Outbreak Investigation Video Series
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https://www.youtube.com/playlist?list=PLvgkamPnkczlPMSlXhNfqaW4KcFWyoW9q

Transcript, Video 5: Source-tracking and Pathogen Fingerprinting

Typically, source tracking and pathogen "fingerprinting" is the last step in the outbreak investigation. By now, an epidemiological investigation should have helped you narrow down the potential causes of the outbreaks, and pointed out the likely suspects. You should have unequivocally identified the pathogen of concern using a combination of culture-based, and nucleic acid-based and immunological techniques. You should have identified the same pathogen in patients and in the potential sources (food or water). Immunological techniques should have demonstrated that the patients (and even some members of the asymptomatic cohort) have been exposed to that pathogen. And even though all evidence points to the fact that the same pathogen that you recovered from a water or food sample and your patients is the same organism, the proof of that will only come from pathogen fingerprinting.

The most conclusive method for establishing that the strains of the pathogen isolated from the source and the patient are the same is to carry out whole genome sequencing of the isolates. Technological advancements made this a fairly straightforward and affordable task, however, currently it still takes at least a few weeks to carry out the sequencing and then genome assembly. In the very near future, it will likely be a much faster process. However, currently, laboratories have to rely on other technologies, such as pulse-field gel electrophoresis (PFGE) or restriction fragment length polymorphism (RFLP) to compare multiple isolates.

The aim of both PFGE and RFLP is to generate a "barcode" that will be unique to each genotype. Remember that PFGE and RFLP cannot provide information on whether the strains are related or not, but they can establish with a reasonable certainty whether the two genotypes are identical or not.

For both PFGE and RFLP, you will need pure cultures of the pathogens, free of other contaminating organisms. This will require culture-based steps. PFGE and RFLP are technically difficult for non-culturable organisms. Once you've cultured your pathogens of interest, you will extract DNA.

PFGE is a technique used by scientists to generate a DNA fingerprint for a bacterial isolate. The first step is to use molecular scissors, called restriction enzymes, to cut bacterial DNA at certain locations known as restriction sites. This will generate a collection of small DNA fragments, which are separated to generate a pattern that looks very much like a barcode. The procedure for PFGE is relatively similar to performing a standard gel electrophoresis, except that instead of constantly running the voltage in one direction, the voltage is periodically switched among three directions; one that runs through the central axis of the gel and two that run at an angle of 60 degrees either side. The pulse times are equal for each direction, resulting in a net forward migration of DNA. However, with periodic changes of the field direction, the various lengths of DNA react to the change at differing rates. Larger pieces of DNA will be slower to realign their charge when field direction is changed, while smaller pieces will do it quicker. Over the course of time with the consistent changing of directions, each band will begin to separate more and more, even at very large lengths. Thus, separation of very large DNA pieces using PFGE is made possible.

RFLP is a less preferred technique. To compare the two strains, typically entire genomic DNA will be digested with a restriction enzyme (or two) to generate a pattern of bands. These patterns could be compared to determine whether they are the same. It is reasonable to hypothesize that if two genomes generate the same banding pattern, they are likely the same, indicative of the same genotype.

Fingerprinting is the last step in the outbreak investigation, which will allow you to link pathogens you have isolated from the patients and from the potential sources of the outbreak.