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Numerical index of the discriminatory ability of biotyping for strains of *Salmonella typhimurium* and *Salmonella paratyphi* B

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SUMMARY

An index of discrimination was used to assess the discriminatory power of biotyping for salmonellas of selected serotypes. Three collections of phage-typed strains of *Salmonella typhimurium* and *Salmonella paratyphi* B, previously tested for biotype, were examined. The results established that the discrimination index was high when full biotyping alone was used for differentiation of strains from international series. When biotyping was combined with phage typing, the discrimination achieved was greater than for either method used alone. These findings confirm that biotyping affords excellent discrimination of strains of *S. typhimurium* and *S. paratyphi* B and indicate that the results obtained from biotyping can be interpreted with confidence.

INTRODUCTION

It is important for the epidemiologist to be able to demonstrate whether strains of a particular bacterial species isolated in the course of an outbreak of infection are different or are the same. For this purpose, a wide range of typing methods is available for subspecies discrimination, i.e. the process whereby different types of strains within any named species can be recognized. The choice of the most suitable method for the satisfactory analysis of the epidemiology of a particular outbreak will be influenced both by the species involved and by the scope of the incident in terms of time and place (1). The available typing methods include not only traditional methods, such as serotyping, phage typing and biotyping that are based on phenotypic markers but also more modern methods, such as plasmid analysis and restriction endonuclease-fragment profiles of plasmid and chromosomal DNA, that are based on genotypic markers (2).

Assessment of the actual efficiency of any such successful typing scheme depends on many different factors (3); three important factors are typability, reproducibility and discrimination. Typability indicates how many of the strains examined are typable by the typing method used and in ideal conditions it should be possible to assign all strains to designated types. Reproducibility reveals how many strains give the same typing result on repeated testing and is a measure of the stability of the typing markers under study. Both typability and reproducibility are readily quantified and are generally expressed in a straightforward way as percentages.

A successful typing scheme should also be discriminating, i.e. it should define an adequate number of types. Thus, discrimination is poor if too few types are recognized, and too fine if too many types are present. But discrimination is less readily assessed than typability or reproducibility; indeed, discrimination is usually presented only as the number, and relative frequencies, of the different types recognized in the typing scheme. The absence of a single numerical value of discrimination has made it difficult to assess the discriminatory power of many typing methods and to compare meaningfully the discriminatory efficiencies of different typing methods. It was of much interest, therefore, that Hunter and Gaston (4) recently proposed an index of discrimination for typing methods, based on the probability that any two unrelated strains in a series belong to the same type. They also suggested that the discrimination index is a valid method for comparing the efficiencies of different typing methods (4).

Some years ago, a new biotyping method was developed and applied to the study of strains of *Salmonella typhimurium* (5). From diverse studies, our subjective assessment was that biotyping, used alone or together with phage typing, provided a highly discriminatory method for typing strains of *S. typhimurium* (5–11). That same biotyping scheme also afforded considerable discrimination among strains of *S. paratyphi* B and, when used in a multiple-typing approach together with data about outer-membrane protein receptors and ribosomal–ribonucleic acid (rRNA) types, allowed definition of three groups of strains and provided evidence that *S. paratyphi* B is a serotype composed of strains of different ancestral origins (12).

It seemed important, therefore, to apply retrospectively the method of Hunter and Gaston (4) and to assess the efficiency of biotyping for the discrimination of strains of *S. typhimurium* and *S. paratyphi* B.

MATERIALS AND METHODS

Bacteria

Two independent series of *S. typhimurium* strains, previously reported elsewhere (6, 10), were examined in the present study.

The first was an international collection of 2092 cultures recovered from 57 different countries between 1920 and 1975 (most of them after 1950) and which had been isolated from man (1292 cultures), cattle (251), 17 other mammalian species (135), 19 avian species (205), other animals (4), and food, the environment and unspecified sources (205). In that series, 2030 of the cultures of *S. typhimurium* had been used for the validation of the new biotyping scheme of Duguid and colleagues (5) who also detailed therein the basis of their collection. A further 62 cultures – namely, 20 cultures of phage type 40 and 3 representative cultures each of phage types 196–209 – had been included when the full series was examined for correlation of phage type, biotype and source (6).

The second was a local series of 2010 cultures that included nearly all of those sent to the Scottish Salmonella Reference Laboratory, Stobhill Hospital, Glasgow, from medical and veterinary laboratories in Scotland in the 3-year period 1974–6. They had been recovered from man (883 cultures), cattle (915), other animals (99), birds (39) and food and the environment (74) and were examined for phage type and biotype (10).

The 338 cultures of *S. paratyphi* B examined by Barker and coworkers (12) constituted an international series which was derived from eight different collections. They had been isolated from man (210 cultures), birds (5), a reptile (1) and from food, the environment and unspecified sources (122). Of these cultures, 73 strains had been isolated before 1965 and the others between 1974 and 1983 (12).

Phage typing

Cultures of *S. typhimurium* were phage-typed according to established methods (13, 14) and phage types were designated as described elsewhere (15). Cultures of *S. paratyphi* B had been phage-typed according to published methods (14, 16, 17) in their country of origin; the latter phage-type information was supplied by the donors.

Biotyping

The primary biotypes (1–32) of cultures were determined by their reactions in five biotyping tests with the substrates: D-xylose, *meso*-inositol, L-rhamnose, *d*-tartrate and *meso*-tartrate. Cultures were assigned to full biotypes on the basis of their reactions in five primary tests and a further ten secondary biotyping tests. Details of the biotyping methods, performance and interpretation of tests and designation of primary and full biotypes have been presented elsewhere (5).

Discrimination index

The discriminatory power of the typing methods, used alone or together, was determined by use of the single numerical index of discrimination recommended by Hunter and Gaston (4). That was based on the probability that two unrelated strains sampled from a test population would be placed in different types; that probability was calculated by Simpson's index of diversity (18). Thus, the index of discrimination (D) is given by

$$D = 1 - \frac{1}{N(N-1)} \sum_{j=1}^{s} n_j(n_j - 1),$$

where N is the total number of cultures in the sample population, s is the total number of types present and n_j is the number of cultures of the 'j'th type. For a full explanation of the application of this index, the reader is referred to (4).

RESULTS

S. typhimurium

Among the 2092 phage-typed strains of *S. typhimurium* in the international series, 204 definitive phage types were recognized, accounting for 1937 strains. In addition, there were 124 cultures which were resistant to all of the typing phages and, hence, were untypable (U). The remaining 31 cultures belonged to type RDNC, i.e. they reacted with the typing phages but gave patterns not conforming with those of any definitive phage type. Although the term RDNC embraces several different patterns and types, we did not have the details of the different RDNC subtypes present. Accordingly, for the purpose of analysis of this series of strains, RDNC and U have each been scored as one type. Thus, the total number

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Table 1. *Discrimination indices of biotyping, used alone or together with phage typing, in the examination of 2092 cultures of* S. typhimurium *from an international series*

Typing method	Number of types	Size (%) of largest type	Discrimination index
Phage typing	206	12·1	0·9669
Primary biotyping	19	20·7	0·8722
Full biotyping	147	12·1	0·9402
Phage typing with primary biotyping	400	9·4	0·9797
Phage typing with full biotyping	612	9·4	0·9844

of types recognized by phage typing was 206 and its discrimination index was 0·9669 (Table 1).

Biotyping, used alone, recognized 19 primary and 147 full biotypes among this same series of strains giving discrimination indices of 0·8722 and 0·9402, respectively (Table 1). Phage typing and biotyping used together divided the series into 400 different phage-type/primary-biotype combinations and 612 phage-type/full-biotype combinations and the discrimination indices of the joint typing methods were 0·9797 and 0·9844, respectively (Table 1). The full data on which these types have been scored have been detailed elsewhere (5, 6).

Among the series of 2010 Scottish strains of *S. typhimurium* isolated in the years 1974–6, phage typing had divided 1784 of the cultures into 55 definitive phage types and another three types (46a, 49a and 104b) closely related to these definitive types. Of the remainder, 15 cultures were untypable (U) and 211 cultures gave RDNC patterns. For this series of strains, unlike those in the international series (see above), additional information provided by the reference laboratory allowed some of the RDNC subtypes to be scored separately; these were RDNC1 (111 cultures), RDNC2 (55) and RDNC3 (29). The remaining 16 cultures belonged to RDNC subtypes other than RDNC1–3 but, because these subtypes were unknown to us, we grouped them as a fourth (heterogeneous) RDNC type (10). Thus, for the purpose of analysis of this series of strains, it was considered that phage typing recognized 63 types; its discrimination index was 0·9159 (Table 2).

Biotyping, used alone, recognized 14 primary and 45 full biotypes among this same series of strains; the corresponding discrimination indices were 0·8253 and 0·8626, respectively (Table 2). Phage typing and biotyping together divided this series of strains into 112 different phage-type/primary-biotype combinations and 137 different phage-type/full-biotype combinations and the discrimination indices of these methods used together were 0·9208 and 0·9228, respectively (Table 2). The full data on which these types have been scored have been detailed elsewhere (10). The numbers of types, the sizes of the largest types recognized by each method, and the discrimination indices obtained when the different techniques were applied to both the international and local series of *S. typhimurium* strains are summarized in Tables 1 and 2.

Table 2. *Discrimination indices of biotyping, used alone or together with phage typing, in the examination of 2010 cultures of* S. typhimurium *recovered in Scotland (1974–6)*

Typing method	Number of types	Size (%) of largest type	Discrimination index
Phage typing	63	15·2	0·9159
Primary biotyping	14	24·9	0·8253
Full biotyping	45	23·6	0·8626
Phage typing with primary biotyping	112	14·4	0·9208
Phage typing with full biotyping	137	14·3	0·9228

Table 3. *Discrimination indices of biotyping, used alone or together with phage typing, in the examination of 338 cultures of* S. paratyphi *B from an international series*

Typing method	Number of types	Size (%) of largest type	Discrimination index
Phage typing	40	13·9	0·9354
Primary biotyping	13	30·5	0·7998
Full biotyping	56	10·4	0·9566
Phage typing with primary biotyping	80	9·5	0·9712
Phage typing with full biotyping	150	3·8	0·9897

S. paratyphi B

The series of 338 strains of *S. paratyphi* B included 280 strains belonging to the 10 phage types recognized by Felix and Callow (17), to 23 subtype variants of some of these types and to another four phage types described later (14). Of the remaining strains, 36 were resistant to all typing phages and were untypable (U), 9 reacted to give RDNC patterns and phage-type information was not specified by the donors for 13 strains. That latter group of 58 strains was considered, therefore, to comprise a further three types. For the analysis of this series of strains, phage typing was considered to recognize 40 types; its discrimination index was 0·9354 (Table 3).

Biotyping divided the *S. paratyphi* B strains into 13 primary and 56 full biotypes and the discrimination indices were 0·7998 and 0·9566, respectively (Table 3). Phage typing and biotyping together discriminated 80 different phage-type/primary-biotype combinations and 150 different phage-type/full-biotype combinations and their discrimination indices were 0·9712 and 0·9897, respectively (Table 3). The full data on which these types have been scored have been detailed elsewhere (12). The numbers of types, the sizes of the largest types defined by each method, and the discrimination indices of the different methods, used alone or together, for the typing of *S. paratyphi* B are summarized in Table 3.

DISCUSSION

Phage typing has proved to be an excellent method for the discrimination of different strains within the serotype *S. typhimurium* (14) and the scheme (15), as now expanded, recognizes 232 definitive phage types. The discrimination index for phage typing was high when applied to both the international and the local (i.e. Scottish) series of strains. If, then, phage typing is so highly discriminatory for strain differentiation of *S. typhimurium* in communities and countries over both long and short periods, are additional typing methods really required? It must be recognized that changes in the typing characters of strains can occur in the course of their epidemic spread with the result that strains happening to share the same typing character may be thought to belong to the same clone whereas the relationships among strains of common lineage may be overlooked because of on-going variations in the typing characters used to detect them (9, 11). In these situations, the true relationships among isolates will be revealed only by recourse to a multiple-typing approach whereby, for example, phage-typed strains of *S. typhimurium* are further differentiated by the application of additional typing methods.

Biotyping has long been a popular method for subdividing different strains within common salmonella serotypes (19), and early attempts at biotyping strains within the serotype *S. typhimurium* met with some success (20–22). However, it was only with the establishment of definitive times of reading for each of the 15 biotyping tests, with the introduction of new biotyping tests and with the proposal of a two-tier biotyping system – based on five primary and ten secondary tests – that Duguid and colleagues (5) brought order to what had been a confused, and somewhat random, approach to biotyping. That two-tier system provided a finer, and more reliable, differentiation of *S. typhimurium* strains; furthermore, it allowed for continuing expansion of the scheme by the incorporation of new biotyping tests at the secondary level (5).

It was already apparent that biotyping afforded considerable strain differentiation because, even when used alone, it has allowed thus far the recognition of 24 primary and 187 full biotypes among *c* 5000 strains of *S. typhimurium* examined (9). Its value is greatly increased when used with phage typing, and > 600 phage type/full biotypes have been differentiated (Table 3). Again, the conjoint use of biotyping and phage typing has shown that many of the phage types (e.g. 10, 12, 141, 170 and 193) commonly found among, for example, isolates in the UK (23) contain strains belonging to diverse primary biotypes (9). Likely explanations for that finding are that the phage types are heterogeneous, containing different biotype lines that have acquired by chance the same phage type-determining character, or that the phage-type lines themselves have undergone considerable variation in biotype characters in the course of their extended epidemic spread.

Whilst it was clear that biotyping fulfilled most of the requirements of a successful typing scheme (5), it was more difficult to assess numerically its discriminatory power and to compare it with other established systems. In this study, we have made use of the discrimination index of Hunter and Gaston (4) to quantify for the first time the discriminatory ability of biotyping for *S. typhi-*

murium. In developing the *S. typhimurium* biotyping scheme, care had been taken to validate it with a large and representative series of strains isolated from a wide range of human, animal and other sources from many countries and spanning many years. The discrimination index of full biotyping was high (0·9402) and compared favourably with that of phage typing for that same series. It was also pleasing to note the considerable discrimination (0·8722) achieved by primary biotyping alone.

Although the acceptable level of discrimination achieved by any typing method depends on many factors (3, 4), it has been suggested that a discrimination index of $> 0{\cdot}9000$ is desirable if the typing results are to be interpreted with confidence (4). Biotyping, it is clear, meets the challenge of that high level of discrimination and must, therefore, be considered as a valuable tool for strain differentiation in *S. typhimurium*. Again, when biotyping is used together with phage typing, as we have always recommended that it should be (5–11), the degree of discrimination achieved by the combined methods is considerably enhanced (0·9797–0·9844) depending on whether primary or full biotyping procedures are applied.

It has been emphasized that the index of discrimination is of most value for large and non-local collections of distinct strains (4). In this study, therefore, it was not surprising to find with the Scottish series of strains that the discrimination indices for both phage typing and biotyping were less than those with the international collection (see Tables 1 and 2). Nevertheless, the discrimination indices, for phage typing (0·9159) and full biotyping (0·8628) alone or combined (0·9228), seem remarkably good, especially when it is remembered that four distinct phage-type/full-biotype combinations (the clones 1/2a, 49/26a, 56/17g and 141/9f) comprised 52% of the 2010 cultures in that circumscribed Scottish series (10).

Although the use of biotyping by Barker and colleagues (12) in their study of *S. paratyphi* B was directed more to understanding the phylogeny than the epidemiology of that serotype, biotype discrimination of strains in that series was high (0·9566); again, biotyping enhanced discrimination when it was used with phage typing. Whilst it may seem unusual that the discrimination index of full biotyping for strains of *S. paratyphi* B was even higher than that achieved for strains of *S. typhimurium*, the serotype for which it was originally devised (5), the explanation of that apparent anomaly probably resides in the collections themselves. Thus, whereas nearly two-thirds of the *S. typhimurium* strains had been collected at random with only single strains taken from different epidemic episodes, Duguid and colleagues (5) had deliberately included in their series some groups of strains from different patients, animals or other sources in the same epidemic episode in order to establish the stability of the biotype characters of strains during growth and spread in their natural and inanimate environments. The *S. paratyphi* B collection, on the other hand, was chosen for phylogenetic study only and it had been more stringently selected to ensure that similar strains were excluded (12). That being so, it is all the more remarkable that the index of discrimination of full biotyping for *S. typhimurium* strains was so high.

Hunter and Gaston (4) considered that their index of discrimination would greatly aid comparison between different typing systems; that it did so with the methods used in this study is clear. They also believed that an index of

discrimination should be included in the description of each new typing method. Although that was not possible when our biotyping method was first published (5), we have been greatly encouraged by its application in this study and consider that the present findings, based on the use of their mathematical model (4), have vindicated biotyping as a valuable method for discrimination among strains of *S. typhimurium* and *S. paratyphi* B.

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REFERENCES

1. Wachsmuth K. Molecular epidemiology of bacterial infections: examples of methodology and of investigations of outbreaks. Rev Infect Dis 1986; **8**: 682–92.
2. Hawkey PM. Molecular methods for the investigation of bacterial cross-infection. J Hosp Infect 1987; **9**: 211–18.
3. Anderson ES, Williams REO. Bacteriophage typing of enteric pathogens and staphylococci and its use in epidemiology. J Clin Pathol 1956; **9**: 94–127.
4. Hunter PR, Gaston MA. Numerical index of the discriminatory ability of typing systems: an application of Simpson's index of diversity. J Clin Microbiol 1988; **26**; 2465–6.
5. Duguid JP, Anderson ES, Alfredsson GA, Barker R, Old DC. A new biotyping scheme for *Salmonella typhimurium* and its phylogenetic significance. J Med Microbiol 1975; **8**: 149–66.
6. Anderson ES, Ward, LR, de Saxe MJ, Old DC, Barker R, Duguid JP. Correlation of phage type, biotype and source in strains of *Salmonella typhimurium*. J Hyg 1978; **81**: 203–17.
7. Barker RM, Old DC. Biotyping and colicine typing of *Salmonella typhimurium* strains of phage type 141 isolated in Scotland. J Med Microbiol 1979; **12**: 265–76.
8. Barker RM, Old DC. Biotypes of strains of *Salmonella typhimurium* of phage types 49, 204 and 193. J Med Microbiol 1980; **13**: 369–71.
9. Barker RM, Old DC. The usefulness of biotyping in studying the epidemiology and phylogeny of salmonellae. J Med Microbiol 1989; **29**: 81–8.
10. Barker RM, Old DC, Sharp JCM. Phage type/biotype groups of *Salmonella typhimurium* in Scotland 1974–6: variation during spread of epidemic clones. J Hyg 1980; **84**: 115–25.
11. Old DC, Barker RM. Persistent and transient clones of *Salmonella typhimurium* of phage type 141 recognized by biotyping. Epidemiol Infect 1989; **102**: 113–18.
12. Barker RM, Kearney GM, Nicholson P, Blair AL, Porter RC, Crichton PB. Types of *Salmonella paratyphi* B and their phylogenetic significance. J Med Microbiol 1988; **26**: 285–93.
13. Callow BR. A new phage typing scheme for *Salmonella typhimurium*. J Hyg 1959; **57**: 346–59.
14. Anderson ES. The phage typing of salmonellae other than *S. typhi*. In: van Oye E, ed. The world problem of salmonellosis. The Hague: Dr W Junk, 1964: 89–110.
15. Anderson ES, Ward LR, de Saxe MJ, de Sa JDH. Bacteriophage typing designations of *Salmonella typhimurium*. J Hyg 1977; **78**: 297–300.
16. Felix A, Callow BR. Typing of paratyphoid B bacilli by means of Vi bacteriophage. Br Med J 1943; **ii**: 127–30.
17. Felix A, Callow BR. Paratyphoid-B Vi-phage typing. Lancet 1951; ii: 10–14.
18. Simpson EH. Measurement of diversity. Nature 1949; **163**: 688.
19. Kauffmann F. The bacteriology of Enterobacteriaceae. Copenhagen: Munksgaard, 1966: 400.
20. Kristensen M, Bojlén K, Faarup C. Bakteriologisk-epidemiologiske Erfaringer om Infektioner med Gastroenteritisbaciller af Paratyfusgruppen. Bibliot f laeger 1937; **129**: 310–39.

21. Hansen AC. Die beim Haüsgeflügel in Danemark festgestellten Salmonellatypen. Zentralbl Bakteriol (Orig) 1942; **149**: 222–35.
22. Harhoff N. Gastroenteritisbaciller af Salmonellagruppen i Danmark. Copenhagen: Nyt Nordisk Forlag – Arnold Busck, **1948**: 243.
23. Palmer SR, Rowe B. Trends in salmonella infections. PHLS Microbiol Digest 1986; **3**: 2–5.