

An Innovative Program for Standardized Molecular Subtyping of *E. coli* 0157:H7 and Other Foodborne Pathogens by Pulsed-Field Gel Electrophoresis*



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Introduction

Molecular subtyping based on genomic DNA macrorestriction fragment-length polymorphisms using pulsed-field gel electrophoresis (PFGE) has become an indispensable tool for unraveling the epidemiology of foodborne diseases and for tracing sources of food contamination. However, much of the potential of this molecular subtyping method remains unutilized due to the lack of standardized methods and the consequent inability to compare DNA restriction fragment patterns between laboratories. There is no universal nomenclature for the RFLP patterns of each foodborne pathogen, so pattern designations used in one laboratory have little meaning elsewhere. Currently, an innovative program is in development for standardizing molecular subtyping of *E. coli* 0157:H7 and other foodborne pathogens by PFGE at the Centers for Disease Control and Prevention in Atlanta, Georgia.

Program Goals and Objectives

The overall goal of this new program is to develop standardized molecular subtyping protocols for all foodborne and diarrheal-disease-causing bacteria in a priority order based on the burden of disease caused by the pathogen, the severity of disease, the propensity of the pathogen to cause outbreaks and the estimated impact of molecular sub-typing in preventing and controlling human disease caused by the pathogen. Standard image acquisition and analysis protocols will be developed to analyze PFGE data obtained in different laboratories, facilitate inter-laboratory comparisons of PFGE patterns and establish a unified database of PFGE subtype patterns for each pathogen that will be readily accessible to public health and food regulatory laboratories. A standardized nomenclature for PFGE patterns of each pathogen will be developed for use by state and territorial health departments, CDC and the food regulatory agencies. A central computer server will then be established at the CDC to house the PFGE pattern libraries for each pathogen. The CDC will assume responsibility for maintaining and updating the server. Laboratories participating in this program will be able to access the server electronically, submit new patterns with appropriate information and retrieve information on preexisting patterns in the databases.

The Value of PFGE

In the 1980s, simple methods were developed to isolate chromosomal and extrachromosomal (plasmid) DNA, cut them into defined fragments with restriction endonucleases and separate them on the basis of their size and electrophoretic mobility by agarose gel electrophoresis. The value of these genetic typing techniques and their advantages over phenotypic typing methods were recognized immediately by the public health community. Restriction fragment-length polymorphism (RFLP) of plasmid and chromosomal DNA has become an indispensable tool for molecular epidemiology in the 1990s. Polymerase chain reaction (PCR)-based subtyping methods (random amplified polymorphic DNA analysis) are much faster and simpler but often present intra- and interlaboratory reproducibility problems that are particularly problematic when subtyping data are used to track strains over long periods and in different laboratories.

DNA macrorestriction analysis utilizes restriction enzymes that cut genomic DNA infrequently and thus generate a small number (usually 10–20) of restriction fragments. These fragments are usually too large to separate by conventional agarose gel electrophoresis. However, these fragments can be effectively resolved by pulsed-field gel electrophoresis (PFGE), which was developed in 1984 to separate yeast chromosome-sized DNAs. PFGE facilitates the differential migration of large DNA fragments through agarose gels by constantly changing the direction of the electrical field during electrophoresis. PFGE has been applied successfully to the subtyping of many pathogenic bacteria. It does not involve Southern hybridization with probes and thus is simpler to perform. Furthermore, PFGE has been repeatedly shown to be more discriminating than methods such as ribotyping for many bacteria. PFGE in the same basic format can be applied as a universal generic method for subtyping of bacteria. Only the choice of the restriction enzyme and conditions for electrophoresis need to be optimized for each species. DNA restriction patterns generated by PFGE are stable and reproducible at the intra- and interlaboratory levels. In summary, PFGE is currently the method of choice for epidemiologic subtyping of pathogenic bacteria.

Image Analysis and Databasing PFGE Patterns

To efficiently manage the information inflow, it will be necessary to standardize image acquisition and analysis protocols and establish a server computer at the CDC that will contain the *E. coli* O157:H7 PFGE pattern database. All participating laboratories will be able to acquire and analyze PFGE patterns and then electronically submit patterns to the server and query the server on whether a pattern is already in the database.

For pre-existing patterns, the participating laboratories will be able to immediately retrieve information on the frequency of any specific PFGE pattern in the *E. coli* O157:H7 PFGE pattern database, geographic distribution and source (patient, specific food types, environment, toxin type, etc.). New patterns will be held in the server in a separate location until confirmation by the Foodborne and Diarrheal Diseases Branch (FDDB). The server will be programmed to alert FDDB personnel about any unusual activity (submission of the same or highly similar patterns from one or more laboratories over a short time span, signaling a potential outbreak). This information will be available to epidemiologists and public health laboratory personnel so that prevention and intervention measures can be rapidly instituted.

Expanding the Program to Other Foodborne Diseases

Standardization of PFGE methods for other foodborne bacterial pathogens will be continued at the FDDB laboratory at an accelerated pace. Evaluation and standardization of PFGE subtyping methods for other foodborne pathogenic bacteria are planned in the following priority: *Salmonella* serotype Typhimurium, other nontyphoidal salmonellae, Enterohemorrhagic *E. coli* other than serotype O157:H7, *Shigella sonnei*, *Listeria monocytogenes*, *Campylobacter jejuni/coli*, *Clostridium botulinum*. Transfer of a standardized PFGE typing method for *Salmonella* serotype Typhimurium to area laboratories and initiation of routine PFGE subtyping of *S. Typhimurium* is the next high priority for FDDB. Because *S. Typhimurium* is usually the most frequently encountered serotype in human disease, the present serotype-based surveillance system may be missing all but large outbreaks. Routine PFGE subtyping of *S. Typhimurium* by area laboratories will facilitate the recognition of smaller outbreaks and is likely to be very valuable in helping CDC achieve its stated objective of reducing *Salmonella* infections to 16 per 100,000 by the year 2000. Eventually, the consortium of CDC/FDDB and the area laboratories will evolve into a formal body for identifying public health laboratory needs, developing, adapting and evaluating diagnostic and subtyping techniques and protocols as well as recommending adoption of collaboratively validated laboratory techniques by the public health laboratories.

* Excerpted from the CDC Manual on Standardized Molecular Subtyping of *Escherichia coli* O157:H7 by Pulsed-Field Gel Electrophoresis (1996). For additional information, contact Dr. Tim Barrett (TJB1) or Dr. Bala Swaminathan (BAS5) at CDC (E-mail: _____@CDC.GOV; PH: 404 639-3813; Fax: 404 639-3333).



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