SM.SA.	Seldom, found in <i>Vibrio</i>
	TC.SM.SA	Common in <i>Yersinia</i>
	SM.SA, SM or SA	Not common
	CM.TC	Very seldom
	CM.TC.SM.SA.AP.	Common in <i>Salmonella</i>
	SM.SA.AP.	Common in <i>Bordetella</i>
	CM.TC.SM.SA.KM.	Common in opportunists
	KM (KMA, NM, PM)	Rare
	KM (NM.PM.LM)	Not rare
	KM (DKB.GM)	Seldom
	CM.TC.SM.SA.AP.KM.	Moderate in opportunists
	TMP	Rare
	Miniplasmids	TC.SM.SA
Mac.PC		Common in <i>Staphylococcus</i>
CM.TC.SM		Common
PC.(Hg)		Found in <i>Neisseria</i>
Mac.TC.SM.		Not rare

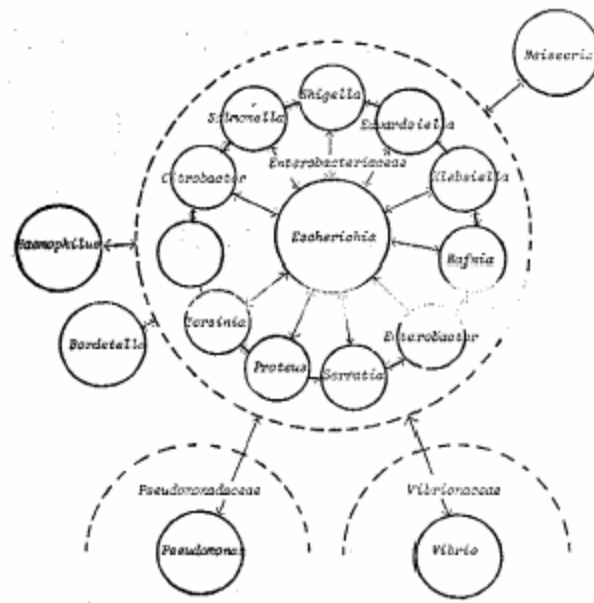


Figure 4. Intra and interfamily transfer of R plasmids

An epidemiological survey in Japan reveals that nearly 50% of clinical isolates of enteric bacilli and pseudomonas carry R plasmids resulting in multiple drug-resistance (Figure 5). *Vibrio cholerae*, however, is exceptional. The microbe is moderately sensitive to SM and sulfonamide (SA) and highly susceptible to chloramphenicol (CM) and tetracycline (TC) even today. Very few strains are resistant to the latter two drugs.

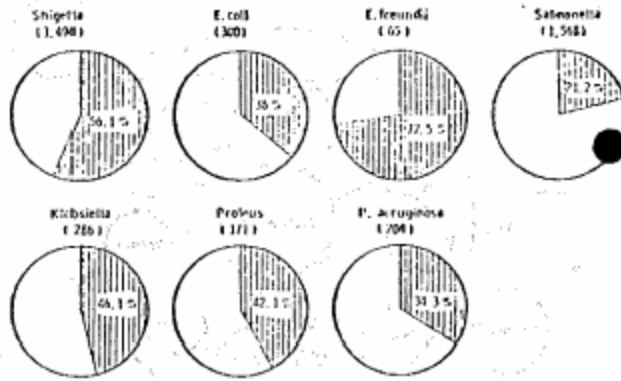


Figure 5. Isolation frequency of R strains in clinical isolates (By Mitsuhashi et al, 1975)

It is noteworthy that drug-resistant clinical isolates bearing plasmids are rare in *V. cholerae*. Only a few percent of clinical strains of *V. cholerae* are multiple drug resistant even in this year, and those carrying transferable R plasmids are more seldom. The reason of low frequency of R+ *V. cholerae* depends upon the instability of most R plasmids in this microbe, and R plasmids are easily eliminated from cholera vibrios when microbe multiplies under a free environment. There are, however, a few plasmids which can be stably maintained even in the *V. cholerae*; the distribution is fortunately limited. Only J and C group plasmids can reside in cholera vibrios for a long time.

Grouping of plasmids is based on the so-called incompatibility test (Figure 6). If genetically different plasmids are introduced into one bacterial cell, they can stably co-exist on different attaching sites of the cytoplasmic membrane, although two plasmids belonging to the same genetic group cannot live together in the same cell because of competition of one attaching site. According to the incompatibility test, B plasmids are classified into more than 16 groups (Table 3). It seems that the incompatibility groups have some relations with host ranges of plasmids, i.e. only H and C or J group plasmids are able to reside in *Salmonella typhi* and *V. cholerae*, respectively.

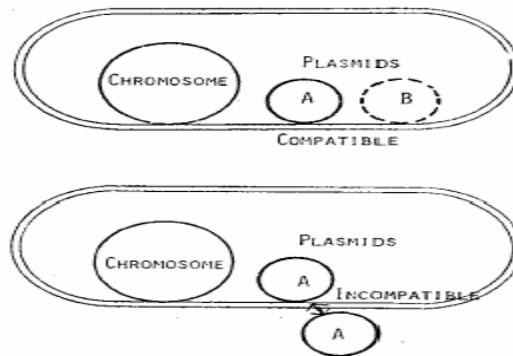


Figure 6. Incompatibility test

Since genetic groups of R plasmids capable of existing long in *V. cholerae* are limited, most of the antibiotics are still effective for the chemotherapy of cholera. Among them, TC seems to be an excellent remedy for that purpose, because it shows average minimal growth inhibitory concentration of 0.13 ug/ml to cholera vibrios, and even after receiving R plasmids *V. cholerae* becomes hardly resistant to more than 50 ug/ml of TC.

Table 3. Natural Distribution of Various Incompatibility Group R Plasmids

Group	Host bacteria
FI	E. coli, Proteus
FII	Shigella, E. coli, Proteus
FII	E. coli
C	Pseudomonas, V. cholerae
H	Salmonella (S. typhi etc.)
I	Salmonella
J	Proteus, V. cholerae
L	Serratia, Yersinia, Serratia, Proteus
O	E. coli
P	Pseudomonas, Bordetella
S	Serratia
T	Proteus
W	Shigella, E. coli, Proteus

Enterotoxin plasmids and drug-resistance (transposons) in enteropathogenic bacteria

Since several years ago, gastroenteritis due to enterotoxigenic *E. coli* has become a topic in infectious diseases.

It has been confirmed that enterotoxigenic *E. coli* produces a low molecular, heat-stable (ST) and a high molecular, cholera-like, heat labile (LT) toxin. The production of both toxins is controlled by a transferable plasmid called ENT but not by the chromosome. This evidence suggests that enterotoxigenic bacilli may increase in variety by transfer of DNA and ENT plasmids.

Further important finding in this area is transposons in both R and ENT plasmids. Fragments of DNA of genes controlling drug-resistance or enterotoxigenicity have a pair of characteristically repeating sequences of purines and pyrimidines on both ends are called insertion sequence. For this reason, the genes can make a small loop of DNA with a stalk of paired insertion fragment and be cut off by its own endonucleases. The fragment moves to other DNA replicons, such as chromosomal DNA, other plasmid DNA and bacteriophage DNA. If the replicons are homologous to the insertion sequence, the genes are integrated into new DNAs. This fact indicates that bacterial drug-resistance and enterotoxigenicity can be widely spread not only by plasmid transfer but also by transposition of the gene itself.

It may be of interest to know what happens when one bacterial cell receive both R and ENT plasmids, since drug-resistant and enterotoxigenic genes are transposons and they can easily transpose to the partner. It may be assumed that a plasmid carrying both genes will emerge. In fact, such ENT-R plasmids are not only artificially made but are also found in clinical isolates of enteropathogenic bacteria.

When the genes of drug-resistance and enterotoxigenicity are picked up from the DNA of ENT-R plasmids, electronmicrography taken by heteroduplex technique revealed three transposons figures: drug-resistance, ST-production and LT-production genes (Figure 7).

DISCUSSION

In this paper, the role of plasmids in bacterial drug-resistance and enteropathogenicity is summarized with special reference to clinical isolates of enteropathogenic bacteria at San Lazaro Hospital. It is fortunate that multiple drug-resistant vibrios are very rare even at the present time (Tables 4 and 5) and that we have still powerful chemotherapeutic agents for the treatment of vibrio infections.

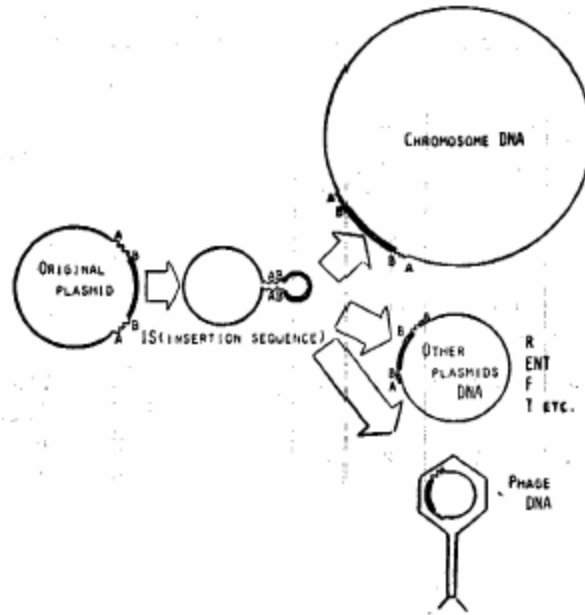


Figure 7. Transposons. Drug-resistance genes and enterotoxigenic genes

Table 4. Emergence of Multiple Drug-Resistant Strains of *Vibrio cholerae* Isolated in the Philippines

Year	Number of strains tested	Multiple drug-resistant strains			
		Number	%	Resistance patterns	Conjugative R plasmid
1964-1965	1,500	4	0.3	Cm.Tc.Sm.Su: 1	1
				Cm.Sm.Su: 3	0
1969	1,109	11	1.0	Cm.Tc. Sm.Su: 2	0
				Cm.Sm.Su : 9	0
1973	225	9	4.0	Cm.Tc. Sm.Su: 2	0
				Cm.Sm.Su : 5	1 (pJY1)
				Cm.Su : 2	0
1978	143	2	1.4	Cm,Sm,su: 1	0
				Cm,Tc,Sm,Su: 1	1

Table 5. Recently Obtained R Plasmids from Clinical Isolates of *V. Cholerae*

Plasmid	Isolation			Resistance markers	Incompatibility group	Megadalton of plasmid DNA
	Place	Year				
pJY1	Caloocan Philippines	1973		cml, str, sul	J	?
R994	Astrakhan U.S.S.R.	1970		cml, tet, str	C	98
R153	Mered Algeria	1911		cml, tet, str sul, kan, amp	C	unknown
pJT17	Nigeria	1977		cml,tet,str, sul ,amp	C	90
pJT45	Nigeria	1977		cml, tet, str, sul, kan, amp	C	99
RI143	Thailand	1975		cml, tet, str kan,amp	C	unknown
RI145	Indonesia	?		cml, tet, str, sul	C	unknown

For enteropathogenic *E. coli* and *S. typhi*, however, it is a different story. Since enteropathogenic *E. coli* carrying plasmids, which harbor both drug-resistance and enterotoxigenicity and *S. typhi* carrying multiple drug-resistance plasmids, have been isolated in neighboring, countries, (Table 6) it may be helpful for public services to continue the field survey of these enteropathogens.

Table 6. Distribution of Multiple Drug-Resistant and Enterotoxigenic *E. coli* in Southeast Asian Countries

Place	R+/ total	R+ ENT+/ total	Common R patterns	Cotransfer of R-ENT
San Lazaro	54/73	2/73	Cm.Tc.Sm. Su (Ap)	0/2
Thailand	157/197	9/197	Cm.Tc.Sm.Su (Ap)	0/9
Taiwan	5/5	5/5	Cm.Tc.Sm.Su (Ap)	5/5
Indonesia	22/22	4/22	Cm.Tc.Sm Su.Ap	2/4

Summarized from a paper by Echeverria et al