METHODS in MICROBIOLOGY

Edited by
T. BERGAN
J. R. NORRIS

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PREFACE

Growing awareness of the factors controlling the spread of disease-causing bacteria in various environments has led to the proliferation of methods for typing individual isolates of human pathogens. For some organisms these are highly developed, and there is a correspondingly detailed understanding of their epidemiology; in other cases understanding is still rudimentary. Although the main typing techniques, biochemical, serological, phage and bacteriocin typing, are applied across a wide spectrum of bacterial types, the details of the technical modifications needed to adapt them to different genera are important and numerous and we are responding to several suggestions from microbiologists in bringing together the typing techniques and discussion of their results in a series of four Volumes of "Methods in Microbiology" (Volumes 10–13). We hope that the Volumes will thereby contribute to a standardization and optimalization of methods.

The collection of various typing methods for many different species also contributes to the general understanding of the problems involved in typing and interpreting the results. Often, the research worker may think that difficulties with the methods and species with which he is working are unique. Consideration of the Chapters contained in the Volumes shows that many problems are universal. We hope that collecting the information together will serve to demonstrate the areas that might benefit from further attention and inspire development and research.

We have tried, as far as possible, to follow the order of genera as they appear in Bergey's "Manual of Determinative Bacteriology", 8th Edition. Volume 10 opens with two general Chapters dealing with the application of typing methods to epidemic disease in general and to the particular problems involved in studying hospital infections. Other Chapters deal with *Pseudomonas*, *Brucella*, *Francisella*, *Haemophilus*, *Pasturella* and *Neisseria*.

It is a pleasure for us to acknowledge the friendly co-operation and patience of our contributors. As in earlier Volumes we have, as far as possible, allowed individual authors considerable freedom in selecting material for presentation and in choosing the way in which the Chapters are constructed. Nevertheless, we have ensured that methods are described in full detail and that references are adequate for the effective orientation of newcomers to a particular group of organisms. It is our hope that these Volumes will make a significant contribution to an important and rapidly expanding area of microbiology.

T. BERGAN
J. R. NORRIS

November, 1977

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CHAPTER I

Usefulness, Applications and Limitations of Epidemiological Typing Methods to Elucidate Nosocomial Infections and the Spread of Communicable Diseases

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I. GENERAL INTRODUCTION

The use of modern epidemiological typing methods in the survey of communicable diseases is fully recognized today. A very important place in epidemiological typing methods is held by phage typing. These methods (phage typing, serotyping, bacteriocin (geno) typing, biochemical typing,

typing by antibiogram and R factors) are used either separately or together to obtain a more detailed level of differentiation within bacterial species.

The history of the practical use of these methods runs parallel to the history of the development of phage typing as a supplementary and very necessary approach to epidemiological investigation. The discovery of the bacteriophage by Twort (1915) and d'Herelle (1917) and of lysogeny by Bordet and Ciucă (1921, 1921a) opened up new ways for research in the field of bacterial genetics and into the ecological, pathogenic and epidemiological characteristics of the host–parasite relationships involved in many communicable diseases.

Today, control of many communicable diseases is not possible without an active follow up of the epidemiological process, control of the spread of pathogenic agents, sources of infection, mechanisms of transmission and features of the spread of infection in terms of the susceptible population. Repeated follow up over several years of these factors by modern methods of epidemiological typing within the framework of national and international programmes, is of particular importance in disease control.

The present Chapter describes the study of the applications and limitations of these methods, with examples from current epidemiological practice. We will discuss, from this point of view, the principal methods of epidemiological typing among which phage typing occupies a foremost place. It is designed primarily for the subdivision of microbial species whose serotype has usually been previously determined. Serotyping and the other approaches mentioned above are also applicable to a series of microbial species of medical interest.

II. PHAGE TYPING

A. Introduction

Phage typing is a method of bacterial differentiation based upon the sensitivity of strains to certain bacteriophages.

Bail (1921) seems to have been the first to be interested in the differentiation of bacterial strains with the aid of bacteriophages. Subsequently, attempts were made for some time to determine certain antigens with the help of phages (Burnet, 1927) or to use them for diagnostic purposes (Sonnenschein, 1925, 1928, 1929; Schmidt, 1932), but phage typing actually began with the investigations of Marcuse (1934) concerning the subdivision of *Salmonella typhi* strains.

The methods of phage typing include:

(a) Direct phage typing with:

– empirically found, unadapted phages (e.g. phage typing of *S. typhimurium* according to Lilleengen, 1948);

– adapted phages (e.g. phage typing *S. typhi* according to Craigie and Yen, 1938).

(b) Indirect phage typing (lysogenotyping) based upon the lysogeny of bacteria, i.e. detection and identification of temperate phages present as prophages in bacteria (for instance phage typing of *S. typhimurium* described by Boyd, 1950, and Boyd *et al.*, 1951).

(c) Combined phage typing: The concomitant use of direct and indirect phage typing.

B. Applications and usefulness

The increasingly widespread use of phage typing in various countries throughout the world for the subdivision of different microbial species has shown that the principal applications and possibilities of this method might be summed up as follows:

1. *Determination of the spread of some organisms in time and space*

There are universal or widespread phage types while others are only of regional interest. Among widely disseminated *S. typhi* phage types A, E_1, F_1, D_1 may be cited; phage types such as B, O, 38 are less widespread. Other *S. typhi* phage types occur only in certain regions of the world; such as phage type M occurring especially in the Far East, in Indonesia or Vietnam. Likewise phage type G is seen in the Far East, in Egypt and Congo, phase types L_1 and L_2 are encountered in North Africa, and so forth.

Ziesché and Rische (1973) report in East Germany *Shigella sonnei* phage types spread throughout the whole country, occurring every year, and others which are only of local importance.

For the different *Escherichia coli* serotypes O_{111}: $K_{58}(B_4)$, O_{26}: $K_{60}(B_6)$, O_{55}: $K_{59}(B_5)$ Nicolle *et al.* (1960) also showed the presence of phage types either with a general or a local distribution. Similar remarks were made for the same *E. coli* serotypes according to the phase typing scheme of Eörsi *et al.* (1953, 1957) and Milch and Deak (1961).

Corynebacterium diphtheriae phage type XVI (Saragea and Maximescu, 1966) was identified in a collection of strains isolated in Australia in 1938 (Gibson *et al.*, 1970), and the same phage type was identified later in Europe (Italy, Jugoslavia, Hungary) and Canada (Vancouver). *C. diphtheriae* phage type I "a" was identified first (by the same authors) in the U.S.A. and never in Europe, except incidentally in Hungary where it was introduced by some relatives coming from the U.S.A. (Saragea, 1975). Saragea *et al.* (1973) showed that in a certain geographical area the same *C. diphtheriae* phage type will persist until all healthy carriers are cleared up.

2. *Differentiation between endemic and epidemic strains*

The existence of a great number of phage types (for instance in *Sh. sonnei* and *Sh. flexneri* (Meitert *et al.*, 1975) within a given territory allows for differentiation of the endemic from the epidemic strains isolated from foci and which, in most cases, belong to only one predominant phage type.

Another example is the differentiation, by phage typing, of ubiquitous *Staphylococcus aureus* strains from epidemic strains with an increased virulence—of real importance from the viewpoint of public health (Meyer, 1966).

3. *Spread of epidemic foci from one area to another*

For certain phage typing schemes, for instance that used for typing *Vibrio cholerae eltor* biotype, the main usefulness is "in tracing the spread of the epidemic from one place to another, rather than tracing chronic carriers and the spread of infection through individuals" (Mukerjee, 1973). It has thus been possible to demonstrate the spread of an infection from Calcutta to neighbouring districts and states (Mukerjee, 1963).

The original source of the cholera outbreak in Afghanistan, in 1960, was an infection in West Pakistan, likewise confirmed by phage typing of *Vibrio* strains isolated in two areas. Similarly, the 1958 cholera outbreak in Nepal could be linked up with the Bihar outbreaks.

Moreover, by phage typing, it was possible to detect the penetration of new phage types in a region, particularly when strains with these characteristics had not yet been encountered in the area.

4. *Determination of the sources of infection in epidemics and epidemic foci*

Phage typing fulfils a valuable function when establishing the source of numerous epidemic foci, since it allows us to exclude apparent sources and reveal the real source of infection. Many examples have been reported in this connection showing that in epidemics or epidemic foci with a single source of infection, the same phage type is isolated from all patients and contacts. Phage typing within the focus also makes it possible to establish the extent of the epidemic area, the persistence in the carriers of the pathogenic agent and differentiation of the epidemic strains from those of carriers or sporadic cases unrelated to the local outbreak.

Within an epidemic focus, corroboration of the epidemiological data with the phage typing results, makes it possible to establish the relationship of cases, thus excluding sporadic cases from a chain of epidemic ones. It is known that a phage typing scheme may be considered reliable when all strains of an epidemic focus, with a single source of infection, belong to the same phage type. For instance, the various cholera epidemics in India and

in Thailand in 1966 were demonstrated by phage typing to start from a single source of infection (Mukerjee, 1973). Numerous similar examples are also found for phage typing of different other pathogenic agents: *Salmonella*, *Shigella*, *E. coli*, *C. diphtheriae* (Rische, 1973).

Moreover, phage typing also offers the possibility of establishing two or even more sources of infection within certain epidemic foci and the extent of the spread of different phage types by patients and carriers.

5. *The role of carriers in epidemic outbreaks*

Not all carriers initiate epidemic foci. Phage typing not only makes it possible to determine the carrier or carriers that caused an epidemic focus by contact or other mechanisms of transmission, but also permits the exclusion of certain carriers (with different phage types) as sources of an epidemic outbreak.

In the follow up of carriers, especially for *Staphylococcus aureus* (Popovici, 1968), *Pseudomonas aeruginosa* (E. Meitert and T. Meitert, 1973) and *C. diphtheriae* strains (Saragea *et al.*, 1973), phage typing may further contribute to determine:

– Carriage in time of a certain phage type and its disappearance (spontaneous or after treatment);
– Reinfection of carriers with the same or another phage type;
– The appearance of new carriers with identical or different phage types;
– The increased spread by contacts of certain epidemic phage types;
– The existence of a mixed carrier with several phage types;
– The role of carriers in alimentary food poisoning.

Many examples can be found in this connection in the Chapters dealing with specific pathogenic agents. We shall recall only the predominance, at a given moment, of certain *C. diphtheriae* strains (Saragea *et al.*, 1973). Thus, *C. diphtheriae* phage type XIV displayed a quicker spread, but shorter persistence in the community and caused milder clinical cases. Phage type XIV "a" showed a higher infectivity, but shorter persistence (duration of carriage in healthy subjects is about 2–3 years). Phage type IX caused sporadic cases, but the clinical picture has been more severe (Saragea *et al.*, 1973). It is of interest to note that at a given moment, *C. diphtheriae* phage type XIV produced about 20–35 healthy carriers around a patient, whereas with phage type XIV "a" there were only 10–12 carriers. With phage type IX no more than 2–3 carriers occurred around one index case.

6. *Retrospective elucidation of certain epidemiologic processes*

In 1957, in a camp on the Baltic Sea, a number of cases of typhoid fever were not identified as such. From the campers, who returned home to

different regions of the country (East Germany), strains with the same phagolytic pattern (uncharacteristic Vi-strains) were isolated. Thus, it was possible to point out the epidemic focus retrospectively by phage typing (Sedlak and Rische, 1968).

By determining the phage type of some strains collected during the years 1927–1947, Blair and Carr (1960) identified 15·6% of 276 *Staphylococcus* strains as phage type 80, the test phage 80 being isolated later.

7. Characterization and follow up of the spread in hospital of pathogenic and opportunist agents

(a) *Determination of the epidemiological significance of certain strains of the same microbial species.* By phage typing of *E. coli* O:111, 55, 26 according to Eörsi *et al.* (1953, 1957), Milch and Deak (1961), it was possible to differentiate the following categories of strains:

- strains arising from a concurrent hospital outbreak;
- strains from a previous hospital outbreak;
- strains introduced more recently into the hospital (Milch, 1973).

Numerous examples may also be given for other microbial species.

(b) *The existence of a phage type in specimens from several patients and the increased incidence of the same phage type in a hospital ("hospital strains").* The selection of certain strains with an increased ability to spread and pathogenicity, often resistant to antibiotics in hospital conditions, is more easily followed up by phage typing.

Williams and Jevons (1960) showed that certain *Staph. aureus* staff strains may spread throughout a hospital without causing infections, while others always cause disease. The predominance in hospital of *Staph. aureus* phage type 80 over a certain period is well known (Nestorescu *et al.*, 1956, 1957a; Meyer, 1957; Meyer *et al.*, 1973; Ciucă *et al.*, 1958).

Similar observations have been made for other opportunists. Thus, Meitert and Meitert (1971) observed that *P. aeruginosa* strains belonging to the same phase type, isolated from hospital infections, were associated with increased properties of spread and virulence. Vieu (1969) showed that the diversity of *P. aeruginosa* phase types, which appear endemically in hospitals, contrast with the uniformity and virulence of the phage types isolated in extensive hospital outbreaks.

(c) *The occurrence of hospital strains in the environment.* Meyer *et al.* (1973) noticed that penicillin-resistant, phage group III staphylococci were at first seldom encountered outside hospitals, but were after a while gradually penetrating into the environment.