A comparison of typing methods for *Serratia marcescens*

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SUMMARY

A simple method for the bacteriocine typing of *Serratia marcescens* without the use of induction was sought. The results of a mutual inhibition experiment with 89 unrelated cultures indicated that a bacteriocine-susceptibility method would give more discrimination between strains than would a bacteriocine-production method. A cross-streaking technique for bacteriocine-susceptibility typing without previous induction was developed, and its performance was compared with that of another susceptibility-typing method in which cell-free lysates of the producer strains were obtained by induction with mitomycin C.

Replicate typing of the same collection of cultures by both methods indicated that small variations in pattern were common and that larger variations occurred occasionally. Differences in pattern of less than two strong reactions in the mitomycin-C induction method, and of less than three strong reactions in the cross-streaking method, should therefore not be taken as evidence that strains can be distinguished.

Sets of cultures of *Ser. marcescens*, 178 in total, from a number of supposed incidents of infection in hospitals, were used to evaluate the two bacteriocine-typing methods; all of the cultures were also O serogrouped. Comparison of the typing patterns of members of the same O serogroup from clear-cut incidents of infection confirmed that results of acceptable reliability could be obtained by either bacteriocine-typing method by the application of the appropriate 'difference' rule. When so interpreted, the cross-streaking method appeared to be slightly the more discriminatory.

The greatest discrimination between strains was obtained by the use of a 'hierarchical' typing system in which the strains were first O serogrouped, and the cross-streaking method of bacteriocine typing was then used to make subdivisions within O serogroups.

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INTRODUCTION

Infection of patients with *Ser. marcescens* does not yet appear to have become widespread in British hospitals, but the frequency with which it has been reported in some other countries (Ewing, Johnson & Davis, 1962; Wilfert, Barrett & Kass, 1968; DuPont & Spink, 1969; Altemeier, Culbertson, Fullen & McDonough, 1969; Wilfert *et al.* 1970; Wilkowske, Washington, Martin & Ritts, 1970; Baumann & Emmrich, 1974, Daschner & Senska-Euringer, 1975) indicated the desirability of having available a typing method that would provide adequate means of investigating sources and routes of infection should the disease become more widespread in our hospitals.

Typing methods for *Ser. marcescens* that have been described were based on antigenic structure (Ewing, Davis & Reavis, 1959), bacteriophage susceptibility (Pillich, Hradečná & Kocur, 1964), bacteriocine susceptibility (Traub, Raymond & Startsman, 1971) or bacteriocine production (Farmer, 1972). In the bacteriocine-sensitivity typing method of Traub *et al.* (1971), the production of bacteriocines by a set of producer strains was induced by mitomycin C; the susceptibility of clinical strains to cell-free bacteriocine preparations was compared and a series of 'type' patterns was designated. Farmer (1972), on the other hand, performed mitomycin-C induction on the clinical strains and tested the resulting preparations for activity against a set of indicator strains.

The first objective of this investigation was to see whether a simple 'cross-streaking' method either of bacteriocine-susceptibility typing or of bacteriocine-production typing, without preliminary induction, would give acceptable results. We then elaborated such a method of bacteriocine-sensitivity typing and compared its performance with that of the bacteriocine method of Traub *et al.* (1971), and with O-serological typing (Ewing *et al.* 1959), in the study of a series of cultures from suspected outbreaks of infection in British hospitals.

MATERIALS AND METHODS

Cultures of Serratia marcescens

We assembled a heterogeneous collection of 89 cultures thought to include few if any that were epidemiologically related. These comprised: 28 cultures each representative of a different O or H serotype in the system of Edwards & Ewing (1962); the 10 producer and 4 indicator strains of Traub *et al.* (1971); 14 strains maintained in the National Collection of Type Cultures (NCTC) and 33 submitted for identification to the Computer Trials Laboratory of the NCTC. In addition we received 178 strains from patients in eight hospitals or groups of related hospitals, each series from a hospital being collected within a limited time ($\leq$ 10 months). All duplicates and sequential strains had been excluded, so that each represented an infected patient or a separate environmental site.

Biochemical identification

Strains were accepted as being *Ser. marcescens* if they fermented glucose, liquefied gelatin rapidly, were oxidase and phenylalanine-deaminase negative and did not acidify arabinose and raffinose in peptone water incubated aerobically.

Media

Nutrient broth was Nutrient Broth no. 2 (Oxoid) and nutrient agar was this broth solidified by the addition of 1 % (w/v) agar.

Cross-streaking methods of bacteriocine typing

After some preliminary experiments (see Results) the following technique was used. A single colony of the strain under test as a bacteriocine producer was subcultured to nutrient broth, which was then incubated at 37 °C for 4 h. An agar plate was seeded from this broth with a sterile cotton-wool swab in a streak, *ca.* 1 cm wide, across the diameter. After the plate had been incubated at 32 °C for 18 h, the visible bacterial growth was removed carefully by scraping with the edge of a glass slide and the residual micro-organisms on the agar were killed by exposing the plate to chloroform vapour for 30 min. The plate was then exposed to the air for 10 min, and broth cultures of the strain under test as an indicator (previously incubated at 37 °C for 4 h) were diluted 1 in 10 in broth and streaked with a loop at right angles to the line of the original streak. The plates were incubated at 37 °C for 18 h.

Clear zones of inhibition of the indicator strains with no resistant growth were scored as +, the presence of some resistant growth (up to 10 colonies) in an area of inhibition as ±, and no distinct inhibition as −. When comparing the patterns of reaction given by different strains, or by the same strain on different occasions, a difference in strength of reaction (between − and ±, or between ± and +) was ignored, and only the difference between − and + reaction was termed a strong-reaction difference.

In the initial assessment of the cross-streaking method for bacteriocine-susceptibility and bacteriocine-production typing, each of the 89 strains was tested against all the others as producer and as indicator. From the results, a 'chequer-board' table was constructed and this was analysed by means of the computer program DTA3 (Willcox & Lapage, 1972), which had initially been designed to choose biochemical tests for use in diagnostic tables. The results of this analysis led us to adopt a cross-streaking method of bacteriocine-susceptibility testing with 12 producers as test reagents, and this was performed as described above.

Bacteriocine-susceptibility typing with mitomycin-C induced lysates

The method of Traub *et al.* (1971) was followed, and the 10 bacteriocine-producing strains recommended by these authors were used. Induction was with mitomycin C obtained from BDH Chemicals Ltd, Poole, Dorset, and filtered lysates were applied as drops to the surface of a freshly seeded plate of the strain to be typed.

Serological typing

Antisera were prepared according to the method of Ewing *et al.* (1959) against the O-serotype strains obtained from Dr W. H. Ewing, Center for Disease Control, Atlanta, U.S.A. The highest serum dilution that showed easily visible agglutination in tube-agglutination tests was termed the titre. For use, the sera were diluted to approximately half their homologous titre. At this dilution the sera gave no cross-reactions when tested against the heterologous type strains.

RESULTS

Development of a cross-streaking method without induction

Preliminary experiments indicated that bacteriocine production was definitely less on agar plates incubated at 37 °C than at 32 °C, and the latter temperature was used in cross-streaking tests for mutual inhibition by the heterogeneous collection of 89 cultures of *Ser. marcescens* (see Materials and Methods).

Two test selections were made by means of the computer. In the first, the action of the bacteriocine producers was looked upon as the test ('producers as tests'); this was considered to be a model for a test in which the strain to be typed was subjected to the action of bacteriocines produced by a set of other cultures ('bacteriocine-susceptibility typing'). In the second, susceptibility to the action of bactericines was looked upon as the test ('indicators as tests'); this was considered to be a model for a test in which the pattern of susceptibility of a set of indicator strains to the bacteriocine or bacteriocines of the strain to be typed was determined ('bacteriocine-production typing').

With producers as tests, only two pairs of cultures could not be separated by their pattern of susceptibility, and three further strains were resistant to all the bacteriocine preparations. The computer selected 12 producers that differentiated 84 distinct patterns among the 89 strains by at least one strong-reaction difference; if at least two strong-reaction differences were required to distinguish between strains, 76 patterns would have been recognized. If the three least separative of the 12 producers were omitted, 73 patterns were distinguishable by one strong-reaction difference. On the other hand, with indicators as tests, three sets of cultures (respectively of two, three and four strains) gave identical patterns, and 16 strains failed to produce bacteriocine active against any of the indicators. At least 17 indicators were needed to make the greatest possible number of separations (67).

These results indicated that a bacteriocine-susceptibility test was likely to be a more discriminating method of typing than a bacteriocine-production test, and we decided to use the 12 producers selected by the computer as a means of bacteriocine-susceptibility typing.
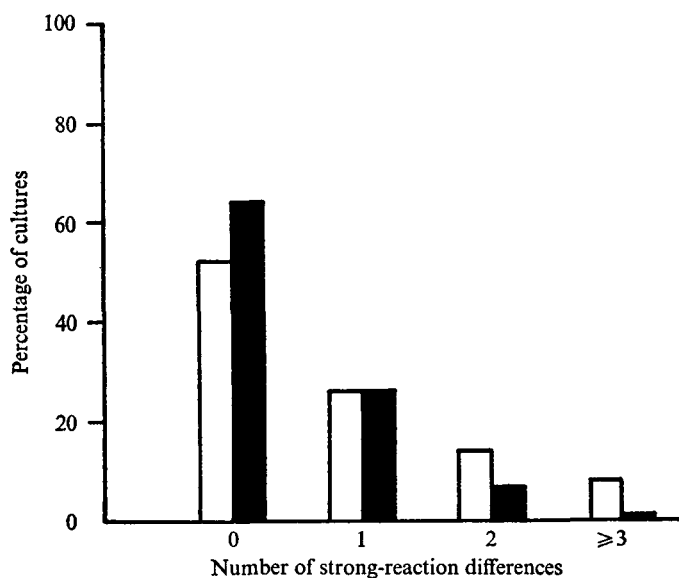
Fig. 1. Reproducibility of bacteriocine-susceptibility typing by the cross-streaking method (without mitomycin-C induction) and by the mitomycin-C induction method of Traub *et al.* (1971); percentage of 89 cultures giving the indicated number of strong-reaction differences by the cross-streaking method (open bar) and by the induction method (solid bar).

Reproducibility of the cross-streaking and the mitomycin-C induction methods of bacteriocine-sensitivity testing

The 89 cultures used for the 'chequer-board' experiment described in the previous section were typed twice, at an interval of 1 week, by (1) our cross-streaking method with the 12 selected producers and (2) by the mitomycin-C induction method of Traub *et al.* (1971) with their 10 producers. The percentages of pairs of tests that showed no, one, two, and three or more strong-reaction differences by each method were calculated (Fig. 1). Only 52% of the strains gave identical patterns of reaction when typed twice by our cross-streaking method; a further 26% showed a difference of one, 14% of two, and 8% of three or more strong reactions. The mitomycin-induction method appeared to give somewhat more reproducible results; 73% gave identical results in the two tests, but 9% of the pairs were untypable on both occasions, so the percentage giving identical patterns was 64. Of the remainder, 26% showed a difference of one reaction, 7% of two reactions and 1% of three or more reactions. Thus, to obtain an accuracy of greater than 90% in the interpretation of results, a difference in pattern of three or more strong reactions would be required in the cross-streaking method, and of two or more strong reactions in the mitomycin-induction method.

Typing of clinical strains

We had received 178 strains from eight hospitals or administratively related groups of hospitals, as follows: establishment A–59 from a variety of clinical sources in three hospitals over a period of 10 months; establishment B–17 from
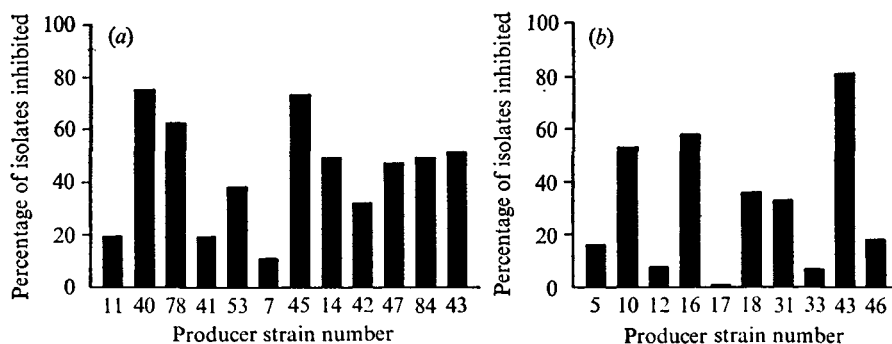
Fig. 2. Proportion of strains sensitive to the bacteriocines of each producer strain when tested by (a) the cross-streaking method (178 strains tested) and (b) the mitomycin-C induction method (137 strains tested). The producer-strain numbers in (b) are those of Traub *et al.* (1971).

Table 1. *Distribution of* O *serogroups among* Serratia marcescens *isolated from patients and related environmental sources in eight medical establishments*

O serogroup no.	Number (and percentage) of strains belonging to the stated serogroup
14	62 (35)
6	46 (26)
3	25 (14)
8	24 (13)
5	6 (3)
4	2 (1)
12	2 (1)
2	1 (1)
9	1 (1)
15	1 (1)
NT	8 (4)
Any	178 (100)

the premature baby unit and 3 from blood cultures in an unrelated ward in the same hospital; establishment C–17 from patients with infected bone flaps in a neurosurgical unit and 4 from an arterial monitor associated with these patients; establishment D–38 from the intensive-care unit and several other wards in one hospital during 9 months; establishments E, F and G (unrelated hospitals) – respectively 11, 4 and 3 from various clinical sources, each set obtained during a short period of time; and establishment H–22 from babies and environmental sources in a special-care baby unit over a period of 10 months. The available epidemiological evidence suggested that epidemics due to single strains of *Ser. marcescens* may have occurred in single wards or departments in three of the establishments (B, two incidents, C and H), but in others (establishments A, E, F and G) the evidence was less clear. In establishment A, there was good evidence that most of the infections were sporadic.

The 178 strains were all typed serologically and by our cross-streaking method; 137 of them were also typed by the mitomycin-induction method. The distribution

of the strains among O serogroups is shown in Table 1. All but 4 % were groupable, and members of 10 of the 15 recognized O serogroups were identified; of these, the most common were serogroup O14 (35 %) and O6 (26 %). On the other hand, five serogroups (O2, O4, O9, O12 and O15) were represented by one or two isolates. Serogroup O14 was responsible for three or more infections in six of the eight hospitals. The relative predominance of a few serogroups suggested that O typing alone would be insufficiently discriminating for use in epidemiological studies.

All 178 strains tested by the cross-streaking method, and 134 of 137 strains tested by the mitomycin-induction method, were typable. The frequency of sensitivity to the bacteriocines of the individual producer strains used as test reagents in the two systems is shown in Fig. 2. These results indicate that the patterns of sensitivity given by the cross-streaking method were considerably more varied than those given by the mitomycin-induction method. This might be looked upon as evidence that the former method had greater discriminatory power than the latter, but it might also be attributed to differences in the reproducibility of the two methods.

The two bacteriocine-susceptibility methods as means of subdividing O serogroups

Inspection of the bacteriocine-susceptibility patterns of the groups of isolates from individual medical establishments showed numerous instances of overlapping patterns, and it was often difficult to allocate these to clearly defined groups. The picture became much clearer however, when we considered the patterns given by members of a single O serogroup from the same establishment. Table 2 shows, for each establishment, the variability of the patterns among groups of three or more isolates of the same O serogroup, expressed as the number of strong-reaction deviations from the modal pattern. Again, the greater heterogeneity of pattern given by the cross-streaking method than by the mitomycin-induction method was apparent. However, the cross-streaking method gave much more uniform patterns in epidemiologically clear-cut incidents than in incidents composed of apparently sporadic infections in a variety of hospital departments, especially if these were spread over a long period of time.

For example, in hospital B, the 17 serotype O3 strains all came from the premature-baby unit (15 from infected infants and 2 from humidifiers); all strains by the induction method and all but one by the cross-streaking method gave patterns within one strong reaction of the mode. The three O-group 14 strains were from a different department of the hospital and were isolated from blood cultures of older patients; by both methods, two of the three strains gave identical patterns and the third showed a small deviation from the mode. In hospital C, with the 18 O-group 6 strains from the neurosurgical unit (17 from the patients and one from the tubing of an arterial monitor) all patterns were within one strong-reaction difference of the mode by the induction method, and all but two were within two strong-reaction differences by the cross-streaking method. The 22 strains from establishment H, although obtained over a long time period, were from a single hospital sub-department, and all except one had reactions within one strong reaction of the mode by the cross-streaking method. A similarly close

Table 2. *Differences in bacteriocine-sensitivity patterns, obtained by the cross-streaking method* (C) *and the mitomycin-C induction method* (I), *among members of the same* O *serogroup of* Ser. marcescens *from the same medical establishment*

Establish-ment	O sero-group	Bacteri-ocine-typing method	Number of strains	Number of strong-reaction differences from modal patterns			
				0	1	2	≥ 3
A	8	C	23	2	10	8	3
		I	23	22	0	1	0
	14	C	18	4	4	1	9
		I	18	14	3	1	0
	6	C	6	2	1	2	1
		I	6	4	0	1	1
	5	C	3	2	0	0	1
		I	3	3	0	0	0
B	3	C	17	8	8	0	1
		I	17	15	2	0	0
	14	C	3	2	1	0	0
		I	3	2	0	1	0
C	6	C	18	8	6	2	2
		I	18	17	1	0	0
	14	C	3	2	0	1	0
		I	3	3	0	0	0
D	14	C	36	15	7	7	7
		I	18	14	1	1	2
E	3	C	7	4	3	0	0
		I	7	7	0	0	0
	5	C	3	1	1	1	0
		I	3	...*	...	...	...
F	14	C	4	4	0	0	0
		I	4	3	1	0	0
G	14	C	3	3	0	0	0
		I	3	3	0	0	0
H	6	C	22	13	8	0	1
		I	0	...	...	...	...
All establish-ments		C	166	70	49	22	25
		I	123†	107	8	5	3

* Untypable.   † Three untypable strains excluded.

conformity of patterns was observed within O serogroups in establishments F and G.

On the other hand, much greater departures from the modal pattern were observed in establishment A (especially with O-serogroup 14 and 8 strains) and in the O-serogroup 14 strains from establishment D. These departures were almost all seen in the results obtained by the cross-streaking method. Establishment A comprised a London teaching hospital and two other associated hospitals in the

Table 3. *Comparison of interpretation of results obtained by the two bacteriocine-sensitivity typing methods on members of the same O serogroup isolated from the same establishment*

Results by the cross-streaking method indicating	Number of comparisons with the modal pattern by the mitomycin-C induction method indicating			
	Similarly (0–1)*		Difference (≥ 2)*	
Similarity (0–2)*	Establishment A	35	Establishment A	1
	Other establishments	66	Other establishments	1
	Total	101	Total	2
Difference (≥ 3)*	Establishment A	11	Establishment A	3
	Other establishments	3	Other establishments	3
	Total	14	Total	6

* Number of strong-reaction differences from the modal pattern.

same district. Inter-hospital transfer of patients was said to be rare. All strains of *Ser. marcescens* isolated during 10 months were collected; these came from a number of different wards and from a variety of sources, but mainly from specimens of urine. Careful retrospective investigation by Dr S. Tabaqchali (personal communication) suggested that most of the infections had been sporadic, and the largest group of clearly related infections numbered four. Less information was available about establishment D, but the isolations had been made from patients in several different wards over a number of months.

Our laboratory study of repeated typing had indicated that some allowance for variability would have to be made in interpreting bacteriocine-susceptibility patterns in epidemiological studies, and that greater latitude would have to be permitted in the cross-streaking than in the induction method. As an empirical rule, we decided to require a difference of two or more strong reactions as evidence of distinguishability by the induction method, and three or more by the cross-streaking method. Table 3 compares the interpretations that would have been placed on the results of typing by the two methods (for the 123 strains that were typed by both) in terms of departure from the modal pattern for all members of the same O group from the same establishment. The two methods gave concordant interpretations in 107 of 123 comparisons. In 16 comparisons the results obtained by the two methods were discordant, and in 14 of these the cross-streaking results suggested distinguishability. Eleven of these 14 comparisons were on strains from establishment A; it should be noted that most of the serogroup O14 strains from establishment D that showed departure from the modal pattern by the cross-streaking method had not been tested by the induction method. If we exclude establishment A from the comparisons, the same interpretation would have been placed on the results of typing by the two methods in 69 of 73 instances.

The use of a 'difference rule' in interpreting pattern reactions leads to some reduction in the discriminatory power of a typing system. We explored this further by comparing the ability of the two bacteriocine-susceptibility typing methods to distinguish members of the same O serogroup isolated from different establish-

Table 4. *Distribution of modal patterns in establishments represented by at least three cultures of serogroups O14 or O6*

| | Cross-streaking method | | | | | | | | | | | | | | Mitomycin-C induction method | | | | | | | | | | | |
| | Number examined | Number of strains of pattern | Pattern: inhibition by producer strain no. | | | | | | | | | | | | Number examined | Number of strains of pattern | Pattern: inhibition by lysate of strain no. | | | | | | | | | |
Establishment			11	40	78	41	53	7	45	14	42	47	84	43			5	10	12	16	17	18	31	33	43	46
Serogroup O14																										
A	18	9	+	+	−	−	−	−	+	+	−	+	−	−	18	17	−	+	−	+	−	−	−	−	+	−
		4	−	+	−	−	+	−	+	−	−	+	+	−												
		4	+	+	+	−	−	−	+	+	−	+	+	+												
C	3	3	−	−	+	−	−	−	−	−	+	−	+	+	3	3	−	+	−	+	−	+	+	−	+	+
D	36	29	−	+	+	−	−	−	+	+	+	−	−	+	18	15	−	+	−	+	−	+	−	−	+	+
		3	−	−	+	−	+	−	−	−	−	−	−	+												
F	4	4	+	+	+	−	−	−	+	+	−	+	−	+	4	4	−	+	−	+	−	−	−	−	+	−
G	3	3	±	+	+	−	−	−	+	±	−	−	−	+	3	3	−	+	−	+	−	−	+	+	+	−
Serogroup O6																										
A	6	5	−	+	−	−	+	−	+	+	−	+	−	−	6	4	−	±	−	+	−	−	−	−	+	−
C	18	16	−	+	+	−	+	−	+	+	+	−	+	+	18	18	−	+	−	+	−	+	+	−	+	+
H	22	21	−	+	+	+	−	−	+	+	−	+	+	+	...	...	...	...	...	...	...	...	...	...	...	...

+, Strong inhibition; ±, partial inhibition; −, no inhibition; ..., not examined.

ments, making the reasonable assumption that strains from geographically well separated establishments are probably of different immediate origin. The results, for the common serogroups O14 and O6, are shown in Table 4. In this table, we have applied the appropriate 'difference rule' to the bacteriocine-susceptibility patterns, and have grouped together patterns given by groups of three or more strains that differed from other groups of strains from the same establishment by more than the prescribed number of strong reactions. If we consider all the patterns given by members of one O serogroup, it is apparent that both bacteriocine-sensitivity typing methods have considerable potential ability to make further subdivisions, even when differences between patterns are interpreted according to a 'difference rule'; and despite the loss of discrimination that resulted from applying the rather 'permissive' three-difference rule to the cross-streaking method, it permitted the recognition of three distinct patterns among the serotype O14 strains from establishment A.

DISCUSSION

Many different bacteriocine-typing methods have been used to study the epidemiology of infection with gram-negative bacteria, such as *Shigella sonnei* (Abbott & Shannon, 1958), *Pseudomonas aeruginosa* (Wahba, 1963; Gillies & Govan, 1966; Farmer & Herman, 1969; Jones *et al*. 1974) and *Proteus* (Cradock-Watson, 1965). According to the nomenclature used in this paper, some of these are methods of bacteriocine-production typing (e.g. Abbott & Shannon, 1958) and others of bacteriocine-susceptibility typing (e.g. Farmer & Herman, 1969); in either class of method, the strains used to produce bacteriocines may or may not be treated with an inducer, such as mitomycin C.

The bacteriocines of *Ser. marcescens* were first studied by Prinsloo (1966), who demonstrated the existence of two groups of inhibitory substances. Group A bacteriocines were heat-stable, resistant to chloroform and trypsin, and active on *Ser. marcescens* and some other gram-negative bacteria. Group B bacteriocines were heat-labile, sensitive to chloroform and trypsin and, while active on *Escherichia* and other enterobacteria, were inactive against *Ser. marcescens*. In a typing method in which strains of *Serratia* inhibit the growth of other strains of the same organism, bacteriocines of group A serve as markers.

Of previously described bacteriocine-typing methods for *Serratia*, one was a 'production' (Farmer, 1972) and the other a 'susceptibility' method (Traub *et al*. 1971); both made use of induction with mitomycin C. Our first concern in attempting to develop a simple cross-streaking method was to decide whether production typing or susceptibility typing was the more appropriate. A study of mutual inhibition among 89 *Ser. marcescens* strains of diverse origins was made. Computer analysis of the results indicated that a susceptibility-typing method was preferable and selected 12 producer strains as the most useful typing reagents. A cross-streaking method of bacteriocine-susceptibility testing, technically similar to Abbott & Shannon's (1958) method of bacteriocine-production typing but used in 'reverse' form, was then developed and was compared with the susceptibility-typing method of Traub *et al*. (1971) in which the typing reagents were filtered lysates obtained after induction with mitomycin C.

7-2

It was no surprise to us that we did not obtain identical results on repeated typing by either of the bacteriocine-sensitivity methods, though Traub *et al.* (1971) had stated that their method gave completely reproducible results. Some degree of variability is an accepted feature of most phage-typing and bacteriocine-typing methods (see, for example, Williams & Rippon, 1952; Asheshov, 1974; Rampling, Whitby & Wildy, 1975). This is the main reason why it is unwise to accept un-critically that any difference in typing pattern, however small, constitutes evidence of distinguishability between strains, and to designate each recognizable pattern as a phage or bacteriocine 'type'. In practice, however, both phage- and bacterio-cine-typing methods give valuable assistance if the range and frequency of varia-tions in pattern have been previously determined by the examination of groups of strains for which there is strong epidemiological evidence of relatedness. The results of these studies can conveniently be expressed as a percentage of pairs of strains that show variability greater than a given amount; this corresponds to the per-centage of instances in which the typing method will indicate that strains are 'different' when they are not.

Williams & Rippon (1952), in a study of the phage-typing of *Staphylococcus aureus*, used a two strong-reaction differences rule that had been arrived at as follows: all variations in strength of reactions were ignored, and a strong reaction was defined as a difference between a + + and a − reaction; strains were con-sidered to be distinguishable only when two such differences were observed (see also Blair & Williams, 1961). A similar three strong-reaction differences rule has been proposed for the phage-typing of *Ps. aeruginosa* (Asheshov, 1974). Our studies of intra-laboratory variation suggested that, as a first approximation, a two strong-reaction difference rule would be appropriate for the method of Traub *et al.* (1971) and a three strong-reaction differences rule for our cross-streaking method.

We typed the strains from supposed incidents of infection in a number of hospitals or groups of related hospitals by both of the bacteriocine-sensitivity methods and obtained a number of overlapping patterns of reactions. When allowance had been made for the expected degree of variability by the application of the appropriate 'difference' rule, we had some difficulty in recognizing clear-cut groups of related strains by their bacteriocine-typing patterns alone. Assistance was therefore sought from an independent typing system for *Serratia*-O sero-grouping. Meitert & Meitert (1966) used O serogrouping for the primary classifica-tion of *Ps. aeruginosa* strains and subdivided the serological groups by phage typing. In our laboratory we have found that this hierarchical arrangement of serological and phage typing provides an admirable solution to the problem of making fine subdivisions within *Ps. aeruginosa* despite a considerable amount of variability in the phage-typing results (Asheshov, 1974).

We have not been able to evaluate fully the reproducibility of our O-serogrouping of *Ser. marcescens*, but it is apparent from other studies (Wilfert *et al.* 1970; Ewing *et al.* 1962) that epidemiologically valid results are obtainable by this method. On serogrouping 50 strains previously examined by Dr J. Washington, of the Mayo Clinic, Rochester, U.S.A., only six of the results did not conform with those originally obtained, and three of these were on strains that were ungroupable in

one or other laboratory (unpublished results). For the purpose of our study of clinical isolates, therefore, we assumed that cultures belonging to different O serogroups are different, whatever their bacteriocine-sensitivity pattern.

The bacteriocine-typing patterns of members of the same O serogroup that had been isolated in the same hospital or group of hospitals were usually identical or similar. The chief exceptions were from two establishments (A and D) in which strains of *Serratia* had been collected in a number of different hospital departments over many months. Among these, there were at least three groups of strains belonging to a single serogroup that showed considerable heterogeneity of bacteriocine susceptibility when typed by the cross-streaking method but not when typed by the mitomycin-C induction method. In one of these establishments (A), there was good epidemiological evidence that most of the infections were in fact sporadic; information about the other (D) was less adequate.

In the rest of the incidents in which comparison could be made (in establishments B, C, E, F and G), nearly all the cultures belonging to the same serogroup had identical or similar patterns when typed by either method. If we make the assumption that each group of cultures represented a single strain of *Serratia* and apply the appropriate 'difference' rule only three of 58 cultures typed by the cross-streaking method would have been considered distinguishable from others from the same incident; this suggests that the error consequent on the use of this rule was 5 % or less. The corresponding potential error for the mitomycin-C induction method was 2 % or less. Although we are here applying the 'difference' rules to departures from a modal pattern, and not to differences between pairs of patterns, as in our study of intra-laboratory variation, the results obtained appear to be of acceptable accuracy. We may therefore have some confidence in using them for the subdivision of groups of cultures that belong to the same O serogroup. There is some evidence that, when used in this way, the cross-streaking method had a somewhat greater ability to discriminate between strains than did the mitomycin-C induction method.

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