DNA Fingerprinting: Application During Foodborne Outbreaks

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Introduction

Every year, foodborne infections in the United States are known to cause millions of illnesses and thousands of deaths. Even though most foodborne infections are self-limiting and resolve after a few days, some progress to more severe infections. These severe infections include meningitis, miscarriages, and hemolytic-uremic syndrome.

Several factors have changed the direction of foodborne diseases in the United States (1, 4, 6). In addition to the traditional outbreaks traced to food-handling error in the kitchen, diffuse and widespread outbreaks involving many counties and states are being identified and investigated. One factor contributing to this new scenario of foodborne diseases is the low-level contamination of a widely distributed food product. Since these cases may not be geographically clustered, these diffuse outbreaks are much more difficult than traditional outbreaks for the epidemiologist to investigate.

From 1993 to 1997, a total of 2,751 outbreaks of foodborne disease were reported to the Centers for Disease Control and Prevention (CDC) (3). Of these outbreaks, only 32% had a known etiology. This indicates that there is a need for improved epidemiologic and laboratory investigations.

Foodborne Outbreak Investigations

The fundamental goal of epidemiologic investigations is to understand the nature and cause of disease in populations (5). With this information strategies can be developed to control their occurrence. For foodborne investigations, these strategies may be in the areas of food production, food processing and storage, food distribution, and food preparation.

A foodborne disease outbreak may be defined as the occurrence of two or more cases of a similar illness resulting from the ingestion of a common food (3). Several steps are involved in the investigation of foodborne outbreaks. The first step would be the detection of cases. This may occur through surveillance programs that monitor pathogens or by the self-reporting of cases by consumers or clinicians. Surveillance for foodborne pathogens in the United States has been conducted for many years. State public health epidemiology offices have primary responsibility for the surveillance records. These offices share this information with the CDC.

Once the cases have been identified, the pathogen and food vehicle must be determined. If the detection of cases was by surveillance, then the pathogen is known. Otherwise, submission of stool isolates from infected persons to the laboratory is necessary. Implicated food items may be submitted for determination/confirmation of the pathogen. Unfortunately, in many cases all of the implicated food has been consumed or is otherwise unavailable.

The final step in outbreak investigations would be the comparison of the human pathogens to each other and if possible to the food organism. This phase of the investigation is completed in the laboratory by subtyping the isolates. This allows for the separation of isolates from individuals that are a part of the outbreak cluster from individuals that have unrelated infections.

Epidemiologic Subtyping Systems

Outbreak situations usually involve either person to person spread or simultaneous infection from a common source. Knowing this, isolates in a series from an epidemiologic cluster or during the course of an infection in a single patient are clonally related. Typing systems are based on the premise that clonally related isolates share characteristics that can be differentiated from unrelated isolates.

The utility of a particular characteristic for typing is related to its stability within a strain and its diversity within a species. A typing system may be categorized by its typeability (ability to obtain an unambiguous positive result for each isolate), reproducibility (ability of a technique to yield the same result when the same strain is tested repeatedly), discriminatory power (ability to differentiate related strains among unrelated strains), ease of interpretation, and ease of performance (2).

Pulsed-Field Gel Electrophoresis

Pulsed-field gel electrophoresis (PFGE) is a molecular typing technique that may be used to physically characterize strains by analyzing microbial DNA. Since its description in 1984, PFGE has been proven to be a highly effective typing technique for investigating community outbreaks, nosocomial

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outbreaks, and investigations involving contamination of foods or food products.

PFGE is a technique that is divided into three main activities: plug production, restriction and electrophoresis of the DNA, and banding pattern analysis. For plug production, a bacterial isolate is grown, cells are harvested, mixed with agarose and placed into a plug mold (this prevents shearing of the DNA molecule). The cells embedded in the agarose are subsequently lysed. Next, a small slice of agarose "plug" is removed and incubated with a restriction enzyme. The enzyme is selected based on the guanine/cytosine percentage found in the isolate's DNA. Then, the restricted DNA embedded in the agarose is loaded into an agarose gel and electrophoresed. After a designated electrophoresis time, the gel is removed and analyzed.

Analysis of the DNA banding patterns involves comparing the patterns of each isolate. If the banding patterns are the same, the isolates are indistinguishable. With supporting epidemiologic data, the isolates are the same strain. If the banding patterns are different, an assessment is made to determine just how different the patterns are from each other. Certain questions should be asked: i) can the difference be related back to one or two genetic events, ii) are the isolates part of a cluster, and iii) is there any epidemiologic association?

PulseNet

Due in part to the change in foodborne infections mentioned earlier, the CDC in collaboration with state health departments and federal food regulatory agencies are expanding and enhancing the national surveillance of foodborne pathogens. These agencies are using PFGE to strain type pathogens.

Each participating laboratory develops a DNA banding pattern bank of their isolates. When a cluster of isolates occurs, the laboratory may compare the banding patterns with the national pattern bank at CDC and other PulseNet laboratory pattern banks. This comparison may be done since the PFGE procedure has been standardized among the participating laboratories.

Currently, the organisms routinely tested by PulseNet laboratories include *Salmonella* (mainly serotype Typhimurium), *Shigella sonnei*, *Escherichia coli* O157, and *Listeria monocytogenes*.

Conclusion

Each link in the production, preparation, and delivery of food can contribute a health hazard. This makes determination of the source of an outbreak a real challenge for epidemiologists. The new dynamics of foodborne outbreaks has only made the epidemiologist's task more difficult.

PFGE is a technology that is being utilized on a local and national level to aid epidemiologists in their investigations. The implementation of PFGE by PulseNet laboratories can be used to prevent and contain widespread foodborne outbreaks. This in turn contributes to the improvement of the safety of the food supply.

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