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Old and new techniques together resolve a problem of infection by *Salmonella typhimurium*

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SUMMARY

Isolates of *Salmonella typhimurium*, recovered over a 9-month period from a child with gastroenteritis, were characterized by biotyping, phage-typing and plasmid-profile analysis. Because the data from the different methods were discrepant, it was difficult to establish whether her infection was due to a single strain that had changed character *in vivo* or was due to recurrent infections with different, unrelated strains. Restriction-enzyme fingerprinting of the plasmids from the different isolates provided an explanation for the initial discrepancy and highlighted a source of potential confusion in epidemiological studies.

INTRODUCTION

Many approaches to the recognition of different strains within a species have been used successfully for epidemiological purposes over the years. Among the more established techniques used for discrimination of strains of *Salmonella typhimurium*, phage typing (Callow, 1959; Anderson *et al.* 1977) and biotyping (Duguid *et al.* 1975) have been applied in the investigation of both outbreaks and individual cases. The combined use of biotyping and phage typing provides additional differentiation (Anderson *et al.* 1978; Barker & Old, 1979) that is useful, for example, in indicating relationships among strains of the same biotype that belong to different phage types which are interconvertible by acquisition or loss of phage type-determining factors (Barker, Old & Sharp, 1980; Barker, 1986).

More recently, molecular techniques, including plasmid profiles and restriction-enzyme fingerprinting, have also been applied successfully to the investigation of the epidemiology of nosocomial and community outbreaks due to *S. typhimurium* (Bezanson, Khakhria & Lacroix, 1982; Brunner, Margadant & Peduzzi, 1983). Furthermore, it is clear that these latter techniques may be as specific as the more established systems in identifying epidemiologically related isolates as belonging

to the same or different strains of clones (Holmberg *et al.* 1984; Brown, Munro & Platt, 1986).

There may, however, be occasions when a combination of both old and new methods will be required to resolve particular epidemiological problems. This paper describes a clinical episode in which discrepancies among the results from established typing methods were clarified by the application of molecular methods.

PATIENT AND METHODS

Patient

From birth this female child (SD) had grown and developed appropriately (height and weight between the tenth and twenty-fifth centiles) until at 10 months of age she developed gastroenteritis. At that time, and intermittently for the subsequent 11 months, *S. typhimurium* was recovered from her stools on 12 occasions. No other bacterial or protozoal enteropathogens were recovered from the child; salmonellae were not recovered from any other member of her family throughout this period. From the age of 11 months, her weight showed a progressive, relative decline. However, evidence that her failure to thrive was associated with her chronic carriage of salmonellae was not convincing.

Isolation and typing of bacteria

Specimens of faeces were examined by the enrichment and selective culture methods routinely employed in the Microbiology Department, Ninewells Hospital, Dundee for the recovery of enteropathogenic bacteria. Isolates behaving biochemically and serologically like *S. typhimurium* were biotyped by the method of Duguid *et al.* (1975). Single-colony subcultures of isolates of *S. typhimurium* were sent to: (i) the Scottish Salmonella Reference Laboratory, Stobhill Hospital, Glasgow for confirmation of identify, full serotyping and for phage-typing by established methods (Callow, 1959; Anderson *et al.* 1977); (ii) the Plasmid Research Laboratory, Royal Infirmary, Glasgow where their sensitivities to 16 antibacterial agents were assessed by previously described methods (Platt, Brown & Munro, 1986); the plasmid profiles and restriction-enzyme fingerprints of the isolates were determined by previously described methods (Platt *et al.* 1986). The restriction endonucleases used were *Pst* I, *Sma* I and *Ava* II.

Antibiotic resistance transfer

Transfer of resistance determinants was tested for by standard methods and the recipient strains used were *Escherichia coli* strain K12 and *S. typhimurium* strain LT2 (Platt, Sommerville & Gribben, 1984).

RESULTS AND DISCUSSION

Isolates of *S. typhimurium* were recovered from the patient on 12 occasions over a period of 11 months; 7 of them (SD1–SD7) were available for detailed examination. All were of biotype 2a. Whereas the first isolate SD1 was resistant to streptomycin (Sm), sulphonamides (Su) and tetracycline (Tc) of the 16 agents tested and belonged to the definitive phage type 193, the subsequent six isolates

Table 1. *Characters of isolates of* Salmonella typhimurium

Isolate no.	Time of isolation (days)	Biotype	Phage type (DT)	Antibiotic resistance*	Plasmid profile (MDa)
SD1	1	2a	193	Sm, Su, Tc	2, 60
SD2	15	2a	110	—	2, 46, 60
SD3	48	2a	110	—	2, 60
SD4	51	2a	110	—	2, 46, 60
SD5	75	2a	110	—	2, 60
SD6	79	2a	110	—	2, 60
SD7	258	2a	110	—	2, 60

* The antibiotics tested were: amikacin, ampicillin, carbenicillin, cefamandole, cefazolin, chloramphenicol, colistin, gentamicin, kanamycin, nalidixic acid, rifampicin, streptomycin (Sm), sulphamethoxazole (Su), tetracycline (Tc), tobramycin and trimethoprim.
—, fully sensitive.

were of phage-type 110 and were fully sensitive to all the antibiotics tested. Plasmid-profile analysis indicated that each isolate contained two plasmids of molecular weight (MW) 2 and 60 megadaltons (MDa), respectively; isolates SD2 and SD4 possessed an additional plasmid of MW 46 MDa (Table 1). These results indicated discrepancies among the three typing methods.

When the plasmids harboured by each isolate were digested by the restriction enzymes *Pst* I and *Sma* I, the sum of the restriction fragments from isolates SD3, SD5, SD6 and SD7 corresponded with the MWs as determined by plasmid-profile analysis. In contrast, the sum of fragments from isolate SD1 (120 MDa) was about twice the expected value. This finding suggested that isolate SD1 harboured two plasmids of very similar MW that co-migrated in agarose gels (Fig. 1). This suggestion was confirmed by transfer of the resistance determinants from isolate SD1 to *E. coli* strain K12 with selection for resistance to streptomycin, sulphonamides or tetracycline. The resulting transconjugants possessed one plasmid (60 MDa; designated pOG671) that specified resistance to all three antibiotics. After digestion of the transconjugant plasmid with *Pst* I or *Sma* I, the fragments obtained corresponded to fragments present in digests of isolate SD1 but absent from isolates SD2–SD7. When this SmSuTc R-plasmid was re-transferred from *E. coli* strain K12 to *S. typhimurium* strain LT2 of phage type 4, the recipient no longer conformed to any standard phage type (i.e. was of phage-type RDNC). Likewise, when *S. typhimurium* of phage type 110 (either an isolate from patient SD or an unrelated isolate) was used as the recipient strain, the phage types converted from 110 to 193. Thus, the observed differences in phage type among isolates from this patient were probably attributable to loss of the SmSuTc R-plasmid.

The restriction fragments obtained after digestion with *Pst* I or *Sma* I of the plasmids of isolates SD2 and SD4, which it will be recalled possessed an additional plasmid, differed only slightly from those of isolates SD3, SD5, SD6 and SD7; a diffuse band was seen in that region of the gel containing material of high MW (and low resolution). However, after digestion of the plasmids from isolates SD2 and SD4 with *Ava*II, seven fragments in addition to those seen with preparations from

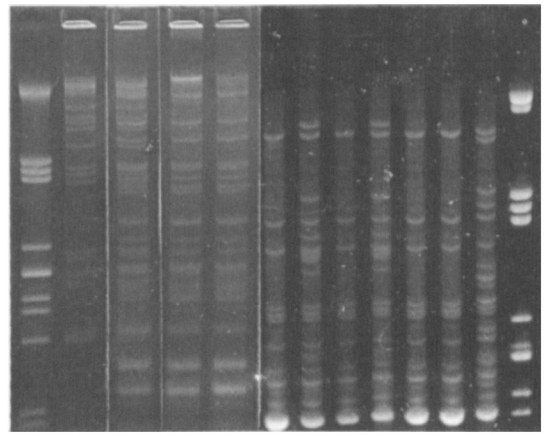

Fig. 1. Restriction enzyme fingerprints of plasmid DNA from *S. typhimurium* (from left to right): bacteriophage lambda digested with *Pst* I (λ/*Pst*), *Sma* I digests of pOG671 in *E. coli* K12, SD1, SD2 and SD3. *Ava* II digests of SD7, SD4, SD6, SD2, SD5, SD1 and λ/*Pst*. (Molecular weight (kb) of λ/*Pst* fragments: 13·8, 11·5, 5·0, 4·7, 4·5, 2·8, 2·6, 2·4, 2·1, 2·0, 1·7, 1·2, 1·1).

isolates SD3, SD5, SD6 and SD7 were found (Fig. 1), a finding consistent with the presence of the plasmid of 46 MDa in these two isolates of phage type 110.

Thus, this patient yielded three distinct lines of *S. typhimurium*. A likely sequence of events would seem to have been initial infection with an isolate of *S. typhimurium* of phage type 193 followed by elimination of the R-plasmid *in vivo* resulting in conversion of the phage type to 110. The intermittent isolation of strains of phage type 110 harbouring an additional cryptic plasmid of 46 MDa indicates either acquisition of the plasmid *in vivo* or heterogeneity in the infecting population. Because only one colony from each specimen was investigated, it was not possible to establish retrospectively whether the patient harboured strains of more than one plasmid type at any one time.

About the same time as the episode with SD, there were eight other Dundee patients whose index isolates of *S. typhimurium* were of phage types 110 or 193 and from whom multiple isolates were available for further examination. The isolates from each patient belonged to only one phage type, either 110 (5 patients) or 193 (3 patients). Furthermore, the 19 isolates of phage type 110 were of biotype 2a (2a/110), were fully sensitive and probably related epidemiologically to those from SD. Sixteen of the 17 isolates of phage type 193 were of biotype 3z (3z/193) and one was of biotype 1a (1a/193); they were fully sensitive and both lines were, therefore, readily distinguishable from the only isolate of phage type 193 (2a/193) from SD. There were only two other known Scottish episodes in 1984 in which isolates of *S. typhimurium* of phage types 110 and 193 were recovered together: (i) two isolates from a Glasgow patient; and (ii) six isolates from six members of a Stirling family (Dr J. C. M. Sharp, personal communication). All eight isolates from these two episodes were of biotype 2a (2a/110 or 2a/193) and were fully sensitive. These latter isolates of 2a/193 differed, however, from that isolate of 2a/19 from SD in lacking an R-plasmid that determined SmSuTc resistance.

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Thus, interconversion between phage types 193 and 110 associated with the SmSuTc R-plasmid may be a rare event and the SmSuTc phenotype was uncommon among the strains of 2a/193 available for study. These results provide further evidence, however, that strains of phage type 193 are heterogeneous not only in their biotypes (Barker & Old, 1980) but also in the routes whereby they come to belong to phage type 193 (Frost, de Saxe & Anderson, 1976; Threlfall, Ward & Rowe, 1978). One route demonstrated by these authors involved lysogeny by a temperate bacteriophage. We were unable to demonstrate the presence of lysogenic phage in the DT193 strain either directly or after pretreatment with mitomycin C (J. Taylor, unpublished) and this further supports plasmid mediated alteration of phage type.

In conclusion, the combined use of different methods of typing strains of *S. typhimurium* has provided information valuable not only for gross epidemiology but also for the resolution of a clinical dilemma in an individual patient. Furthermore, although plasmid-profile analysis alone has proved useful in other studies, this example demonstrates that reliance on plasmid profiles in the absence of restriction-enzyme fingerprinting may sometimes afford epidemiological interpretations that are misleading.

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