BIOTYPES OF STRAINS OF SALMONELLA TYPHIMURIUM OF PHAGE TYPES 49, 204 AND 193

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Since June 1977, strains of Salmonella typhimurium of phage types 193 and 204, showing multiple drug resistance, have been responsible for many incidents of infection in cattle and man throughout Britain (Davies et al., 1978; Lancet, 1979). Those of phage type 204 were resistant to chloramphenicol, streptomycin, sulphonamides and tetracyclines (R type CSSuT) whereas those of phage type 193 were resistant to ampicillin, chloramphenicol, kanamycin, streptomycin, sulphonamides and tetracyclines (R type ACKSSuT) (Threlfall, Ward and Rowe, 1978a). Characterisation of the resistance plasmids carried by strains of phage type 204 suggested the derivation of strains of R type CSSuT from one of R type SuT, the probable progenitor of which, in turn, was thought to be a sulphonamide-resistant strain of phage type 49. Furthermore, acquisition of a plasmid (AKS) coding for resistance to ampicillin, kanamycin and streptomycin converted strains from phage type 204 and R type CSSuT to phage type 193 and R type ACKSSuT (Threlfall, Ward and Rowe, 1978b). Thus, interconversion of phage types 49, 204 and 193 was demonstrated.

Fine biotype discrimination of strains of S. typhimurium has been achieved by the use of the scheme of Duguid et al. (1975) which allowed the recognition of 144 full biotypes among 2030 naturally occurring strains. The combined use of biotyping and phage typing, furthermore, afforded greater strain discrimination than either method alone (Anderson et al., 1978). Thus, strains of a single phage type have been subdivided by biotyping, and possible phage-type conversions indicated among related strains of a single biotype belonging to different phage types (Anderson et al., 1978; Barker and Old, 1979). Our understanding of the phylogenetic relationships of strains of S. typhimurium has been considerably enhanced by the use of biotyping data and the genealogical tree proposed by Duguid et al. (1975). The present paper reports on the full biotypes of a series of strains of phage types 49, 204 and 193 isolated in Scotland from 1974 to 1978, i.e., before and after the emergence of the chloramphenicol-resistant strains in mid-1977, and discusses the relationships among strains of these phage types.

MATERIALS AND METHODS

Bacteria. We examined 655 cultures of Salmonella typhimurium of phage types 49 (375 cultures), 204 (189) and 193 (91), which were from cattle (348 cultures), man (227) and other sources (80). These were most of the cultures of these phage types sent originally for confirmation of identification to the Scottish Salmonella Reference Laboratory, Stobhill Hospital, Glasgow, from medical and veterinary laboratories during 1974–1978. We received stock cultures stored on Dorset's egg slants.

Phage typing. The phage types, determined at the Division of Enteric Pathogens, Central Public Health Laboratory, Colindale Avenue, London, are the definitive types of Anderson et al. (1977).

Biotyping. Each culture was biotyped with five primary and 10 secondary tests by the methods described by Duguid et al. (1975).

Antibiotic sensitivity. Sensitivity to the antibiotics on Multodisk No. 2860E (Oxoid) and to chloramphenicol (25 μg) on a disk from Mast Laboratories was determined routinely by conventional means on Sensitivity Test Agar (Oxoid) with 5% (v/v) lysed horse-blood.

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RESULTS

The biotypes of the strains are given in the table. The majority (c. 98%) of the strains of phage types 49 and 204 were of full biotype 26a. The few strains of these phage types of biotypes 26f (trehalose non-fermenting), 26i (inositol non-fermenting) and 26y (auxotrophic for cysteine) were probably mutants in secondary biotype characters derived in vivo during spread of the epidemic lines of phage type/biotype (PB) 49/26a and 204/26a. Chloramphenicol resistance was detected in 18 of the strains of phage type 49, 16 of which were also trimethoprim resistant (Richards et al., 1978), and in 17 strains of phage type 204. Regardless of the different R types

<table>
<thead>
<tr>
<th>Phage type</th>
<th>Number of strains of full biotype</th>
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<tbody>
<tr>
<td>49</td>
<td>0 0 0 0 0 0 363 1 9 2 375</td>
</tr>
<tr>
<td>204</td>
<td>0 0 0 0 0 0 188 0 1 0 189</td>
</tr>
<tr>
<td>193</td>
<td>25 2 4 3 7 5 19 1 23 2 0 0 91</td>
</tr>
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</table>

present, however, the strains of phage types 49 and 204 were remarkably homogeneous in biotype. In contrast, the biotypes of the strains of phage type 193 were diverse. Our finding that biotyping subdivided these 91 strains into six primary and ten full biotypes (table) demonstrates well the discriminatory potential of the combined typing methods. The 15 chloramphenicol-resistant strains of R type ACKSSuT were present among the cultures of PB types 193/26a and 193/26f.

DISCUSSION

The biotyping data reported here confirm previous observations of the regular association in strains of S. typhimurium of phage types 49 and 204 with biotype 26 (Anderson et al., 1978; Barker, Old and Sharp, 1980) and provide additional evidence of a close phylogenetic relationship between strains of these phage types. These are probably also related to the minority of strains of PB type 193/26. The origin of our strains of PB type 193/26a (and 26f) of R type ACKSSuT probably occurred by the pathway proposed by Threlfall et al., (1978b), i.e., from strains of PB type 204/26a and R type CSSuT by acquisition of the (AKS) plasmid, which seems to be the phage type-determining plasmid in these multiresistant strains of phage type 193. Detailed plasmid analysis will, no doubt, in time explain the route of descent of strains of PB type 193/26 that lack the (AKS) plasmid. Similarly, relationships with other clones of S. typhimurium and alternative lines of descent should be sought for strains of phage type 193 of primary biotypes 1, 2, 3, 9 and 17 (table).

Previous studies of 2010 cultures of S. typhimurium isolated in Scotland from 1974 to 1976 suggested regular associations of certain phage types with certain biotypes (Barker et al., 1980). Thus, cultures of biotype 9f were usually of phage type 141; the first exceptional culture was one of PB type 193/9f isolated from a child in a family, the other members of which were excreting cultures of PB type 141/9f. That epidemiological evidence and the biotyping data strongly indicated an interconversion of these two phage types. Whether the four additional cultures of PB type 193/9f were descendants of that original culture or examples of further distinct conversions, we are not able to say. Similarly, our observations on these 2010 cultures revealed another association, viz., that between phage type 56 and biotype 17g. Our findings that in at least three epidemiologically discrete incidents, the PB types 56/17g and 193/17g were simul-
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Simultaneously present, suggest possible conversion of these phage types. Because strains of primary biotypes 1a, 2a and 3a are found in association with many different phage types, the lines of descent of PB types 193/1a, 193/2a and 193/3a will be less easily predicted.

Multiresistant strains of phage type 193 have been subdivided by an ancillary phage-typing method into subtypes of different compatibility groups. Some geographical distribution of the subtypes, which seemed to be clonal in nature, was indicated (Frost, de Saxe and Anderson, 1976). Biotyping might have provided more information about these strains. In view of that diversity, our demonstration of biotype heterogeneity among a more circumscribed series of strains of phage type 193 is of interest.

Although it is true that the precise epidemiological investigation of infection with S. typhimurium requires initially the use of phage typing to discriminate between strains, and that relationships between phage types may be demonstrated by detailed plasmid analysis, we feel that a complete understanding of the epidemiology of clones of S. typhimurium will be greatly facilitated by a knowledge of their biotypes.

SUMMARY

Biotyping provided evidence of the phylogenetic relationships between strains of Salmonella typhimurium of phage types 49 and 204 and certain strains of phage type 193, which were interconvertible in phage types. All of 564 strains of phage types 49 and 204, 35 of which were chloramphenicol-resistant, were of biotype 26, whereas those of phage type 193 (91 strains) belonged to six different primary biotypes. Lines of descent are suggested for strains of phage type/biotype: 193/26a, 193/17g and 193/9f.

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