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Incidence and Elimination of R Plasmids in *Vibrio cholerae*

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Of 124 strains of *Vibrio cholerae*, 32 were multiply resistant to antibiotics. This resistance appeared to be determined by R plasmids on the basis of their effective elimination by sodium dodecyl sulfate, acridine orange, ethidium bromide, and ultraviolet radiation.

Naturally occurring R plasmids in vibrios generally appear to be stable, although some transferred R plasmids have been reported to be unstable (5, 6, 8, 9). The present study was undertaken to examine the incidence and elimination of naturally occurring R plasmids after treatment with several "curing" agents in vibrios. In this study, 124 human isolates were examined for multiple resistance to penicillin, cloxacillin, chloramphenicol, erythromycin, polymyxin, kanamycin, streptomycin, and tetracycline as well as for bacteriocinogeny. Resistance to each antibiotic was determined by spotting 10^5 colony-forming units of a culture on nutrient agar containing 0 (control), 10, 25, 50, 100, 200, 400, or 800 μg of an antibiotic per ml and examining for growth up to 48 h. Thirty-two strains exhibited resistance to at least three of these antibiotics (Table 1). The results showed that, whereas resistance to cloxacillin and polymixin was encountered most often and was highest in comparison with all other antibiotics tested (200 to 800 and 100 to 200 μg/ml, respectively), resistance to chloramphenicol and tetracycline was lowest (<10 μg/ml for 115 and 120, respectively, of 124 strains tested). Resistance to other antibiotics varied between 25 and 100 μg/ml.

Five multiply resistant strains representing five different combinations of antibiotic resistance and belonging to different bacteriocin types (3; Table 2) were chosen for studying the effects of sodium dodecyl sulfate, acridine orange, ethidium bromide, and ultraviolet radiation on these resistances and bacteriocinogeny. The minimum inhibitory concentrations of sodium dodecyl sulfate, acridine orange, and ethidium bromide were first determined. Other procedures were as described (2, 4), and the "curing" effects with ultraviolet radiation were as in reference 6. The treated clones were tested for loss of antibiotic resistance as well as bacteriocinogeny (4). The effects of these agents on the antibiotic resistance and bacteriocinogeny are described in Table 2. Only one colony each of *Vibrio cholerae* 1369 treated with sodium dodecyl sulfate and ethidium bromide lost bacteriocinogeny. These elimination studies suggest that the antibiotic resistance determinants in *V. cholerae* are probably plasmid linked, like

TABLE 1. *Distribution pattern of multiple resistance among strains of V. cholerae*

Multiple resistance pattern[a]	No. of strains[b]	Multiple resistance pattern[a]	No. of strains[b]
$Pe_{25}Cx_{400}Cm_{25}Ka_{25}$	1	$Pe_{25}Cx_{200}Po_{100}$	2
$Pe_{25}Cx_{400}Er_{50}Po_{50}Ka_{25}Sm_{50}$	1	$Pe_{50}Cx_{200}Ka_{50}$	1
$Pe_{100}Cx_{400}Er_{25}Ka_{25}Sm_{100}$	2	$Cx_{200}Cm_{25}Er_{100}Ka_{100}$	1
$Pe_{25}Cx_{400}Er_{50}Po_{100}Ka_{50}$	1	$Cx_{400}Er_{25}Po_{100}Ka_{25}Sm_{25}$	1
$Pe_{100}Cx_{400}Er_{50}Po_{100}$	3	$Cx_{400}Er_{100}Ka_{100}$	2
$Pe_{50}Cx_{400}Er_{100}Po_{100}Tc_{50}$	1	$Cx_{400}Po_{100}Ka_{25}Sm_{25}$	2
$Pe_{100}Cx_{400}Er_{25}$	6	$Cx_{400}Po_{100}Sm_{25}$	1
$Pe_{100}Cx_{400}Po_{100}Ka_{25}Sm_{25}$	2	$Cx_{400}Ka_{25}Sm_{25}$	2
$Pe_{100}Cx_{400}Po_{100}Ka_{50}$	3		

[a] Pe, Penicillin; Cx, cloxacillin; Cm, chloramphenicol; Er, erythromycin; Po, polymyxin; Ka, kanamycin; Sm, streptomycin; Tc, tetracycline; subscripted numbers denote levels of corresponding antibiotic resistance in micrograms per milliliter.

[b] When more than one strain belonged to a particular resistance pattern, resistance described belong to a representative strain.

TABLE 2. *Co-elimination of antibiotic resistances in V. cholerae resulting from the action of several agents*

V. cholerae	Wild-type resistance pattern[a]	Curing agent	Resistances eliminated
K-23	$Pe_{25}Cx_{400}Er_{50}Po_{50}Ka_{25}Sm_{50}B^5$	SDS	PePo(1)[b], Pe(44)
		AO	PePo(2), PeEr(1), Pe(47)
		UVR	PeEr(1), Pe(43)
		EB	PePo(1), Pe(49)
601	$Pe_{100}Cx_{400}Po_{100}Ka_{50}B^{2A}$	SDS	Pe(46)
		AO	PePo(31), Pe(16)
		UVR	Pe(50)
		EB	Pe(48)
805	$Pe_{100}Cx_{400}Er_{50}Po_{100}B^3$	SDS	PeCxPo(10), PeCxEr(9), CxPo(5), Er(7)
		AO	PeCxEr(50)
		UVR	PeCxPo(14), PeCx(36)
		EB	Pe(50)
824	$Pe_{25}Cx_{400}Er_{50}Po_{100}Ka_{50}B^6$	SDS	PePo(2), PeEr(1), Pe(46)
		AO	
		UVR	
		EB	Pe(49)
1369	$Pe_{50}Cx_{400}Er_{100}Po_{100}Tc_{50}B^8$	SDS	PeCxErPoB[8](1), Pe(27)
		AO	Pe(47)
		UVR	Pe(39)
		EB	PeErPoB[8](1), PeEr(46), Pe(2)

[a] Pe, Penicillin; Cx, cloxacillin; Er, erythromycin; Po, polymyxin; Ka, kanamycin; Sm, streptomycin; Tc, tetracycline; subscripted numbers denote levels of resistance (micrograms per milliliter) to respective antibiotics; B^5, B^{2A}, B^3, B^6, and B^8 represent bacteriocin types 5, 2A, 3, 6, and 8, respectively (3); SDS, sodium dodecyl sulfate; AO, acridine orange; UVR, ultraviolet radiation; EB, ethidium bromide.

[b] Numbers within parentheses indicate number of colonies "cured" of resistance(s) of 50 colonies tested; broth cultures after treatment with one of the "curring" agents were plated out for isolated colonies, which were then transferred to different antibiotic-agar and nutrient agar plates. Antibiotic(s) in these plates contained 25% of the original level of resistance.

that of bacteriocinogeny (1, 4), and that independent elimination of each plasmid for antibiotic resistance and bacteriocinogeny occurs; this is most readily interpreted on the basis that each of these is determined by an independent plasmid.

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