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Letter to the Editor

Epidemiological Interpretation of Chromosomal Macro-Restriction Fragment Patterns Analyzed by Pulsed-Field Gel Electrophoresis

Thal et al. (4) have recently reported data comparing the macro-restriction fragment patterns of clonal *Enterococcus faecalis* isolates differing in the chromosomal location of the conjugative transposon Tn*916*. They observed, in relation to the parental strain FA2-2, one to two restriction fragment differences resulting from a single Tn*916* insertion and two to four differences associated with two Tn*916* insertions (i.e., two different insertions in the same isolate). However, when the transconjugants were compared with each other, up to seven restriction fragment differences were noted, which the authors found problematic in light of consensus guidelines for the epidemiological interpretation of such data recently published by Tenover et al. (2). In fact, the data reported by Thal et al. (4) are entirely consistent with the consensus guidelines. In comparing macro-restriction fragment patterns in a nosocomial setting, it is important to remember that one is attempting to assess the probability that different isolates represent the patient-to-patient transfer of an organism whose chromosome may have been altered by a genetic event during the course of the outbreak. The degree to which this may be accurately determined depends in large part on when the epidemiological window of assessment opens. For example, early in the outbreak diversity is likely to be low, while later in the outbreak there is greater opportunity for random changes to occur, thus increasing the diversity of the macro-restriction patterns. As we emphasized in the consensus guidelines, the key initial step in interpreting the fragment patterns is identifying the epidemic or parental type, which is the most-common restriction fragment pattern present among the isolates. It is only then that closely related potential subtypes (differing from the epidemic type by one genetic event; usually a difference of ≤ 3 restriction fragment positions) may be accurately recognized (3). In the absence of a clear epidemic pattern, as is implied by Thal et al. (4), one may be comparing multiple subtypes with each other where, as illustrated in the consensus guidelines (Fig. 1, lanes B and C [2]), differences from the epidemic type may have an additive effect, obscuring their true relationship. Subtypes obviously have the potential to differ from each other to a greater extent than each differs from the parental type, thus making epidemiological assessment difficult (1). In other words, if the parental type is designated A and the subtypes are A1 and A2, comparing A1 directly to A2 will mask the relationship of A to A1 and A to A2. For this reason, attempting to assign epidemiologically relevant subtypes to a group of isolates (none of which exhibit identical macro-restriction patterns) is premature. With the example of Thal et al. (4) in a true nosocomial setting, a series of isolates with identical macro-restriction fragments collected within a limited time frame from different patients during a presumed nosocomial outbreak would be expected to identify *E. faecalis* FA2-2 as the parental or epidemic type. The consensus guideline protocol would then clearly categorize isolates with Tn*916* insertions as epidemiologically related to FA2-2 even though two subtypes exhibit seven restriction fragment differences from each other. Identification of the epidemic pattern (e.g., FA2-2) is where the typing algorithm begins. This information directs the rest of the protocol and should avert the problem of comparing one subtype with another rather than with the true parental type.

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Authors' Reply

Strain typing is an essential part of the epidemiologic investigation of some nosocomial infections. Pulsed-field electrophoresis (PFGE) has become the method of choice for strain delineation of various organisms. We agree with the recently published consensus guidelines for interpretation of gels (1). These guidelines make the following assumptions about isolates representing an outbreak strain. (i) They are the recent progeny of a single or common precursor; such isolates will have the same genotype. (ii) Epidemiologically unrelated strains will have different genotypes. (iii) Guidelines for interpretation of gels are similar for different genera of organisms. (iv) The parental strain type can be determined. Determination of the parental strain type may not always be possible, unless strain typing is accompanied by careful epidemiologic investigation. In an ongoing outbreak, isolates may demonstrate considerable genetic variability. It may not be possible to establish the parental strain type when small numbers of isolates are evaluated, epidemiologic information is not available on patients, or the outbreak is ongoing for a long time.

In our study (2), we found that the PFGE *Sma*I digestion patterns of two isolates that were very closely related to each other differed by seven restriction fragments. Both isolates were the same FA2-2 *E. faecalis* isolate, differing by only the Tn*916* (tetracycline resistance transposon) insertions in the chromosome. Our study is specific for *E. faecalis*. The results of our study provide information on fragment differences in PFGE patterns due to transposon insertion in the chromosome. We agree with Drs. Goering and Tenover that if these transconjugants were compared to the parental type (*E. faecalis* FA2-2) the strains would be classified as epidemiologically related. We also agree that, when possible, the parental strain

type should be determined and other isolates should be compared to this strain type. We do, however, suggest caution when using six restriction fragment differences as the basis for relatedness of strains when not all the guideline assumptions are met.

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