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Persistent and transient clones of *Salmonella typhimurium* of phage type 141 recognized by biotyping

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SUMMARY

Among the 81 cultures of *Salmonella typhimurium* of phage type 141 examined, 72 had been isolated from Sheffield incidents in 1984–5 and 9 were Scottish isolates from 1986–7. All of these cultures from diverse sources belonged to primary biotype 31; 79 were of full biotype 31beg and 2 anaerogenic cultures were of full biotype 31begj. This is the first known occasion on which an epidemic strain of *S. typhimurium* of phage type/biotype 141/31beg has been implicated in outbreaks of human or animal infection in the UK. Because previous epidemic strains of *S. typhimurium* of phage type 141 in the UK belonged to biotypes 1f and 9f which are phylogenetically unrelated to biotype 31beg, the likely origin of this most recent epidemic *S. typhimurium* strain of phage type/biotype 141/31beg is discussed.

INTRODUCTION

Isolation of strains of *Salmonella typhimurium* of phage type 141 was relatively uncommon in the UK before 1972. At that time, the few isolations of strains of that phage type were generally associated with sources such as poultry and cattle (Scottish Home and Health Department, 1973). Between August 1972 and February 1973, Scottish outbreaks occurring in Edinburgh, Midlothian and Peeblesshire were associated with the consumption of contaminated food (Bone, 1975) or raw milk (MacLachlan, 1974; Bone, 1975). Thereafter, there were reports of localized outbreaks, and numerous sporadic cases, of infections in man and of outbreaks among cattle from various districts in south-east and central Scotland (for details, see Barker & Old, 1979). Although strains of *S. typhimurium* of phage type 141 were found infrequently elsewhere in the UK between 1972 and 1975, most isolations, it seems, were reported from Scottish laboratories (Dr J. C. M. Sharp, personal communication).

There was a relatively quiet period between 1977 and 1983, when very few isolations of phage-type 141 strains were reported anywhere in the UK. However, in 1984 this became the second most common of the phage types of *S. typhimurium* responsible for infections in man in England and Wales and was found particularly in the Yorkshire and Trent regions (Communicable Disease Surveillance Centre Report, 1986). Most (74%) of the animal incidents in 1984 that were associated with strains of *S. typhimurium* of phage type 141 were from

poultry or from liquid egg suggesting that poultry and egg products were major vehicles of human infection (Communicable Disease Surveillance Centre Report, 1986). Five outbreaks and a number of sporadic cases which occurred between 1 January 1984 and 31 July 1985 in the Sheffield area were traced to the consumption of contaminated hens' eggs (Chapman, Rhodes & Rylands, 1988).

Subdividing common salmonella phage types on the basis of biotype characters may help in epidemiological studies and, when available, is a valuable supplement to phage typing for the identification of subtypes present in any major phage type. The biotyping scheme of Duguid *et al.* (1975), based on the use of 15 biotype markers, allowed assignment of strains to both primary and full biotypes. Thus far, 24 primary and 184 full biotypes have been identified. When biotyping was used in conjunction with phage typing each system complemented the other and together they characterized 574 distinct phage type/biotype groups (Anderson *et al.* 1978). Strains of several phage types were subdivided by biotyping; for example, strains of *S. typhimurium* of phage type 141 included representatives of three distinct biotypes; 1f, 9f and 31bd (Barker & Old, 1979). These three biotypes probably reflected the acquisition of the same phage type-determining characters by different biotype lines of *S. typhimurium*.

The resurgence in 1984 of *S. typhimurium* of phage type 141 prompted us to examine some of these recent cultures isolated since our last report describing strains of this phage type. We did this because we wished to determine which, if any, of the biotype clones that had been previously identified were still represented and to consider whether biotyping might again contribute to the epidemiological study of strains of *S. typhimurium* of phage type 141.

MATERIALS AND METHODS

Bacteria

Of the 81 cultures of *S. typhimurium* of phage type 141 examined, 72 were received from Mr P. A. Chapman as representatives of those isolated in the Sheffield area in 1984 and 1985. These cultures included: 10 human isolates representative of those present in the five outbreaks described by Chapman *et al.* (1988); 3 cultures from 3 different food sources associated with one of the outbreaks; 3 from samples of liquid egg from 3 separate farms; and 56 from sporadic cases of human infection. The other 9 cultures were received from the Scottish Salmonella Reference Laboratory, Stobhill Hospital, Glasgow; they had been isolated from diverse animal sources (cattle, dog, horse, man, poultry) in Scotland in 1986 and 1987.

Biotyping

We determined the primary biotypes (1–32) of cultures by their reactions in five tests with: D-xylose, *meso*-inositol, L-rhamnose, *d*-tartrate and *meso*-tartrate. Strains were assigned full-biotype designations on the basis of their reactions in a further ten secondary tests. Details of the biotyping media, and performance and interpretation of tests have been presented elsewhere (Duguid *et al.* 1975).

Colicins

Cultures were screened for the production of colicins by the agar-overlay method as described by Barker (1980).

RESULTS

The 81 cultures of *S. typhimurium* of phage type 141 belonged to primary biotype 31, i.e., they did not ferment D-xylose in Bitter's medium, did not ferment *meso*-inositol or L-rhamnose in peptone water, did not utilize *d*-tartrate in peptone water but they did utilize *meso*-tartrate and, hence, they were not inhibited by *meso*-tartrate in the plate-inhibition test; thus, they were $Bxyl^-$, Inl^-, Rha^-, dTa^-, mTa^+. In the secondary biotyping tests, the 72 cultures from Sheffield reacted similarly. They were non-fimbriate (full biotype character, b), fermented D-xylose late (i.e., in > 24 h) in peptone water (e) and were unable to utilize glycerol in Stern's medium (g) but were positive in the other secondary tests; thus, their full biotype was 31beg (see Duguid *et al.* 1975). Of the 9 Scottish cultures examined, 7 also belonged to biotype 31beg; the other 2 were, in addition, anaerogenic (character j) and, hence, belonged to biotype 31begj.

Only 2 of the 81 cultures produced colicins; they carried ColIb plasmids and came from sporadic cases of human infection.

DISCUSSION

For *S. typhimurium*, one of the two principal serotypes involved in infections in the UK (Palmer & Rowe, 1986), phage typing provides an excellent method for the discrimination of different strains within the serotype. The system is fast and economical and, performed by reference laboratories, provides accurate information about the epidemiology of prevalent phage types present in any community. The phage-typing system of Callow (1959) for *S. typhimurium* has been progressively expanded (Anderson *et al.* 1977) so that it currently recognizes 232 definitive phage types thereby providing a very fine degree of strain discrimination within that serotype. Again, by long-term surveillance, it indicates the presence of major, related and often interconvertible phage types present in a country (Anderson, 1964; Palmer & Rowe, 1986).

The application of other typing schemes to the study of phage-typed strains of *S. typhimurium* may provide further discrimination allowing identification of additional strains within major, apparently homogeneous, phage-typed groups of strains. We have always preferred to use biotyping to supplement phage typing and have established that strains of many major phage types can be subdivided by biotyping and that strains of most major biotypes can be subdivided by phage typing (Anderson *et al.* 1978; Barker & Old, 1979; Barker *et al.* 1980). Combined phage typing-biotyping studies have helped to characterize variant strains that arose from epidemic strains in the course of their spread and have indicated likely phage-type conversions (Barker *et al.* 1980; Barker, 1986); biotyping has also provided the basis for phylogenetic studies (Old, 1984).

Whilst it would seem reasonable to conclude that the sudden upsurge of

strains of a previously uncommon phage type, such as 141, had resulted from the emergence of only one kind of strain, the conjoint use of biotyping with phage typing readily disproved that assumption by revealing the existence of three major phage type/biotype groups: 141/1f, 141/9f and 141/31bd (Anderson *et al.* 1978; Barker & Old, 1979; Barker *et al.* 1980). Thus, our earlier observations on 623 cultures recovered from Scotland between 1965 and 1977 (551 cultures), some representative strains from England and Wales between 1958 and 1975 (63), and a few representative isolates from Australia and the Netherlands in 1960–63 (9) showed the following distribution of strains: biotype 1f and related biotype variants (75); 9f and related variants (530); and 31bd and variants (18); these results are summarized in Table 1.

Naturally occurring strains of *S. typhimurium* of biotype 31 are phylogenetically distant from strains of biotypes 1 or 9 (Old, 1984); strains of biotype 31bd differ from those of biotypes 1f and 9f in, respectively, seven and six biotype characters, so that interconversion of biotype 31bd to either of these types is unlikely (Barker & Old, 1979). Although strains of phage type 141 of biotypes 1f and 9f differ only in the inability of the latter to utilize *meso*-inositol, that character was, nevertheless, stably inherited and cultures from individuals in sporadic cases or from infected persons or animals in outbreaks always belonged to biotype 1f or 9f and never to both. Furthermore, the distribution of biotypes of the phage-type 141 strains was of interest in that outbreaks in cattle were generally associated with biotype 9f strains whereas biotype 1f strains, when found, were more usually associated with human infections (for rare exceptions, see Barker & Old, 1979).

Although chicken had initially been suspected as the likely vehicle of infection responsible for human infections by phage-type 141 strains in Scotland (MacLachlan, 1974; Bone, 1975), we demonstrated convincingly that neither biotype 1f nor biotype 9f strains were chicken-associated at that time and that the chicken type (141/31bd) was a member of the FIRN group of strains long known to be poultry-associated (Duguid *et al.* 1975; Anderson *et al.* 1978). However, in the period (1969–77) of our earlier surveys, there were very few isolations of type 141/31bd; thus, for example, only 12 cultures of 141/31bd were found among the large Scottish series of 1965–77 (Table 1) and 10 of these were isolated from imported Danish chicken. Overall, therefore, the available evidence indicated that type 141/31bd had not been a major source of human or animal infections in the UK.

It was of great interest, therefore, to find that all of the 81 representative cultures of phage type 141 from England and Scotland from 1984–7 belonged to the primary FIRN biotype 31 and that the biotypes 1f and 9f, previously so dominant among strains of phage type 141 in the UK, were not represented among these recent cultures (Table 1). This is the first demonstration of any large-scale involvement of phage-type 141 strains of the FIRN biogroup in human or animal infections in the UK. Epidemiological investigations had implicated poultry and poultry products as the likely vehicles in the human infections with type 141 strains that reappeared in England and Wales in 1984 (Communicable Disease Surveillance Centre Report, 1986); this may have been because of the increased heat-resistance of phage-type 141 strains (Chapman *et al.* 1988). It was, therefore, encouraging to obtain the additional information that all English strains from

Table 1. *Numbers of cultures of* S. typhimurium *of phage type 141 examined and their primary biotypes*

Country of isolation	Years of isolation	Numbers of cultures in primary biotype		
		1	9	31
England and Wales	1958–74	36	21	6
Australia and Netherlands	1960–3	9	0	0
Scotland	1965–77	30	509	12
England	1984–5	0	0	72
Scotland	1986–7	0	0	9

Table 2. *Diversification in secondary biotype characters among strains of* S. typhimurium *of phage type 141 and primary biotype 31*

Full biotype (number of cultures)	Source	Country and year of isolation
31bdeg (2)	Cattle	Scotland 1965
	Man	England 1969
31bde (13)	Man	England 1971
	Man, poultry	Scotland 1972
	Man	England 1973
31bey (2)	Cattle, man	England 1971
31be (1)	Poultry	Scotland 1972
31beg (79)	Man, poultry	England 1984–5
	Cattle, dog, horse, man	Scotland 1986–7
31begj (2)	Poultry	Scotland 1987

sporadic or outbreak cases in man, from diverse food sources and from poultry products such as liquid egg belonged to the same biotype (31beg). Phage-type 141 strains of *S. typhimurium* have been infrequently isolated in Scotland in recent years with, for example, only 21 cultures reported in 1985 and most of these from sporadic incidents (Barker *et al.* 1987). It was surprising, therefore, to find that strains of this same type (141/31beg) were so widely distributed in hosts other than man and poultry among the few recent Scottish isolates available for study.

It is difficult to know whether the present line of 141/31beg has been derived from any of the diverse FIRN lines of biotype 31 strains known to be present in the UK earlier. Re-examination of 18 pre-1974 cultures available in our collection showed that all of them, like the current epidemic strain, fermented D-xylose in peptone water after incubation for $\geqslant 30$ h (their previous classification as prompt fermenters of D-xylose, i.e. in < 24 h, was incorrect, see Anderson *et al.* 1978); their modified full biotypes are given in Table 2. All 18 of these early cultures were non-fimbriate and xylose late-fermenting strains (be); 15 of them were *l*-tartrate non-fermenting (d); 2 did not ferment glycerol in Stern's medium (g); and 2 required cysteine as growth factor (y). The current epidemic strain of type 141/31beg differs, therefore, from these putative ancestral types in only one or two minor biotype characters (Table 2) and, hence, its descent from these types is not difficult to envisage.

However, we do not even know whether *S. typhimurium* of type 141/31beg reappeared in the UK in 1984 from the pool of indigenous strains or whether it was imported from another country at that time. It may even be that a strain of biotype 31beg belonging to some other phage type (which we do not know) acquired the phage type-determining factors that converted it to type 141. Answers to these intriguing speculations would perhaps be revealed by the continuous monitoring of all strains by biotyping as well as phage typing.

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