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New Virulence-Associated Plasmid in *Yersinia enterocolitica*

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In a study of 103 strains of *Yersinia enterocolitica*, 10 strains were found to be lethal for mice and to possess 42- and 82-megadalton plasmids. This association was statistically significant ($P \ll 0.001$). Serotypes of *Y. enterocolitica* previously considered avirulent were found to possess these plasmids and to be lethal for mice. A spontaneous derivative of one strain contained only the 82-megadalton plasmid and was lethal for mice anyway. This virulence-associated plasmid is a potential diagnostic tool for the clinical or public health laboratory which must delineate pathogenic strains of *Y. enterocolitica*.

The recent interest in *Yersinia enterocolitica* and the difficulty in distinguishing pathogenic from nonpathogenic strains have stimulated research in this area. The virulence of *Y. enterocolitica* has been associated with several properties, including the ability to penetrate guinea pig conjunctiva (4, 11, 12), invade tissue culture cells (9, 12), kill adult mice (1, 4, 5), and autoagglutinate under appropriate conditions (7, 10). Although these assays for virulence may be important in defining pathogenicity for humans, they remain tools of the research laboratory. A more important observation to the clinical laboratory is the occurrence of a 42-megadalton (Mdal) plasmid in assay-positive *Y. enterocolitica* (1, 4, 5, 9–12). Screening for the presence of a specific plasmid could be a simple and rapid epidemiological and diagnostic procedure. To test this hypothesis, we are currently studying the phenotypic and genotypic characteristics of *Y. enterocolitica* strains of human origin from the United States. We have examined 100 strains for characteristics which include virulence and plasmid profile.

Three outbreak-associated *Y. enterocolitica* strains served as positive controls for studies of virulence and the presence of the 42-Mdal plasmid; their avirulent derivatives served as negative controls. Plasmid DNA was isolated routinely by the method of Birnboim and Doly (2), purified by cesium chloride-ethidium bromide density centrifugation (3), and analyzed by agarose gel electrophoresis (8). Molecular sizes were determined in relation to the migration of plasmids whose molecular sizes range from 23 to 140 Mdal.

We found that 7 of the 100 study strains and the 3 positive controls were lethal for adult mice and that each contained one 42- and one 82-Mdal plasmid. Of the 93 strains not lethal for mice, only 1 contained 45- and 82-Mdal plasmids, and 2 contained only a 42-Mdal plasmid (Tables 1 and 2). Although the 82-Mdal plasmid has not been reported in previous studies of *Y. enterocolitica*, the data from those studies reflected the use of a sodium dodecyl sulfate-salt precipitation method described by Guerry et al. (6). In our hands, this procedure failed to detect the presence of the 82-Mdal plasmid, which was visible at low intensity in both routine and gradient preparations. One explanation for this is the sensitivity of large plasmid molecules to phenol and chloroform, which are used to extract protein in the method of Guerry et al. Another explanation is the possibility that this large plasmid is present in low copy numbers or in a relatively small percentage of the cell population. We did not find a reciprocal relationship between any other assay and the detection of plasmid DNA in the 100 study strains (unpublished data).

We believe that the 82-Mdal plasmid is a unique entity, not a dimer or an artifact of alkaline denaturation, for the following reasons. (i) It was present in gradient preparations, which select for covalently closed circles of DNA. (ii) Two strains contained only the 42-Mdal plasmid. (iii) A spontaneous derivative of strain 1223-75-1 contained only the 82-Mdal plasmid. (iv) Enzyme digests of strains 1223-75-1 and 1223-75-2 demonstrated DNA fragments unique to the 82-Mdal plasmid: these were of uniformly low intensity and were present as part of the parent strain digest (Fig. 1).

We also believe that this 82-Mdal plasmid may be associated with virulence for the following reasons. (i) It was present in all study and control strains which were lethal for mice. (ii)

TABLE 1. Association of lethality with plasmid DNA for *Y. enterocolitica* strains

Strain	Serotype	Lethality	Plasmid size(s) (Mdal)
A2627	O:8	+	36, 42, 82
A2628	O:8	+	36, 42, 82
3973-76	O:40	+	42, 82
874-77	O:20	+	42, 82
1137-77	O:8	+	42, 82
1324-77	Rough	−	42
9312-78	O:13, 18	−	45, 82
9385-78	O:5	−	42
1209-79	O:13	+	42, 82
1223-75-1	O:20	+	42, 82
1223-75-2[a]	O:20	+	82
WA+[b]	O:8	+	42, 82
WA−[a]	O:8	−	None
A2635[b]	O:8	+	42, 82
TAMU-75[a]	O:8	−	None
Y7P[b]	O:8	+	36, 42, 82
Y7N[a]	O:8	−	None

[a] Derivative which has spontaneously lost plasmid DNA.
[b] Control strain (4).

The association of this plasmid with lethality was statistically significant ($P \ll 0.001$ [Fisher exact test]). (iii) The two strains containing only the 42-Mdal plasmid were not lethal for mice. (iv) A derivative, strain 1223-75-2, contained only the 82-Mdal plasmid and was as lethal for mice as was the parent strain, 1223-75-1. (v) Previous reports have shown the presence of a 42-Mdal plasmid in an avirulent *Y. enterocolitica* strain (9). (vi) These previous studies have not shown complete homology among 40- to 48-Mdal plasmids isolated from virulent *Y. enterocolitica* strains (9). (vii) The correlation between this plasmid and lethality in *Y. enterocolitica* serotypes other than O:3, O:8, and O:9 was not expected but is consistent with the potential mobility of plasmid DNA.

Further work must be done to compare the 42- and the 82-Mdal plasmids in all of these strains and especially to determine the degrees of homology among the 82-Mdal molecules. Both plasmid species are statistically associated with lethality for mice, and one or both may be necessary to determine the virulence of *Y. enterocolitica*. However, the initial evidence suggests that the newly observed 82-Mdal plasmid is an excellent candidate for both plasmid profile screening and genetic probe construction. Either technique offers a potential diagnostic and epidemiologic tool not available at present.

TABLE 2. Summary of results for 103 strains of *Y. enterocolitica*[a]

Lethality	No. of strains with:		
	82-Mdal plasmid only	42-Mdal plasmid only	82- and 42-Mdal plasmids
Yes	1	0	10
No	0	2	1

[a] The association of the 42- or 82-Mdal plasmid with lethality for mice was significant for the 103 strains studied ($P \ll 0.01$ [Fisher exact test]).

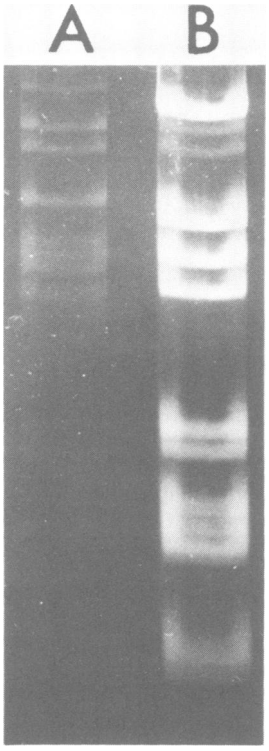

FIG. 1. Agarose gel electrophoresis of plasmid DNA isolated from *Y. enterocolitica* 1223-75 and digested by *Hin*dIII. Lane A, Derivative strain 1223-75-2; lane B, parent strain 1223-75-1.

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