Reaction Difference Rule for Phage Typing of *Staphylococcus aureus* at 100 Times the Routine Test Dilution

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Duplicate phage typing tests of 150 isolates of methicillin-sensitive and -resistant *Staphylococcus aureus* at 100 times the routine test dilution showed that the existing reaction difference rule remains satisfactory when isolates are tested on the same day; i.e., two or more reactions must differ before isolates should be considered distinct. However, if typing is done on different days, a three-reaction-difference rule is needed.

For 50 years, phage typing has been used to investigate the relationships between isolates of *Staphylococcus aureus*, predominantly to assist with the interpretation of outbreaks of infections in hospitals. The technique was improved with the development of the Lidwell applicator (3) and the expansion of the number of phages (4), but patterns have continued to be interpreted by using the reaction difference rule of Williams and Rippon (5), namely, that one major phage reaction should be allowed before isolates from the same incident of infection can be considered distinct. However, this rule was defined for methicillin-sensitive isolates tested at the routine test dilution (RTD). Methicillin-resistant *S. aureus* (MRSA) isolates are often nontypeable at this dilution, so the *S. aureus* Reference Service at the Central Public Health Laboratory now tests all *S. aureus* isolates at 100 times the RTD. With a much stronger phage concentration, it would not be surprising to find greater variations in results. To assess this and to determine the amount of variation obtained at this concentration, some of the original experiments of Williams and Rippon (5) were repeated with representatives of currently available strains, as well as with the propagating strains of phages currently in use.

Apart from the propagating strains, 150 isolates were selected from those submitted to the Reference Service for epidemiological typing between October 1998 and February 2000 as representatives of six epidemic MRSA (EMRSA) strains and their recognized variants; strains known as “widespread sporadic” strains because they are common but are rarely associated with cross infection; strains that react with the so-called group II phages and that are highly likely to be skin associated (4); distinct strains, i.e., those with an unrecognized phage pattern so they are likely to be sporadic strains; and strains confirmed to be mecA negative (Table 1). Isolates nontypeable at 100 × the RTD were not included. When isolates with the same pattern were included, e.g., because they were representatives of the same EMRSA strain or a widespread sporadic strain, isolates from different hospitals and different times were chosen in order to minimize the possibility of epidemiological relatedness. They were maintained on nutrient agar at room temperature.

All isolates were grown in nutrient broth at 37°C overnight rather than the more conventional 4 h. This was so that the method corresponded with current Reference Service practice, introduced several years ago to cope with the rapidly increasing numbers of referrals for MRSA detection. The broths were used to flood two nutrient agar plates with additional sodium and calcium chloride (3); after the plates dried they were spotted with the 23 phages of the Basic International Set (4) at 100 × the RTD plus the four experimental phages from the United Kingdom, phages 88A, 90, 83C, and 932 (1). After overnight incubation at 30°C, the plates were read and the reactions were recorded as inhibition; confluent lysis; strong lysis; weak lysis with 1 to 5, 6 to 20, or 21 to 50 plaques; or negative (i.e., one of six categories of reaction strength plus no reaction). Inhibition reactions are those that result in no or thin growth as a result of bacterial cell death without phage infection and lysis, e.g., as a result of bacteriocin activity. They are much more susceptible to dilution effects than strong lytic reactions and therefore have the same status for interpretation purposes. Several days later, the isolates were again phage typed by producing duplicate plates on the same day, but this time after the inoculation of two broths. To minimize person-to-person variation, the same experienced individual read all plates on both occasions in a blinded manner.

The protocol described above allowed three analyses: analysis of duplicate plates from the same broth on the same day, analysis of duplicate plates from different broths on the same day, and analysis of duplicate plates from different broths on different days. The results of each comparison were recorded as showing no difference in phage result, differing only in reaction strength, differing in the loss or gain of weak or inhibition reactions, or differing in the loss or gain of one or more strong reactions (including confluent lysis). The results are shown in Table 2. It can be seen that reproducibility was very similar for duplicate plates made from both the same and different broths on the same day and that 80 to 84% of the isolates gave identical results or results that differed only in the strength of the reaction. A further 9 or 10% of the isolates showed no differences in their strong reactions, other than possibly differences in their reaction strengths, but lost or gained weak or inhibition reactions. When the same isolates...
were typed on different days, reproducibility had dropped so that only 45% were identical or differed only in the strength of the reaction. Ninety percent of the isolates were not included until two strong reaction differences were allowed. It would therefore appear that for phage typing of S. aureus at 100 × the RTD, the existing two-reaction-difference rule is still valid, provided that isolates are typed on the same day. However, if typing is done on different days, a three-reaction-difference rule should be applied; i.e., two strong phage reaction differences should be allowed before isolates are considered distinct strains.

It was notable that the isolates with large numbers of reaction differences were those with long phage patterns, so that despite the number of differences, they still had many reactions in common. When this is seen in epidemiologically related isolates typed on different days, it may therefore be prudent to either retype the isolates on the same day or check them by an alternative typing method such as pulsed-field gel electrophoresis (PFGE). The Reference Service has carried out such a policy for some time now and, as a consequence, has picked up related isolates that would have otherwise been missed.

With the advent of MRSA, epidemic strains are now common. Some of these have persisted for many years, and phage variants with significantly different patterns have been identified from these strains. Consequently, the policy of checking isolates by PFGE is applied to isolates that share some reaction patterns with the classic phage typing patterns as well as three or more differences. This has proved particularly useful with the Irish-2 strain (Table 1), which is characterized only by inhibition reactions and a single variable strong reaction (H. M. Aucken, G. Ó Neill, M. Ganner, N. Dinerstein, M. Ali, and S. Murchan, unpublished data). EMRSA-15 is another epidemic strain that does not have any characteristic strong phage reactions, being characterized by a single weak reaction, although variants have arisen, and these are identifiable by characteristic strong reactions (2). The classic phage pattern of strain 75w is, perhaps surprisingly, remarkably stable. Non-phage-typeable variants of EMRSA-15 do occur, and PFGE studies have shown that they account for about 70% of non-phage-typeable isolates reported by the Reference Service (S. Murchan, M. Carter, and H. M. Aucken, Letter, J. Hosp. Infect. 46:157–158, 2000). When these are added to those with the classic EMRSA-15 pattern, it can be shown that only 10% of EMRSA-15 isolates, in England and Wales at least, are nontypeable. Conversely, apart from those isolates that are similar to known variants, isolates which show phage reactions that are in addition to those shown by strain 75w are rarely confirmed as EMRSA-15 by PFGE (H. M. Aucken and M. Ganner, unpublished data).

In conclusion, therefore, it must be emphasized that reaction difference rules can at best be a useful guide. They are no substitute for interpretation based on experience and epidemiological data. We thank Ana New and Mark Ganner for technical expertise in phage typing and Tyrone Pitt for constructive comments.

REFERENCES