JOURNAL OF CLINICAL MICROBIOLOGY, June 1983, p. 1159–1160
0095-1137/83/061159-02$02.00/0

Vol. 17, No. 6

Plasmid Profiles of Value in Differentiating *Salmonella muenster* Isolates

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Received 3 December 1982/Accepted 3 March 1983

Strains of *Salmonella muenster* displaying identical antibiotic susceptibility and bacteriophage reaction patterns were found to differ in their complements of plasmid DNA. Plasmid profiles were used to separate 40 isolates from diverse sources into four distinct groups, one of which contained 30 strains without plasmids.

In the first half of 1982, one institutional and three family outbreaks of *Salmonella muenster* occurred in the province of Ontario, Canada. A larger than usual number of bovine infections were recorded as well. At present, there exist no specific phage-typing or biotyping schemes for characterizing this serotype. It is difficult, therefore, to relate one isolate to another and to designate unequivocally sources of infection or establish patterns of bacterial dissemination. Thus, in the four human incidents noted above, it can be reported only that they appeared to have originated with contaminated dairy products. For this reason, it was of interest to note the use of plasmid profiles as an epidemiological tool among the species of *Salmonella* (3–6). We conducted a small survey to assess whether such an approach might enable us to distinguish among separate isolates of *S. muenster*.

Thirteen nonhuman (milk, cheese, bovine, and avian sources) and 27 human isolates were examined. All 40 isolates were sensitive to commonly used antibiotics and gave the same pattern with our collection of 60 nonspecific *Salmonella*-typing bacteriophages (data not shown). In addition, a representative number of isolates (six from each source) failed to show any biochemical differences when tested by the method of Duguid et al. (2) for the biotyping of *Salmonella typhimurium*. DNA was extracted from saturated (overnight) broth cultures of each isolate by a scaled-down (to the microfuge level) Casse procedure (1). This procedure has an advantage over others in that it permits the simultaneous recovery of both small and larger, more fragile, plasmid molecules that might otherwise go undetected. Ethanol-precipitated DNA was then characterized by its electrophoretic mobility in 0.75% agarose slab gels. The latter were run in the vertical dimension for 2.5 h at 7 V/cm.

Extrachromosomal (plasmid) DNA was detected in 10 of the isolates screened. A typical plasmid profile is shown in Fig. 1. Plasmids ranging in mass from 3 (lanes f, g, i, j) to 68 (lanes a through d) megadaltons (Md) were separated in this gel. The existence of the 26-Md plasmid (lane k) was confirmed by electrophoresis of a second portion of the DNA preparation in a lower-concentration gel (0.65%).

Using plasmid number and size as a basis, we could separate the 40 *S. muenster* isolates into four groups (Table 1). The bulk of the isolates (75%) did not carry plasmids and were placed, regardless of their source, in group IV. Isolates from the institutional outbreak belonged in this group. Group I strains carried a single large plasmid (68 Md) and were all of animal origin. Four of these isolates came from the same farm: three from individual members of a dairy herd, and one recovered from a sparrow found dead in the cattle barn. The single group II strain was recovered from a sporadic human infection unrelated to any of the other outbreaks sampled here.

The four group III isolates were recovered from individual members of the same family, indicating, not unexpectedly, that the causative agent in this small outbreak was a single variant of *S. muenster*. From an epidemiological viewpoint, it is interesting to note that the characteristic 3-Md plasmid was not detected in the strain of *S. muenster* isolated from the putative source of this particular infection, raw milk (Fig. 1, lane e).

There is no system available for the biotyping of *S. muenster* isolates, and other conventional epidemiological markers (antibiotic resistance, phage typing) were of no use in differentiating

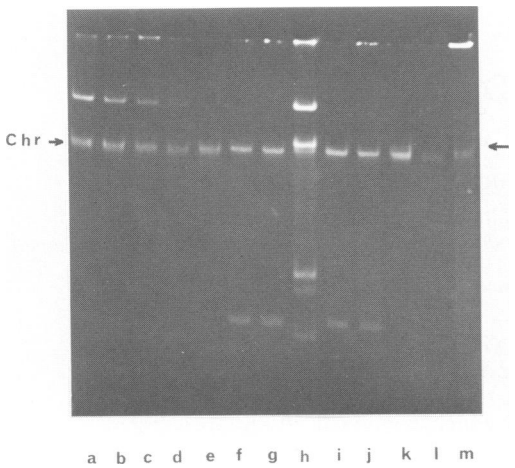

FIG. 1. Electrophoretic analysis of the plasmid contents of strains of *S. muenster*. Lanes: a through e, nonhuman isolates; f, g, and i through m, human isolates; h, molecular weight standards. Arrow indicates 26-Md plasmid in lane k. Chr, Chromosome.

TABLE 1. Differentiation of *S. muenster* strains by plasmid profiles

Group	Plasmid content		Isolates		Comment
	No.	Mass (Md)	No.	Source	
I	1	68	5	Animal	Four bovine, one avian
II	1	26	1	Human	Sporadic infection
III	1	3	4	Human	Single family
IV	0		30	Human/ nonhuman	

the 40 strains examined here. The determination of plasmid profiles, however, did permit their separation into four distinct subgroups. Thus, although the number of strains screened here was limited and only 25% carried plasmids, the analysis of plasmid content does appear to be of use in distinguishing otherwise similar isolates of *S. muenster*. It remains to be seen whether this will also be of value in confirming the source and pattern of spread of other *S. muenster* infections.

We thank A. Borczyk, Enteric Reference Laboratory, Ontario Ministry of Health, for the stimulus and strains he provided for this survey.

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