Interpretation of Pulsed-Field Gel Electrophoresis Patterns in Foodborne Disease Investigations and Surveillance

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ABSTRACT

Since the establishment of the well-known Tenover criteria in 1995 (Tenover et al., 1995), relatively few papers have been published about the interpretation of subtyping data generated by pulsed-field gel electrophoresis (PFGE). This paper describes the approach that has been used in the PulseNet network during the past 10 years. PFGE data must always be interpreted in the proper epidemiological context and PFGE data can not alone prove an epidemiological connection. The Tenover criteria are not generally applicable to the interpretation of PFGE subtyping data of foodborne pathogens. The reproducibility of the method with a particular organism, the quality of the PFGE gel, the variability of the organism being subtyped, and the prevalence of the pattern in question must always be considered. Only isolates displaying indistinguishable patterns should be included in the detection of clusters of infections or the initial case definition in a point-source outbreak. More variability (patterns differing from each other in two to three band positions) may be accepted if the outbreak has been going on for some time or if person-person spread is a prominent feature. If epidemiological information is sufficiently strong, isolates with markedly different PFGE patterns may be included in an outbreak.

INTRODUCTION

Subtyping is the characterization of bacteria below the species and subspecies level. It may be performed by a multitude of phenotypic and genotypic techniques. Subtyping may be done for taxonomic purposes, study of population structure of a species, phylogenetic analyses directed at determining relatedness of the target organism to other organisms or delineating steps in the evolution of the target organism, or for molecular epidemiology. Herein, we focus our attention solely on molecular epidemiologic application of subtyping.

Levin et al. (1999) defined molecular epidemiology as the identification of microparasites responsible for infectious disease and determining their physical sources, their biological relationships, and their route of transmission and those of the genes responsible for their virulence, vaccine-related antigens, and drug resistance. From a public health standpoint, the determination of the “physical source of the microparasite” that caused human illness, particularly in an outbreak setting, and understanding its route of transmission from the source to the affected person are of paramount importance. However, another aspect of the statement of Levin et al. (1999) needs to be emphasized. In considering the subtype of an organism, one must consider all available data such as biochemical reaction profiles, serotype, bacteriophage type, antimicrobial susceptibility profiles, presence and/or type of surface appendages and virulence factors; reliance on a single parameter for characterization should be avoided wherever possible.

An infectious disease outbreak is defined as
a cluster of acute illnesses caused by a pathogen that are geographically and temporally associated, and occur in excess of what is usually expected for that time and place. The smallest outbreak consists of two cases. In a few instances, where a pathogen or its toxin causes extremely severe disease that may result in death (e.g., botulism), even a single case is considered and investigated as an outbreak. Only a small proportion of enteric illnesses manifest as common source outbreaks; nevertheless, these outbreaks contribute greatly to the understanding of the transmission of the pathogens causing foodborne and diarrheal diseases, because they offer the best opportunities to identify the source of the outbreak by providing multiple case histories to compare for common exposure to a specific source or vehicle (Keene, 1999). Further, the identification of the food, water or environmental source of infection and the institution of appropriate public health remedial measures (e.g., recall of food, closing of the water source, closure of an implicated petting zoo) will result in prevention of additional exposure and disease. When the source of contamination of an outbreak is determined to be a widely distributed food, the remedial measures may have long-term implications for an entire segment of the food industry and may lead to significant changes in specific food production and processing protocols for that segment of the food industry (e.g., requirements for more thorough cooking of hamburger patties in fast-food restaurants after the 1993 Western states Escherichia coli O157:H7 outbreak). Findings from some outbreak investigations also stimulate further research on the prevalence and persistence of the pathogen in the vehicle or source of the outbreak that may lead to the development of prevention strategies that may reduce or eliminate the recurrence of similar contamination events in the future.

**BENEFITS TO PUBLIC HEALTH**

Strain identification by subtyping has many public health benefits. While patient diagnosis and treatment are seldom impacted by the additional information provided by subtyping, subtyping has a profound impact on public health surveillance, disease cluster identification, and outbreak investigations. For example, the identification of a diarrheal pathogen as *Salmonella* and determining its antimicrobial susceptibility profile provide adequate information to a physician for the treatment of the patient. However, further characterization of the clinical *Salmonella* isolate by determination of its serotype, bacteriophage type, its resistance to antimicrobial agents, and the determination of its DNA “fingerprint” are necessary and essential for public health officials to study the emergence and disappearance of specific serotypes, and for timely recognition of newly evolving strains, particularly those that may be resistant to treatment by multiple antimicrobial therapies. Further, serotype and DNA “fingerprint” data enable the detection of disease clusters, particularly those that are geographically dispersed. Subtyping also assists epidemiologists in separating outbreak-associated cases from temporally associated sporadic cases of disease caused by the same pathogen, thereby increasing the power of epidemiologic analyses. Once the epidemiologists complete their analysis of an outbreak and implicate a specific food as the cause of the outbreak, isolation of the pathogen from the implicated food and its characterization by subtyping provide independent confirmation of epidemiologic findings.

Subtyping methods may be phenotypic or genotypic and may vary in discriminating power from low to very high. Examples of the phenotyping methods are biotyping, serotyping, antimicrobial susceptibility profiles, and bacteriophage typing. Except for bacteriophage typing, the other phenotyping methods generally have low discriminating power and are used primarily as first level subtyping methods. Among the plethora of DNA-based subtyping methods, macrorestriction analysis by pulsed-field gel electrophoresis (PFGE) has become the gold standard for bacterial pathogen subtyping during the past ten years because of its broad applicability, high discriminating power, and epidemiologic concordance. The PulseNet network has demonstrated that PFGE patterns generated using highly standardized PFGE protocols and their analysis by com-
Computer-assisted pattern normalization techniques results in very high levels of reproducibility of PFGE patterns within and between laboratories (Swaminathan et al., 2001).

Despite its many advantages, the interpretation of PFGE patterns in the context of foodborne disease epidemiology is not simple and there is critical need for guidelines for interpretation of these data. The widely cited Tenover criteria (Tenover et al., 1995) were originally developed as guidelines for the investigation of nosocomial outbreaks but have been universally applied. These criteria may not be appropriate for application to highly clonal enteric pathogens such as Salmonella and E. coli. Further, point mutations were thought to be one of the major contributing factors to PFGE pattern diversity at the time the Tenover criteria were developed. However, Kudva et al. (2002) have demonstrated that the PFGE patterns diversity in E. coli O157:H7 is primarily attributable to insertions and deletions and not to point mutations. Based on our extensive experience with PFGE pattern analysis of E. coli O157:H7, Salmonella, Shigella and Listeria monocytogenes, we have developed guidelines for the interpretation of PFGE patterns of these enteric pathogens in the context of foodborne disease surveillance, disease cluster recognition and outbreak investigations. We hope that these guidelines will serve to stimulate discussion of this topic.

**INTERPRETATION OF PFGE RESULTS**

*First consideration: are observed differences real?*

Even when a carefully standardized procedure for PFGE is followed, artifacts may occur which could lead to erroneous conclusions about the relationship between profiles. It is therefore important to know the nature of these artifacts in order to recognize and correct them. Artifacts in this context are banding patterns that can not be reproduced on subsequent testing in the same or in a different laboratory. When the results of the subtyping of strains generated by a particular method are not reproducible, there are two general explanations, one relating to the subtyping procedure and the other relating to the strain in question. The latter depends on the stability of the typing method, and is simply a reflection of the fact that bacterial strains change over time. If strains change quickly enough, different subcultures of the same strain may show different subtypes (Iguchi et al., 2002). On the other hand, differences due to the subtyping procedure usually manifest as artifacts introduced by small, often unrecognized differences in the subtyping procedure. Sometimes these small differences in procedure manifest as subtle differences in band separation (e.g., one thick band versus two thinner bands), but with PFGE the most common artifact is probably incomplete restriction.

When DNA is restricted, the restriction enzyme will ideally cleave the DNA every time it meets its recognition sequence, cutting the DNA in a well-defined number of pieces of equally well defined size. If the restriction is incomplete, some DNA molecules will be completely restricted whereas others will not be cleaved at all the recognition sequences. This will result in the generation of more fragments, some with the expected size, and some larger fragments containing one or more restriction sites that were not cleaved. In a PFGE gel, this will show up as additional bands, usually at the top of the gel. However, if the incomplete restriction is little pronounced, as may be the case if a restriction site is methylated (McClelland et al., 1994), this may result in the addition of a single or a few extra bands. Bands present due to incomplete restriction are typically of a weaker intensity than their neighbors ("ghost bands"). The presence of such ghost bands should always trigger a repeated restriction and analysis of the isolate in question.

Another common error causing lack of repeatability is working with cultures that are not pure. If a culture contains more than one strain, there is obviously a risk of picking different strains when selecting single colonies for repeating subtyping of the same culture. In case of swarming bacteria such as Campylobacter, it may be difficult to obtain a pure culture or pick a single colony. In this case, the subtyping results will be completely unpredictable and repeated subtyping of such cultures will almost invariably lead to different results. Media or culture conditions preventing swarming should be used for obtaining a pure culture.
If an organism causing an outbreak is part of the normal flora of humans, animals, or food, there is always a risk of overlooking the outbreak strain in a particular specimen if multiple strains are present but only one colony is investigated. This may be the case with *Listeria monocytogenes*, which is widely distributed in the environment and in food (Tham et al., 2000, 2002); the solution to this problem is to pick multiple colonies from the primary cultures for subtyping. Organisms such as *Clostridium perfringens*, which may be either a normal commensal or cause disease if the strain produces enterotoxin, present another problem. For such organisms, only colonies producing enterotoxin should be selected for further subtyping in an outbreak setting (Lukinmaa et al., 2002).

**Tenover criteria and sources of variability in PFGE patterns**

In 1995, Tenover et al. published a paper on the interpretation of PFGE profile differences. According to the authors, profiles differing from each other in the position of up to three bands should be considered closely related and profiles differing by up to six bands should be considered possibly related. The reasoning behind this recommendation is that a single genetic event (a point mutation in a restriction site, a deletion or an insertion) would result in up to three-band differences. Thus, a three-band difference would be the result of one genetic event and a six-band difference the result of two genetic events. A point mutation in a restriction site will lead to the loss of that site, and the two original fragments with the restriction site will merge to one larger fragment, leading to a three-band difference (loss of two fragments, gain of one). Conversely, if a restriction site is gained by a point mutation, a large fragment is split into two smaller fragments, also resulting in a three-band difference. Deletions and insertions will show as a change in the size of one band leading to a two-band difference (loss of a band of one size, gain of a band of another). If the insertion/deletion contains a restriction site, additional fragments may be gained or lost relative to the pattern of the strain without the insertion/deletion.

Over the past 10 years, the Tenover criteria have proven to be useful, especially when comparing profiles of nosocomial pathogens and in suspected settings of ongoing transmission. For example, Aucken et al. (2000) compared PFGE patterns obtained with *XbaI* digestion of genomic DNA from 28 unrelated isolates of *Serratia marcescens* and 29 isolates from two outbreaks. Twenty-six of the 28 unrelated isolates had unique profiles with more than 10-band differences. Twenty-seven of the 29 outbreak isolates could be assigned to the correct outbreak based on PFGE results, with most isolates differing by less than three bands. Three isolates differed by five to seven bands.

As useful as the Tenover criteria have been in many circumstances, they are clearly not applicable to all situations. The criteria are very general and assume that all genomic fragments are visible in the gel and that the plasmid content in the strains is stable, at least for the plasmids visible in the gel. If these assumptions were always correct, all profiles would either be identical or differ from each other in the position of at least two bands (a change in band size being interpreted as loss of one band and gain of another). It is clear that the plasmid content of bacterial cells is not stable; plasmids are often gained or lost. PFGE profiles differing from each other by a single band are common, at least in foodborne pathogens. Thus, these assumptions are not always sound.

One potential cause of a one band difference is the presence of an extra plasmid in one of the strains. Plasmids may be visible in a PFGE gel, often as intense bands. This intensity may be due to the presence of multiple copies of the plasmid versus the single copy of the chromosome. If plasmids are digested by the restriction enzyme used, the resulting fragments will migrate as a function of their size in a PFGE gel, just like the chromosomal fragments. For example, in *XbaI* digested preparations of *Salmonella* Enteritidis, the 57-kb virulence plasmid is visible in the gel and strains lacking this plasmid may differ from strains harboring this plasmid by just this single band (Buchrieser et al., 1994; Powell et al., 1994). However, if the plasmids are not in linear conformation, their migration is unpredictable. Large undigested plasmids will typically move very slowly and may not be visible in a PFGE gel, but they may...
also be visible almost anywhere in the gel (Buchrieser et al., 1994; Barton et al. 1995).

Because of the variability in how plasmids may migrate, it has been suggested that fragments of a size less than 125 kb should be excluded from analysis (Olsen et al., 1994). Even this drastic measure may not solve the problem, however, as plasmids may appear in a higher size range than 125 kb, either because they actually are that large (“megaplasmids”) or because they have not been digested by the restriction enzyme used and are not in a linear conformation (Barton et al., 1995). It is well-known that plasmids, including megaplasmids, are common in foodborne pathogens (Bradbury et al., 1983; Wachsmuth et al., 1991; Olsen et al., 1994; Barton et al., 1995; Itmean et al., 1996; McLauchlin et al., 1997), but the influence of the plasmid content on the PFGE profiles of foodborne pathogens has not been well characterized.

A second cause of one-band differences in PFGE patterns is the possibility that one or more of the fragments generated by deletion, insertion, or point mutation are so small that they migrate off the gel. On the other hand, it should also be kept in mind that even changes affecting larger fragments will not always be readily apparent. Only comparatively large deletions or insertions will be detectable by PFGE. Band size differences of 1–2% (0.5–1 kb at the bottom and <10–15 kb at the top of the gel) are not detectable under most conditions. It has been shown that changes in PFGE profiles of Shiga toxin–producing E. coli O157 are caused primarily by deletions and insertions and not by point mutations in the restriction sites (Kudva et al., 2002). Little is known about the cause of changes in the PFGE profiles of other foodborne pathogens. Even if point mutations are common, the rareness of restriction sites for enzymes used in PFGE would dictate that only a tiny fraction of mutations would be expected to occur within a specific restriction site and thus change the PFGE pattern. It seems likely that in many, if not most cases, several genetic events have likely occurred before the PFGE pattern is visibly altered, and the Ten-over criteria represent a conservative estimate of the number of genetic events behind changes in PFGE profiles.

Interpreting differences in the context of organism diversity

Optimal interpretation of the differences (or lack thereof) between the PFGE patterns of two isolates depends largely on the variability of the organism being typed. Some organisms are highly clonal while others demonstrate extreme variability. Helicobacter pylori, for example, shows so much diversity that molecular subtyping is used primarily to determine if an infection is new or recurring, and contributes little to the understanding of H. pylori epidemiology (Taylor et al., 1995). Even within a genus, species or serotypes

FIG. 1. Pulsed-field gel electrophoresis (PFGE) patterns of S. Baildon (A) and S. Bareilly (B) in the PulseNet USA until 2000.
may vary greatly in genetic diversity. Dendrograms of PFGE patterns of two Salmonella serotypes from the PulseNet database are shown in Figure 1. As of 2000, S. Baildon isolates were all highly similar (Fig. 1a), while S. Bareilly isolates were highly diverse (Fig. 1b). Matching PFGE patterns for isolates of S. Bareilly would thus be more likely to indicate epidemiologic relatedness than matching patterns for S. Baildon, while minor differences between S. Baildon patterns would be more significant than the same differences in S. Bareilly patterns. Recent S. Baildon isolates have begun to show more diversity (Fig. 2), but the primary pattern still constitutes most of the database. S. Bareilly patterns continue to be highly diverse.

In addition to the diversity of subtypes of the organism being tested, one must also consider the prevalence of the particular subtype or pattern. A recent study in our laboratory found considerable diversity in S. Heidelberg PFGE patterns, but 33/60 (55%) apparently unrelated isolates had the same PFGE pattern (Kubota et al., 2000). This pattern represented about half of all S. Heidelberg isolates in our collection from each time period sampled dating back to 1985. The remaining 27 isolates were divided among 21 PFGE patterns. In the absence of epidemiologic information, one would have to conclude that isolates sharing one of the less common patterns are more likely have come from a common source than isolates sharing the predominant pattern.

Ongoing transmission versus a point source outbreak: interpreting differences in different settings

Time is also a critical factor in interpreting typing results. The shorter the duration of an outbreak, the less time the outbreak strain has to undergo mutations that change the PFGE pattern. Foodborne disease outbreaks often result from a single contamination event where all of the patients are exposed to the same contaminated food at the same time, and variability among isolates from patients is expected to be minimal. Hospital or community outbreaks, on the other hand, are frequently prolonged, with strains being passed from person to person with much more opportunity for mutational changes in PFGE patterns. An outbreak of Shigella flexneri serotype 2a in Taiwan township in 1996 illustrates both situations (Chen et al., 2003).

During the month of August, when the outbreak was identified, all outbreak-related isolates had the same PFGE pattern. By the end of December, eight variants with differences of three or fewer bands were detected. By the end of 2000, a total of 50 variations of the original pattern had been seen. A similar situation was reported during a large person-to-person outbreak of S. sonnei in Massachusetts in 1999 (Hackbarth et al., 2000). The outbreak was recognized in June, when isolates from three cases were found to have indistinguishable PFGE patterns. A second pattern had emerged by July, and by December of that year, 21 different PFGE patterns had been seen in isolates from persons who could be epidemiologically connected.

In a multistate outbreak of listeriosis in the United States in 1998, outbreak-associated isolates included variants of those with the major PFGE pattern isolated from patients and the epidemiologically implicated food product. Of 108 cases identified as part of this outbreak, 77 had L. monocytogenes with the major outbreak pattern, 29 were infected with a variant that differed from the major outbreak strain by the absence of an 80-kb Apal restriction fragment, and two were infected with a variant that differed by the absence of the 80-kb fragment and the presence of a 190-kb fragment. Epidemiologic data strongly associated all three types with the outbreak. All three types and an additional variant (containing the 190-kb fragment) were found in foods obtained from the implicated food processing facility. The investigators hypothesized that the major strain may have been
a resident of the processing plant for a duration long enough to produce the genetic variants (Graves, 2005).

In contrast to these descriptions of ongoing transmission, outbreaks with a point source and little secondary transmission are typically yield isolates with a more limited range of patterns. Two other *Shigella* outbreaks illustrate this point. The first outbreak occurred in January, 2000 and involved a contaminated food product (bean dip) (CDC, 2000). The subtyping results were typical of a foodborne (point source) outbreak. *S. sonnei* isolates from 25 persons and two foods were available for subtyping. Isolates from 21 patients and both foods were indistinguishable by PFGE, and the other isolates had highly similar PFGE patterns. A second outbreak occurred in July and August of 1998, and was due to consumption of contaminated parsley at restaurants in the United States and Canada (Naimi et al., 2003). Seventy-seven percent of the isolates had one of two closely related PFGE patterns. These outbreaks illustrate the impact of time and transmission on PFGE patterns, and emphasize the importance of the outbreak setting in interpreting PFGE results.

*Lab results do not tell the whole story*

Perhaps the most important factor to consider in interpreting PFGE results is context: how the laboratory results fit together with epidemiologic and environmental investigations. The simple fact that common PFGE patterns exist for many organisms demonstrates that indistinguishable PFGE patterns alone do not unequivocally demonstrate an epidemiologic connection. Even if PFGE patterns could prove that two isolates were the same strain, it is always possible that two people could have been infected with the same strain by different routes.

It is perhaps less obvious, but equally true that clearly differing patterns do not prove that isolates are epidemiologically unrelated. At least four distinct PFGE patterns (differing by three or more bands) were found among *E. coli* O157:H7 isolates from persons who ate at the same restaurant in Seattle over the course of a few days in 1993 (Jackson et al., 2000). A case/control study implicated salad bar items, apparently due to cross-contamination from raw beef in the restaurant kitchen. If epidemiologists had relied solely on molecular subtyping data rather than patient interviews and environmental observations of the restaurant, the source of this outbreak would likely have been unidentified.

**WATERBORNE OUTBREAKS OF PATHOGENS USUALLY ASSOCIATED WITH FOOD**

Contaminated drinking water has the propensity to cause large outbreaks of diarrheal disease (Hrudy et al., 2003). Molecular subtyping may have less utility in the early detection of some outbreaks caused by contamination of potable water supplies with diarrheal bacterial pathogens if the outbreaks have a very sharp epidemic curve resulting from large numbers of people in a geographically localized area being exposed to the infecting pathogens over a very short window of time. These outbreaks are easily detected by sharp increases in the number of patients seeking medical attention or even by the rapid depletion of over-the-counter anti-diarrheal medications in stores in a specific area (Hrudy et al., 2003; CDC, 1999; Mac Kenzie et al., 1994). Nevertheless, molecular subtyping has provided critical information for the investigation of waterborne outbreaks and for tracking their sources.

In an outbreak in Walkerton, Canada, a single *E. coli* O157:H7 strain was isolated from the case-patients and from the farm that was identified as the source of contamination. In contrast, four *Smal* PFGE types of *C. jejuni* were isolated from outbreak-associated case-patients (Clark et al., 2003). The four outbreak-associated PFGE patterns shared a common backbone and differed in the presence or absence of four DNA fragments in the middle portion of the DNA “fingerprint.” Another large dual-pathogen outbreak of waterborne disease occurred in upstate New York following a county fair in 1999. Once again, *E. coli* O157 and *C. je-
were the two pathogens that were involved. However, in this outbreak, PFGE analysis of *E. coli* O157:H7 showed that 65% of the patient isolates, and 63% of the isolates from the implicated water source shared the same PFGE profile. An additional 27% of the patient isolates were represented by a PFGE profile that differed from the major pattern by two bands. The remaining isolates were also variants of the major PFGE profile with 1–3-band differences from the major pattern (Bopp et al., 2003). These data suggest that the progenitor of the outbreak strain may have persisted on the farm (from which cow manure was suspected to have leached into the well that served as the source of water for the county fair) long enough to diverge into variants observed by PFGE typing. Changes in *E. coli* O157:H7 PFGE patterns over time have been demonstrated *in vitro* (Iguchi et al., 2002).

Interestingly, there was much less genetic diversity among the *C. jejuni* isolates (83% of the isolates had the same PFGE profile) associated with this outbreak when compared with the Walkerton, Canada outbreak. It is possible that the *C. jejuni* may have contaminated the water source from a source other than the farm because no *C. jejuni* was recovered from any of the samples taken from that farm.

A waterborne salmonellosis outbreak of *S. Bareilly* infections in 95 persons spread across 10 states highlights another aspect of interpretation of PFGE data in the context of epidemiologic information. PFGE subtyping of the *S. Bareilly* isolates from case-patients revealed numerous PFGE patterns. However, the epidemiologic investigation revealed that the infections were associated with drinking water from private wells and springs in the southeastern United States and from drinking bottled water from a bottling operation that used water from a spring in that area (Epi-Aid Report 00-61; CDC, unpublished data). Because *S. Bareilly* is an amphibian-associated serotype, investigators hypothesized that multiple *S. Bareilly* strains may have persisted in amphibian populations in and around the different water sources to confound the interpretation of the PFGE patterns associated with this outbreak.

This simplest and probably most useful way to answer this question is to consider patterns that show any discernable differences to be different patterns. This is the approach that PulseNet has chosen. In point source outbreaks (typical of most foodborne outbreaks) the vast majority of outbreak related isolates display the same profile (Mølbak et al., 1999; Naimi et al., 2003). In investigating recognized outbreaks, it is fairly easy and often useful to designate patterns that differ slightly from the primary outbreak pattern as “subpatterns” or “related patterns.” In surveillance for cluster detection, such an approach quickly becomes unwieldy and could easily be misleading. A recent multistate outbreak of *E. coli* O157 illustrates the rationale of this approach (Gerner-Smidt et al., 2005).

In the summer of 2002, a cluster of cases of *E. coli* O157 infections was detected in Colorado. It soon became evident that cases were occurring in other states. In accordance with established PulseNet protocol, only patients with isolates displaying patterns indistinguishable from the original outbreak pattern were included in the initial case interviews. Thirty-seven isolates of the outbreak pattern were uploaded to the PulseNet server during the outbreak period. During the same time period, forty-one isolates of a frequently encountered pattern that differed from the outbreak pattern by a single band were also uploaded. The case-control study pointed to ground beef from one particular manufacturer as the source of the outbreak. If the interviews had included cases infected with the related, frequently encountered pattern in the case definition, the results of the case-control study might have been less conclusive. While some of the patients infected with isolates having the common, non-outbreak pattern may have actually acquired their infection from eating meat from the implicated producer, the fact that this pattern was commonly seen before the outbreak argues that most probably did not. Including these patients in the case interviews would have made iden-
tifying the source of the outbreak much more difficult. At the same time, epidemiologic evidence supported the inclusion of two siblings as part of the outbreak, even though their isolates had the frequently encountered (non-outbreak) pattern. While it has been well documented that a strain may undergo changes during an outbreak causing minor differences in the number or position of the DNA fragments in a PFGE gel (Proctor et al., 2002), including variants in the case definition may complicate the interview studies of the outbreak, especially if one of the variants represents a common pattern. This example illustrates the complexities involved in interpretation of outbreak investigation data and underscores the importance of considering all the available evidence in assessing strain relatedness.

AVOIDING “FALSE ALARMS” IN CLUSTER DETECTION

If subtyping is to be useful for cluster detection and alerting public health authorities to potential outbreaks, there must be a balance between reporting too many clusters, many of which will prove to be “false alarms,” and failing to identify real outbreak possibilities. One of the advantages of PulseNet is that the databases are large and contain data many years of data. These data provides a historical baseline against which possible clustered cases may be compared. The cluster detection level for a common profile should be higher than for a rare or unique profile to avoid reacting to accumulations of common profiles that are to be expected to occur by chance only (“false alarms”). It also helps to identify organisms where PFGE is not sufficiently discriminatory and where alternative subtyping methods should be applied in case clusters are detected.

While cluster profiles should be compared with the full database to determine the frequency of the observed pattern, the time factor should also be considered. The dynamics of subtypes are constantly changing, and a pattern that is common in the database as a whole may not have been common for several years. Such a pattern would suggest reacting to a lower number of reports than if the pattern occurred evenly over the past several years.

RECOMMENDATIONS FOR INTERPRETING PFGE RESULTS

When any differences in PFGE patterns are observable, the patterns should be reported as different. Likewise, patterns that are indistinguishable should be reported as such. Indistinguishable is a more accurate term than “identical,” since it implies only that any differences are not observable under the conditions used. Interpretation of PFGE results, however, should include each of the following steps.

1. The gel should be of sufficient quality to be properly interpreted. A gel that includes partial digestions or other artifacts, or in which the bands are not sharp and clear, should be re-run without attempting to interpret the results. Interpreting a poor quality gel can be misleading, and can actually be worse than having no results at all.

2. Consider the diversity of the organism being tested. Large databases such as those maintained by PulseNet should provide sufficient data to make reasonable determinations of diversity. If the organism displays little diversity, one should be cautious in assuming that closely related patterns, or even indistinguishable patterns, indicate a high likelihood of a common source. PFGE using a second enzyme may be useful. Other available laboratory data such as phage type, biochemical profile, antimicrobial susceptibility profile, or multilocus sequence type should also be considered. If the organism being tested shows substantial diversity, one must still consider whether there are clonal populations within a non-clonal organism, as is the case with S. Heidelberg. If the PFGE pattern in question matches the pattern of a large clonal group, the overall diversity of the serotype is of little relevance and additional data is needed to interpret the PFGE results. On the other hand, when an organism demonstrates extreme variability, such
as that seen with *H. pylori*, any pattern matches are probably significant.

3. After establishing the diversity of the organism and whether or not there are clonal populations, one should then consider the outbreak setting. If it appears to be a point source outbreak without continued transmission, only very minor differences are likely to be observed and isolates that differ by several bands are probably not part of the outbreak (unless it is a multi-strain outbreak). When there is ongoing transmission, such as a community outbreak of shigellosis, more variability should be expected. Isolates that are temporally or geographically related, and have PFGE patterns that are more similar to patterns known to be outbreak-associated than to other patterns in the database, may be considered potentially outbreak-related and worth further laboratory study or epidemiologic investigation.

**CONCLUSION**

Bacterial subtyping has proven useful for outbreak investigations and cluster detection in so many settings over the years that its utility is seldom questioned. However, the interpretation of subtyping results can still prove problematic. It can not be overemphasized that proper interpretation can only be made in context. The reproducibility of the subtyping method, quality of the PFGE gel, variability of the organism being subtyped, and prevalence of a particular pattern are all critical factors. Perhaps the most important factor, however, is how the laboratory data fit with the epidemiologic and environmental information. PFGE results alone can not establish an epidemiologic connection between isolates. All of the available information must be considered in order to interpret subtyping results appropriately.

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