

GUEST COMMENTARY

Interpreting Chromosomal DNA Restriction Patterns Produced by Pulsed-Field Gel Electrophoresis: Criteria for Bacterial Strain Typing

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INTRODUCTION

Clinical microbiologists are often asked to determine the relatedness of a group of bacterial isolates, that is, to type them. During the last decade, traditional methods of strain typing, such as bacteriophage typing and serotyping, have been supplemented or replaced in many laboratories with newer molecular methods, such as plasmid fingerprinting (43), ribotyping (40), PCR-based methods (45), and analysis of chromosomal DNA restriction patterns by pulsed-field gel electrophoresis (PFGE) (4, 14, 27). Although bacteriophage typing is still used in a number of large reference laboratories around the world for epidemiologic studies of *Staphylococcus aureus* (36) and serotyping continues to be a useful tool for epidemiologic surveillance of *Salmonella* species (30), there is a need for a method of strain typing that can be used to type a broader array of bacterial species. At present, PFGE comes closest to satisfying that need (3, 42).

PFGE involves embedding organisms in agarose, lysing the organisms in situ, and digesting the chromosomal DNA with restriction endonucleases that cleave infrequently (14, 27). Slices of agarose containing the chromosomal DNA fragments are inserted into the wells of an agarose gel, and the restriction fragments are resolved into a pattern of discrete bands in the gel by an apparatus that switches the direction of current according to a predetermined pattern. The DNA restriction patterns of the isolates are then compared with one another to determine their relatedness. Currently, there are no standardized criteria for analyzing the fragment patterns. Consequently, different investigators viewing the same PFGE results may come to quite different conclusions as to which isolates should be designated as outbreak related and which should be designated as non-outbreak related.

This guest commentary proposes a set of guidelines for interpreting DNA restriction patterns generated by PFGE. The authors are investigators from the United States who, over the last several years, have correlated epidemiologic data from dozens of outbreaks with strain typing results produced by PFGE. These guidelines are intended to be used by clinical microbiologists in hospital laboratories to examine relatively

small sets of isolates (typically, ≤ 30) related to putative outbreaks of disease. In an effort to make PFGE more easily understood and accessible as a typing method, the use of statistical methods and equipment to digitize patterns has been avoided. Such methods may be appropriate for larger collections of isolates studied in reference laboratories, but they are neither feasible nor necessary for laboratories that will be confronted primarily with short-term outbreaks.

DEFINITIONS

The following vocabulary will be used throughout this commentary.

Isolate. Isolate is a general term for a pure culture of bacteria obtained by subculture of a single colony from a primary isolation plate, presumed to be derived from a single organism, for which no information is available aside from its genus and species.

Epidemiologically related isolates. Epidemiologically related isolates are isolates cultured from specimens collected from patients, fomites, or the environment during a discrete time frame or from a well-defined area as part of an epidemiologic investigation that suggests that the isolates may be derived from a common source.

Genetically related isolates (clones). Genetically related isolates (clones) are isolates that are indistinguishable from each other by a variety of genetic tests (e.g., PFGE, multilocus enzyme electrophoresis, or ribotyping) or that are so similar that they are presumed to be derived from a common parent. (Given the potential for cryptic genetic changes detectable only by DNA sequencing or other specific analyses, evidence for clonality is best considered relative rather than absolute [13]).

Outbreak. An outbreak is the increased incidence of an infectious disease in a specific place during a given period that is above the baseline rate for that place and time frame.

Strain. A strain is an isolate or group of isolates that can be distinguished from other isolates of the same genus and species by phenotypic characteristics or genotypic characteristics or both. A strain is a descriptive subdivision of a species.

Outbreak strain. Outbreak strains are isolates of the same species that are both epidemiologically related (e.g., by time, place, and common source of infection) and genetically related (i.e., have indistinguishable genotypes). Such isolates are pre-

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sumed to be clonally related since they have common phenotypes and genotypes and were isolated within a defined period.

Endemic strain. Endemic strains are isolates that are recovered frequently from infected patients in a particular health care setting or community and that are indistinguishable or closely related to each other by typing methods but for which no direct or epidemiologic linkage can be demonstrated. Such organisms are presumed to be clonally related, but their common origin may be more temporally distant from those of outbreak strains.

ASSUMPTIONS FOR STRAIN TYPING

The goal of strain typing studies is to provide laboratory evidence that *epidemiologically related* isolates collected during an outbreak of disease are also *genetically related* and thus represent the same strain. This information is helpful for understanding and controlling the spread of disease in both hospitals and communities. The use of strain typing results for infection control decisions is based on several assumptions: (i) isolates representing the outbreak strain are the recent progeny of a single (or common) precursor, (ii) such isolates will have the same genotype, and (iii) epidemiologically unrelated isolates will have different genotypes. By chance, some epidemiologically unrelated isolates may have similar or indistinguishable genotypes, particularly if there is limited genetic diversity within a species or subtype (6). For example, most strains of methicillin-resistant *S. aureus* are derived from a small number of ancestral clones (24, 36, 44). Thus, when methicillin-resistant *S. aureus*, which is a common nosocomial pathogen, is endemic in a hospital, it can be difficult to discern when an outbreak of methicillin-resistant *S. aureus* occurs, especially if the endemic strain is responsible.

In practice, typing is most effective as an aid to outbreak investigations when it is applied to small sets of isolates that are epidemiologically related. The isolates should be obtained from patients, fomites, and environmental sources that are related to (i) the area in which infections are occurring, (ii) the period during which the infections occurred, and (iii) a common source of infection. If possible, typing studies should be performed in a blinded fashion to reduce bias. Typing studies performed on isolates for which epidemiologic information is not and will not be available may produce misleading information. Strain typing data do not substitute for epidemiologic data. Rather, the two data sets should be developed independently but analyzed together to determine whether an outbreak has occurred.

THE INTERPRETIVE CRITERIA

To interpret the DNA fragment patterns generated by PFGE and transform them into epidemiologically useful information, the microbiologist must understand how to compare PFGE patterns and how random genetic events can alter the patterns. Ideally, the PFGE patterns of isolates representing the outbreak strain would be indistinguishable from each other and distinctly different from those of epidemiologically unrelated strains. When this occurs, the outbreak strain is easy to identify (19). More commonly, random genetic events, including point mutations and insertions and deletions of DNA, alter PFGE patterns during the course of an outbreak. While this makes interpretation of the patterns a little more challenging, knowledge of how such genetic events affect the patterns enables the microbiologist to correctly assign the pattern of each isolate to one of four categories: indistinguishable from the outbreak pattern, closely related to the outbreak pattern, pos-

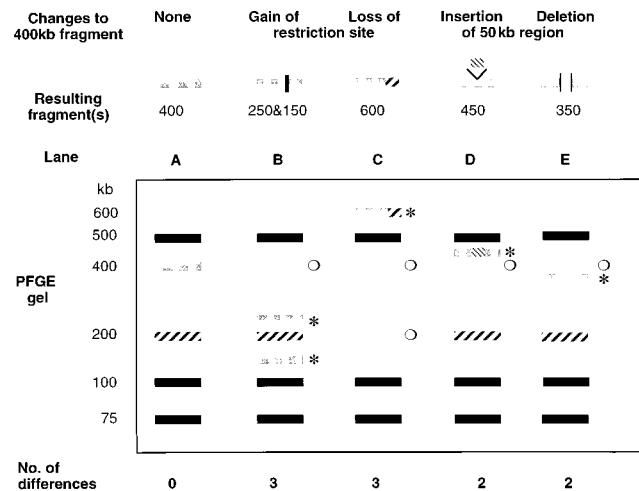


FIG. 1. Schematic diagram showing the changes in the PFGE pattern of an isolate as a result of various genetic events. Lane A, outbreak pattern; lane B, gain of a restriction site; lane C, loss of a restriction site; lane D, insertion of DNA in an existing fragment; lane E, deletion of DNA from an existing fragment. The open circles indicate fragments present in the outbreak pattern and missing from the test isolate after a genetic event; asterisks indicate fragment present after a genetic event but absent from the outbreak pattern.

sibly related to the outbreak pattern, or unrelated to the outbreak pattern. The criteria proposed herein are reliable if PFGE resolves at least 10 distinct fragments. When fewer bands are detected, the robustness and discriminatory ability of the criteria are unknown. We believe that the comparison of restriction patterns remains, in part, a subjective process that cannot be totally reduced to rigid algorithms. However, the process becomes easier and more consistent with experience. As noted earlier, although equipment to perform computer-based image acquisition and analysis is available, such equipment is not widely available in clinical laboratories, and thus, the use of such equipment has not been incorporated into these criteria.

ANALYZING THE RESTRICTION PATTERNS AND ASSIGNING THE ISOLATES TO CATEGORIES OF RELATEDNESS

First, examine the patterns to identify the common or outbreak pattern, which is presumed to represent the pattern for the outbreak strain. If there is no common pattern, then the isolates are most likely unrelated. (Among epidemiologically related isolates, the absence of a common pattern is a rare event.) After identifying the outbreak pattern, the size and number of the fragments in the outbreak pattern are compared with the fragments that make up the patterns of the other isolates. On the basis of pairwise, fragment-for-fragment comparisons, each isolate's pattern is then classified for its relatedness to the outbreak pattern. Patterns that are distinctly different from the outbreak pattern (fewer than 50% fragments in common) are considered unrelated types. Patterns that differ from the outbreak pattern by two or three fragment differences (as described below) are considered to be subtypes of the outbreak pattern. The various restriction pattern changes are illustrated in Fig. 1 and are summarized in Table 1. The criteria for interpreting PFGE patterns are summarized in Table 2 and are discussed in detail below.

TABLE 1. Effects of genetic events on PFGE fragment patterns

Type of genetic event	Resulting changes in PFGE pattern compared with the outbreak pattern
Point mutation resulting in creation of a restriction site.....	The altered pattern will lack one fragment present in the outbreak pattern and, concomitantly, will have two new smaller fragments not present in the outbreak pattern; the sum of the sizes of the two smaller fragments should approximate the size of the larger fragment. This is considered a three-fragment difference (Fig. 1, lane B).
Point mutation resulting in loss of a restriction site	The altered pattern will have a new larger fragment not present in the outbreak pattern and will lose two smaller fragments. This is a three-fragment difference (Fig. 1, lane C).
Insertion of DNA into an existing restriction fragment (new DNA does not have a restriction site).....	The altered pattern will have the same number of fragments as the outbreak pattern, but it will lack one small fragment and will show a new fragment of a larger size. This two-fragment difference is commonly referred to as a fragment shift (Fig. 1, lane D).
Deletion of DNA from a fragment (deleted material does not contain a restriction site).....	The altered pattern will show a new fragment of a smaller size and loss of a larger fragment. This is a two-fragment difference (Fig. 1, lane E).

CATEGORIES OF GENETIC AND EPIDEMIOLOGIC RELATEDNESS

Indistinguishable. Isolates are designated genetically indistinguishable if their restriction patterns have the same numbers of bands and the corresponding bands are the same apparent size (Fig. 1, lane A). The epidemiologic interpretation of these results is that the isolates are all considered to represent the same strain; i.e., isolates demonstrating the common outbreak pattern represent the outbreak strain. For many species, comparative studies indicate that isolates that are indistinguishable by PFGE are unlikely to demonstrate substantial differences by other typing techniques (17, 28, 30, 37, 39, 44).

Closely related. An isolate is considered to be closely related to the outbreak strain if its PFGE pattern differs from the outbreak pattern by changes consistent with a single genetic event, i.e., a point mutation or an insertion or deletion of DNA. Such changes typically result in two to three band differences (Fig. 1; Table 1). For example, a spontaneous mutation that creates a new chromosomal restriction site (a single genetic event) will split one restriction fragment into two smaller fragments. (The sum of the sizes of the two smaller fragments should approximate the size of the larger fragment.) The loss of the original large fragment is a one-band difference, and the appearance of two new smaller fragments represents two additional band differences; thus, there is a three-band difference between the outbreak pattern and that of the test isolate. Such an isolate is considered to be closely related

to the outbreak strain because by PFGE analysis they differ by only a single genetic event (Table 2). Variations of two to three bands have been observed in strains of some species when they are cultured repeatedly over time or isolated multiple times from the same patient (5, 34).

Possibly related. An isolate is considered to be possibly related to the outbreak strain if its PFGE pattern differs from the outbreak pattern by changes consistent with two independent genetic events (i.e., four to six band differences that can be explained by simple insertions or deletions of DNA or the gain or loss of restriction sites). While these isolates may have the same genetic lineage as that of the outbreak strain, they are not as closely related genetically and, consequently, are less likely to be related epidemiologically (Table 2). Such variation has been observed among isolates collected over longer periods (≥ 6 months) or taken from large numbers of patients involved in extended outbreaks. Isolates that are possibly related genetically but that have no epidemiologic link to the outbreak strains are likely to differ by other typing techniques, such as plasmid fingerprinting (3, 44).

Unrelated. An isolate is considered unrelated to the outbreak strain if its PFGE pattern differs from the outbreak pattern by changes consistent with three or more independent genetic events (generally seven or more band differences). Typically, this implies that $< 50\%$ of the well-resolved fragments present in the pattern from such an isolate will be present in the outbreak pattern.

Method of reporting. The DNA restriction pattern that is designated the outbreak pattern is usually reported as type A; the isolates whose restriction patterns are indistinguishable from that pattern are reported as representing the outbreak strain. Patterns that are closely or possibly related to the outbreak pattern are considered subtypes of A and are designated type A1, type A2, etc. Isolates with closely or possibly related restriction patterns are reported as probably or possibly epidemiologically related, respectively. Patterns that differ substantially from the outbreak pattern and that are classified as unrelated are designated type B, type C, etc. Isolates with unrelated patterns are considered unrelated epidemiologically.

TABLE 2. Criteria for interpreting PFGE patterns

Category	No. of genetic differences compared with outbreak strain	Typical no. of fragment differences compared with outbreak pattern	Epidemiologic interpretation
Indistinguishable	0	0	Isolate is part of the outbreak
Closely related	1	2-3	Isolate is probably part of the outbreak
Possibly related	2	4-6	Isolate is possibly part of the outbreak
Different	≥ 3	≥ 7	Isolate is not part of the outbreak

GENERAL CONSIDERATIONS WHEN USING THESE GUIDELINES

The guidelines and interpretive criteria presented here are intended for use in analyzing discrete sets of isolates obtained

TABLE 3. Representative data for bacteria analyzed by PFGE^a

Organism	Restriction enzyme	Approximate no. of restriction fragments	Fragment size range (kb)	Reference
Gram-positive organisms				
<i>Enterococcus</i> spp.	<i>Sma</i> I	15–20	5–400	27, 29
<i>Clostridium difficile</i>	<i>Sma</i> I	10–15	10–900	23
<i>Clostridium difficile</i>	<i>Sac</i> II	10–15	10–900	
<i>Clostridium perfringens</i>	<i>Sma</i> I	12	45–1,460	10
<i>Clostridium perfringens</i>	<i>Sac</i> II	10	45–1,640	
<i>Staphylococcus aureus</i>	<i>Sma</i> I	15–20	10–700	27, 47
<i>Staphylococcus aureus</i>	<i>Csp</i> I	10–15	30–500	
<i>Staphylococcus</i> spp. (coagulase negative)	<i>Sma</i> I	15–20	5–400	15, 27
<i>Streptococcus</i> spp. (group A and B)	<i>Sma</i> I	15–20	5–500	16, 26
<i>Streptococcus pneumoniae</i>	<i>Sma</i> I	10–19	20–300	25
<i>Streptococcus pneumoniae</i>	<i>Apa</i> I	10–19	20–250	
Gram-negative organisms				
<i>Acinetobacter calcoaceticus</i>	<i>Sma</i> I	20–25	5–300	1
<i>Acinetobacter baumannii</i>	<i>Sma</i> I	20–40	5–300	18
<i>Acinetobacter baumannii</i>	<i>Apa</i> I	20–30	10–300	
<i>Bacteroides</i> spp.	<i>Not</i> I	8–10	200–1,200	7
<i>Bordetella pertussis</i>	<i>Xba</i> I	20–30	20–700	12
<i>Borrelia burgdorferi</i>	<i>Sma</i> I	10–20	10–300	8
<i>Burkholderia (Pseudomonas) cepacia</i>	<i>Spe</i> I	20–25	40–700	2
<i>Campylobacter jejuni</i>	<i>Sma</i> I	8–10	40–400	41, 48
<i>Campylobacter fetus</i>	<i>Sma</i> I	10–15	40–400	32
<i>Campylobacter fetus</i>	<i>Sal</i> I	10–15	40–300	
<i>Chlamydia trachomatis</i>	<i>Sse</i> 8387I	17	9–220	9
<i>Coxiella burnetii</i>	<i>Not</i> I	19	10–293	21
<i>Enterobacter</i> spp.	<i>Xba</i> I	ca. 20	10–700	27
<i>Escherichia coli</i>	<i>Xba</i> I	ca. 20	10–500	3, 6, 20, 27
<i>Escherichia coli</i>	<i>Not</i> I	12–15	10–1,000	
<i>Escherichia coli</i>	<i>Sfi</i> I	15–20	10–700	
<i>Haemophilus influenzae</i>	<i>Sma</i> I	10–12	10–500	27
<i>Haemophilus influenzae</i>	<i>Rsr</i> II	10–12	10–500	
<i>Klebsiella</i> spp.	<i>Xba</i> I	ca. 20	10–700	27
<i>Legionella pneumophila</i>	<i>Sfi</i> I	10–15	50–700	37
<i>Legionella pneumophila</i>	<i>Not</i> I	5–10	50–2,000	22
<i>Mycobacterium</i> spp.	<i>Ase</i> I	12–20	10–700	5, 27, 46
<i>Neisseria gonorrhoeae</i>	<i>Spe</i> I	12–17	10–500	31
<i>Neisseria meningitidis</i>	<i>Not</i> I	20–30	5–200	38
<i>Neisseria meningitidis</i>	<i>Bgl</i> II	20–30	5–200	
<i>Proteus mirabilis</i>	<i>Sfi</i> I	7–10	50–700	27
<i>Proteus mirabilis</i>	<i>Not</i> I	6–10	75–700	
<i>Pseudomonas aeruginosa</i>	<i>Spe</i> I	20–25	10–700	27
<i>Pseudomonas aeruginosa</i>	<i>Xba</i> I	40–50	10–300	1
<i>Salmonella</i> spp.	<i>Not</i> I	40–50	5–400	30
<i>Shigella</i> spp.	<i>Xba</i> I	15–23	10–700	27
<i>Shigella</i> spp.	<i>Sfi</i> I	15–20	10–700	
<i>Vibrio cholerae</i>	<i>Not</i> I	20–30	10–400	11
<i>Stenotrophomonas (Xanthomonas) maltophilia</i>	<i>Xba</i> I	ca. 15	10–700	27
<i>Yersinia pestis</i>	<i>Xba</i> I	ca. 20	10–700	27

^a In some instances, restriction fragment numbers and size ranges were estimated from photographs in the published literature. Not all of the studies described here validated each organism-enzyme combination with epidemiologically related and unrelated strains. Review the literature citations for details.

during epidemiologic studies of potential outbreaks in hospitals or communities spanning relatively short periods (1 to 3 months). The criteria for strain identity are stringent and are not appropriate for studies of large populations of organisms collected over extended periods of 1 year or longer. These guidelines are based on the presumption that laboratories have limited time and resources to perform typing studies and will analyze strains by using a single restriction endonuclease. Reference laboratories investigating potential relationships among isolates collected over extended periods may need to modify these criteria to accommodate the use of multiple enzymes and analyses. Restriction enzymes that have proven useful for PFGE analysis of different bacterial species are

listed in Table 3, and the number of fragments typically resolved is indicated.

Before PFGE can be considered reliable for typing a given bacterial species, the technique must be validated by demonstrating that when restriction fragment patterns are analyzed by PFGE, epidemiologically unrelated isolates generate unique fragment patterns and epidemiologically related isolates generate indistinguishable or, on occasion, closely related fragment patterns. Discriminatory power and reproducibility are important attributes in any typing system. For most of the common bacterial pathogens, the validity of PFGE for molecular typing is well established (3).

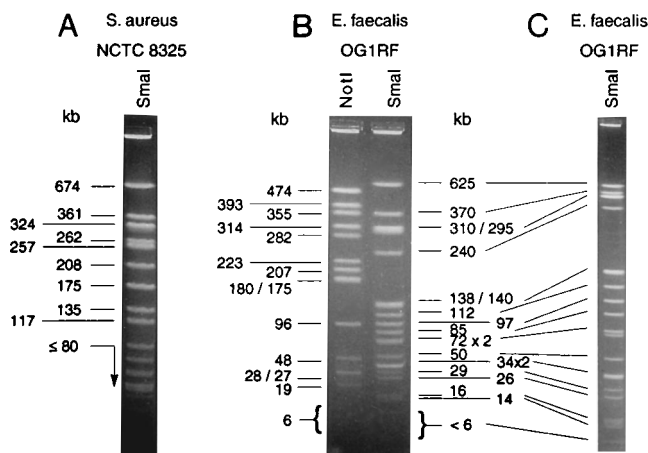


FIG. 2. PFGE patterns of chromosomal DNA restriction fragments resolved in 1.6% Seakem Gold agarose in $0.5\times$ TBE buffer (35) for *S. aureus* NCTC 8325 DNA digested with *Sma*I (pulse times, 2 to 45 s; running time, 29.5 h) (A), *E. faecalis* OG1RF DNA digested with *Not*I or *Sma*I (pulse times, 2 to 45 s; running time, 29.5 h) (B), and *E. faecalis* OG1RF DNA digested with *Sma*I (pulse time, 2 to 21 s; running time, 50 h) (C). The sizes of the fragments are indicated in kilobases.

CONTROLS

A well-characterized control strain should be processed along with the unknown isolates being tested. Obtaining expected results with the control organism affirms that (i) the procedure, including the cell lysis, washing, and endonuclease digestion steps, is working; (ii) the gel and electrophoretic conditions have been appropriate; and (iii) the conditions of the procedure have yielded results that are reproducible from run to run within the laboratory and that are consistent with those obtained by other investigators for the same strain.

A molecular size standard should be run in at least one lane of the gel to provide size orientations of the fragments. (It is often helpful to run standards in one outside lane and in a lane in the middle of the gel.) Standards are needed to evaluate minor profile differences that may result from single genetic events such as deletions, insertions, or mutations. The molecular sizes of unknown fragments can be determined by plotting the distance of migration (in millimeters from the bottom of the sample well in the gel) of the standard against the \log_{10} of the molecular size of the fragments. This plot can be used to convert the migration distance of the bands in the test samples to molecular size. In most cases, visual estimates of fragment sizes are adequate for interpretation of PFGE profile differences, and it is not necessary to calculate the sizes of the fragments for the test strains. Preparations consisting of phage lambda concatamers, referred to as a "lambda ladder," are commonly used as molecular size standards, and some vendors offer preparations that contain an enhanced 48-kb band, which is helpful for size orientation in the gel. Other preparations containing fragments of known size, such as agarose plugs containing restriction endonuclease-digested *Saccharomyces cerevisiae* DNA, are also commercially available, but they are not needed for determining the sizes of bands obtained in most PFGE strain typing protocols. The DNA restriction fragment patterns of several well-characterized organisms, in which the size of each chromosomal fragment has been determined, are shown in Fig. 2 and 3. These include patterns for *S. aureus* NCTC 8325, *Enterococcus faecium* GE1 (ATCC 51558), *Escherichia coli* MG1655 (ATCC 47076), and *Enterococcus faecalis* OG1RF (ATCC 47077). Inclusion of one of these strains in

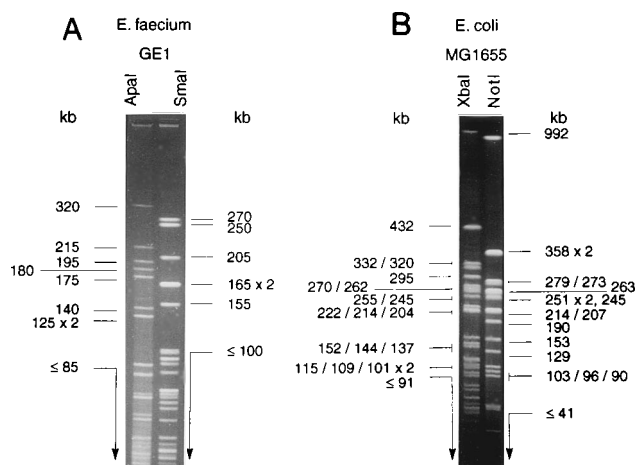


FIG. 3. PFGE patterns of chromosomal DNA restriction fragments resolved in 1.6% Seakem Gold agarose in $0.5\times$ TBE buffer (35) for *E. faecium* GE1 DNA digested with *Apa*I or *Sma*I (pulse time, 2 to 21 s; running time, 50 h) (A) and *E. coli* MG1655 DNA digested with *Xba*I or *Not*I (pulse time, 5 to 75 s; running time, 55 h) (B). The sizes of the fragments are indicated in kilobases.

each group of isolates to be tested provides both a procedure control and a molecular size standard.

When typing a set of isolates suspected of being part of an outbreak, it is helpful to include a sample of epidemiologically unrelated isolates as well to ensure that endemic strains can be differentiated from outbreak strains. This is particularly important for analyzing outbreaks of methicillin-resistant *S. aureus* in which the overall number of PFGE patterns is limited (36, 39).

SAFETY

Three areas of safety that need to be considered before applying PFGE to pathogenic bacteria are the hazards associated with propagating the bacteria, handling and disposing of chemical reagents, and electrical hazards.

Before they are embedded in agarose plugs, bacteria are grown in 1 to 10 ml of broth to the late-log or stationary phase, centrifuged, washed, and adjusted to the appropriate cell density in a buffer. Precautions generally used to prevent aerosols during these steps need to be followed, e.g., centrifugation in appropriate containers fitted with leak-proof caps. Also, precautions to prevent the release of the infectious agent by breakage of tubes, spills, and aerosolization must be used during vortex mixing of the bacteria to resuspend them in buffer (33). These precautions should be taken for all bacteria that require handling at Biosafety Level 2 (33); additional precautions may be necessary to handle bacteria that have low infectious doses (e.g., *Shigella* sp. and *Escherichia coli* O157:H7) or those known to cause laboratory-acquired infections, particularly via the respiratory route.

After the bacteria are embedded in agarose plugs, the mold used to cast the plugs should be soaked in disinfectant. After the chemical lysis step, most gram-negative bacteria embedded in agarose plugs will be inactivated; however, complete killing may not occur for gram-positive spore-forming and non-spore-forming bacteria, including *Mycobacterium tuberculosis*. For these organisms, the efficiency of the lysis procedure for killing the cells should be determined by culturing the plugs after the lysis step.

Two hazardous chemicals may be encountered when performing PFGE. Ethidium bromide, which intercalates between the bases of nucleic acids and fluoresces when it is exposed to

UV light, is widely used to stain gels to resolve the fragments for photography. It is a powerful mutagen (35). Another chemical, phenylmethylsulfonyl fluoride was used in many early PFGE protocols to inactivate proteinase K. It is very destructive to the mucous membranes of the respiratory tract, eyes, and skin and may be fatal if it is inhaled, swallowed, or absorbed through the skin (35). Recent protocols avoid the use of this reagent (27). Laboratory personnel who use these chemicals should be aware of their hazardous properties and should take appropriate precautions.

The electrophoretic separation of large DNA fragments by PFGE is done at higher voltages (5 to 10 V/cm) than those used for conventional agarose submarine gel electrophoresis of smaller DNA fragments (1 to 5 V/cm). Commercially available PFGE apparatuses usually have safety interlocks to prevent opening of the apparatus while it is still connected to the power supply. However, to maintain an even temperature throughout the gel during electrophoresis, many systems recirculate the running buffer through a cooling apparatus. Leaks in the recirculation system may expose the operator to high voltages.

SUMMARY

In summary, we hope that these guidelines will aid microbiologists in interpreting the fragment patterns resolved by PFGE and will serve as the basis for further discussions regarding the use of molecular techniques to identify and differentiate bacterial strains.

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