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R Plasmids from Asian Strains of *Vibrio cholerae*

R. W. HEDGES,* J. L. VIALARD, N. J. PEARSON, AND F. O'GRADY

Bacteriology Department, Royal Postgraduate Medical School, London, W12 OHS England; Bacteriology Department, Pasteur Institute, Paris 75015 France; and Microbiology Department, University of Nottingham, Nottingham, NG5 1PH England*

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Five R plasmids transferred from Asian strains of *Vibrio cholerae* all proved to be members of compatibility group C. A non-self-transmissible plasmid, stable in *V. cholerae*, was mobilized for transfer to *Escherichia coli* K-12 and found to be unstably inherited in that host. Plasmids of group C and P transferred to a wild *V. cholerae* strain were stably inherited.

The seventh cholera pandemic has been the result of the westward spread of the E1 Tor biotype of *Vibrio cholerae* from Indonesia (3). Initially these bacteria were apparently uniformly susceptible to antibiotics but, once the pandemic spread was accomplished, antibiotic-resistant strains were reported from several different parts of the world. In several cases, this resistance was apparently due to the presence of antibiotic resistance plasmids (R plasmids).

The occurrence of these plasmid-carrying (R+) strains was sporadic and the antibiotics to which they conferred resistance differed from case to case, suggesting that the acquisition of an R factor by *V. cholerae* cells has occurred on different occasions and in different parts of the world.

Kuwahara et al. (12) in the Philippines and Rizzo et al. (17) in Apulia seem to have observed the transfer of R plasmids from unknown donor strains into pathogenic strains of *V. cholerae* in infected humans.

Despite this diversity of origins, Hedges and Jacob (11) noted that all the plasmids reported from *V. cholerae* isolates were either known to belong to compatibility group C or determined resistance phenotypes, which suggested that they might belong to this group. This generalization was based on the properties of a very small number of plasmids, so we have sought further antibiotic-resistant cultures of *V. cholerae* to test the conclusion.

MATERIALS AND METHODS

Bacteria. The *V. cholerae* strains are listed in Table 1, and additionally, strain V63P$^-$ was used which requires adenine, leucine, and arginine for growth (6). Also *rif*^{r} and *nal*^{r} derivatives of the above strains resistant to 50 μg of rifampin per ml or 15 μg of nalidixic acid per ml were employed.

The *Escherichia coli* K-12 strains used were: J53, F$^-$ *pro met*; J53-1, F$^-$ *pro met nal*^{r}; J53-2, F$^-$ *pro met rif*^{r}; J62, F$^-$ *pro his trp lac*; C600-2, F$^-$ *thr leu thi lac rif*^{r}.

Plasmids. R40a, R55, and R57b, plasmids of compatibility group C (4), the equivalent of *com6* (1), were used. R40a confers resistance to ampicillin, kanamycin, and sulfonamides and the latter two confer resistance to ampicillin, chloramphenicol, gentamicin, kanamycin, and sulfonamides. RP4, a plasmid of compatibility group P conferring resistance to ampicillin, tetracycline, and kanamycin was used (5).

Techniques for plasmid transfer to *E. coli* and determination of compatibility were described in references 1, 2, 6, and 7. Transfer between strains of *V. cholerae* was attempted using a *rif*^{r} mutant of the donor and a *nal*^{r} mutant of the recipient strain, usually V63P$^-$. Apparent transcipients were tested for susceptibility to rifampin and amino acid requirement.

RESULTS

Resistant strains of *V. cholerae*. Strains of *V. cholerae* resistant to therapeutically used drugs were found during a survey of 1,156 strains from worldwide sources. The origins of the strains are described elsewhere (15). Several of the isolates that were initially found to be resistant to one or more drugs were, upon subsequent testing, found to be fully susceptible. That several R factors are unstable in *V. cholerae* has been reported by Yokota et al. (21). Those strains which retained antibiotic resistance are listed in Table 1.

All these strains were tested for ability to transfer resistances to *E. coli* K-12 strains J53-2 and C600-2. Five cultures, E14, F1, P78, L171, and L172 were effective donors (Table 1). All other strains failed to transfer their resistances.

Transmissible R factors. The antibiotic resistance properties determined by the five plasmids in *E. coli* K-12 are shown in Table 2. As

TABLE 1. *Antibiotic-resistant strains for Vibrio cholerae*

Original strain no.	Nottingham no.	Origin	Minimal inhibitory concn[a]						Resistance pattern[a]	Resistances transferred to *E. coli*
			Am	Cm	Su	Tc	St[b]	Ka[b]		
A27	JA27	Philippines (J)	1	32	>256	0.5	R	S	St Cm Su	
C24	JC24	Philippines (J)	2	32	>256	32	R	S	St Tc Cm Su	St Tc Cm (Su?)[c] R1146
C28	JC28	Philippines (J)	2	32	>256	1	R	S	St Cm Su	
H1	JH1	Philippines (J)	1	32	>256	4	R	S	St Cm Su	
6397	28	Philippines (G)	256	0.25	4	0.5	S	S	Am	
C21	C21	Philippines (G)	2	32	>256	128	R	S	St Tc Cm Su	
C24	C24	Philippines (G)	2	32	>256	128	R	S	St Tc Cm Su	
552	P64	Philippines (S)	2	32	4	2	R	S	St Cm	
3505	P78	Philippines (S)	2	4	>256	64	R	S	St Tc Cm Su	St Tc Cm Su R1144
5506	A80	Calcutta	2	32	>256	2	R	S	St Cm Su	
5482	A148	Calcutta	256	0.5	8	2	S	S	Am	
13/69	BA39	W. Bengal	1	0.5	64	0.5	R	S	St Su	
8/75	F1	Thailand	>256	16	4	64	R	R	Am St Tc Cm Ka	Am St Tc Cm Ka[d] R1143
1249	E14	Indonesia	2	16	>256	32	R	S	St Tc Cm Su	St Tc Cm Su R1145
	L171	Indonesia	2	0.5	>256	32-64	R	S	St Tc Su	St Tc Su Lac R1166
	L172	Indonesia	2	0.5	>256	32-64	R	S	St Tc Su	St Tc Su Lac R1167

[a] Abbreviations: Am, Ampicillin; Cm, chloramphenicol; Su, sulfafurazole; Tc, tetracycline; St, streptomycin; Ka, kanamycin.

[b] Resistances tested by multodisk.

[c] Mobilized by R40a.

[d] Ampicillin resistance is due to production of a TEM-1β-lactamace (13; M. Mathew, personal communication).

TABLE 2. *Minimal inhibitory concentrations (MIC) of antibacterial agents for V. cholerae and E. coli strains*

Strain	MIC (μg/ml)[a] of antibiotics				
	Sp[b]	St	Tc	Cm	Ka
J53-2	10	2.5	<1.25	<1.25	<1.25
J53-2 (R1143)	>100	>100	25	>100	>100
J53-2 (R1144)	>100	25	50	50	<1.25
J53-2 (R1145)	10[c]	25	50	100	<1.25
J53-1 (R40a) (R1146)	100	25	100	100	>100
J53-2 (R1166)	25	10	25	<1.25	<1.25
J53-2 (R1167)	50	10	25	<1.25	<1.25
P78[d]	>100	50	25	10	<1.25
P78 (R40a)[d]	10	5	<1.25	1.25	>100

[a] In Direct Sensitivity Testing agar (Oxoid, CM261).

[b] Abbreviations: Sp, Spectinomycin; St, streptomycin; Tc, tetracycline; Cm, chloramphenicol; Ka, kanamycin.

[c] Since R1145 determines resistance to streptomycin but not to spectinomycin, we presume that it determines synthesis of streptomycin phosphotransferase, whereas the R factors that confer resistance to both antibiotics probably carry genes for streptomycin, spectinomycin adenyltransferase (8).

[d] A spontaneous rifampin-resistant mutant of P78.

may be seen, the level of chloramphenicol resistance determined by these R factors in *E. coli* is very markedly greater than that shown by the original host strains (Table 1).

The compatibility properties of the plasmids were determined by testing their ability to coexist with representatives of the known plasmid compatibility groups. R1143 was shown to be incompatible with R57b, and R1144, R1145, R1166, and R1167 were incompatible with R40a. All five were capable of excluding, and subject to exclusion by, R factors of group C. Thus, all are members of group C (4).

Both R1166 and R1167 confer lactose fermen-

tation ability upon *E. coli* J62. Plasmids of this group which determine this metabolic ability have been described (9).

When J53(R40a) was mated with a rifampin-resistant mutant of P78 the rate of transfer was very low, less than one transcipient per 10^{10} donors, indicating that, as in *E. coli*, R1144 determined powerful surface exclusion. A single R40a$^+$ colony was obtained; this clone had lost tetracycline and chloramphenicol resistances. Thus, we conclude that in *V. cholerae* (as in *E. coli*) R1144 is incompatible with R40a and that the low level of chloramphenicol resistance of P78 is due to the presence of the resident plasmid.

Nontransmissible resistances of strain JC24. *V. cholerae* strain JC24 was resistant to streptomycin, tetracycline, chloramphenicol, and sulfonamides, and these resistances are stably inherited. JC24 was, however, unable to transfer this resistance either to *E. coli* or to other *V. cholerae* strains.

R40a was transferred to a rifampin-resistant mutant of JC24 with an efficiency (10^{-5} to 10^{-6} per donor) sufficiently high to suggest that JC24 has no surface exclusion ability. After entry of R40a into the strain, there was no elimination of the resistance.

When JC24 Rif^r(R40a) was incubated with *E. coli* J53-1 there was efficient transfer of R40a but very little mobilization of the resistances to JC24. However, a single clone of J53-1 was obtained which had acquired tetracycline resistance. The efficiency of transfer of tetracycline resistance was less than one per 10^{10} donors.

This clone was resistant to streptomycin, tetracycline, and chloramphenicol (as well as possessing the ampicillin, kanamycin, and sulfonamide resistances attributable to R40a). It was designated J53-1(R40a)(R1146).

The R40a-determined resistances were stable, but those of R1146 were highly unstable. Cultures grown overnight in drug-free broth at 37°C contained one-quarter to one-third of the cells that lacked the R1146-determined resistances. We therefore conclude that the R1146 replication mechanism functions efficiently in *V. cholerae* but only very inefficiently in *E. coli* K-12.

When J53-1(R40a)(R1146) was allowed to conjugate with J62, the R40a markers were transferred with a frequency approaching 10^{-4} per donor and usually without R1146. Of 24 kanamycin-resistant transcipients, all lacked the R1146 markers. When the mating was performed and selection was made for tetracycline-resistant transcipients, the transfer rate was less than 10^{-6} per donor. All carried R40a as well as R1146.

Stability of plasmids in *V. cholerae*. Yokota et al. (21) introduced *fi*$^+$ and *fi*$^-$ plasmids into a strain of *V. cholerae* and found that most of these were very unstably inherited. We therefore examined the stability of JC24rif^r(R40a) and a strain of JC24rif^r into which RP4 had been transferred. Both plasmids were stably inherited and overnight cultures grown in drug-free broth regularly contained more than 99% R$^+$ cells.

Stability in *V. cholerae* of several plasmids belonging to group C has also been described by Rahal et al. (K. Rahal, G. R. Gerbaud, and D. H. Bouanchaud, Abstr. Annu. Meet. Am. Soc. Microbiol. 1974, G149, p. 44). Conversely, they observed that plasmids belonging to a large number of different groups were lost at high frequency in *V. cholerae* "El Tor."

DISCUSSION

Our results confirm the impression that the R plasmid set of *V. cholerae* is dominated by plasmids of compatibility group C. The five self-transmissible R plasmids from our collection of strains all exhibit this compatibility.

Plasmids of group C have been found in a wide range of genera including *Pseudomonas, Proteus, Providencia,* and *Klebsiella* (20). In *Providencia* they are by far the most abundant type of R factor (9). Thus, it is not surprising that they are to be found in *Vibrio*. More surprising is the failure to find R plasmids of compatibility group P in this genus. RP4 is readily transmissible to, stably inherited in, and able to express resistance in *V. cholerae*. So, there seems no reason to doubt that such plasmids will sooner or later be found in *Vibrio*.

It is hard to avoid the conclusion that certain bacterial groups, notably the coliform bacteria with deoxyribonucleic acid of ~50% guanine + cytosine, were easily able to produce a large range of R plasmid groups. Perhaps, they were preadapted by virtue of carrying a variety of plasmids determining colicinogenicity, fermentation genes, etc., onto which resistance genes could be transposed. Other groups of bacteria, e.g., the *Proteus*-Providence (40% guanine + cytosine deoxyribonucleic acid) group seem to have had greater difficulty in generating a set of R factors. Thus the R factor set of *Proteus rettgeri* (2), *Proteus mirabilis*, and *Proteus vulgaris* (10) contain plasmids of groups imperfectly adapted to these bacteria, e.g., F_{II} plasmids that dissociate.

However, R factors specific for these genera

are evolving. Some are capable of functioning efficiently in other genera, but are, in nature, found only in particular genera. Thus, plasmids of compatibility group T have been found in *P. vulgaris* in Japan (19), *Providencia* in the United Kingdom (10), and *P. rettgeri* in South Africa (2), but never in any strain outside this group.

A more highly adapted class is represented by plasmids of compatibility group V. These have only been detected in strains of *P. mirabilis* in Asia. (They are transmissible to *E. coli* but are unstably inherited; apparently their replication mechanism is not coordinated with that of the alien host [10].)

Finally, a few R factors have been described that are transmissible between strains of *P. mirabilis* but not to *E. coli* (14, 18).

V. cholerae may, in this respect, resemble the *Proteus* group in that its plasmid set contains R factors of a group well known for its wide host range and also plasmids whose replication cycle is so specifically adapted to these bacteria that inheritance is unstable in *E. coli*.

It may not be a coincidence that most of the plasmids of group C have been observed in *V. cholerae* strains at the fringe of the spread of the current pandemic, e.g., Algeria (16) and European U.S.S.R. (11), whereas the strain shown to carry the plasmid more highly adapted to *V. cholerae* was isolated in the Philippines, near the center of distribution of the pandemic.

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