

Sub-typing of isolates

Subtyping (or just typing) refers to the characterisation of bacterial, viral or parasite isolates beyond the species or subspecies level. Typing may be done by a large variety of methods and is an important tool in outbreak investigations. Typing may be used both for outbreak detection and to establish that an outbreak is taking place. For international outbreaks, typing is often essential to link cases from different countries. When an outbreak investigation is ongoing, typing is valuable both for case-definition and for establishing a link between cases and a suspected outbreak source. For international outbreaks, typing has been particularly valuable for salmonella outbreaks, but has also been used recently within Europe for shigella and STEC outbreaks (see table with examples of papers on international outbreak investigations) and can also be of importance for viral infections with Hepatitis A and norovirus. A comprehensive review of bacterial typing methods have been given by van Belkum et al.

Typing as a tool for use in international outbreaks may sometimes be challenging, primarily because different methods are in use. Furthermore, there may be several protocols in use for some of the methods, which makes comparison of results obtained in different countries difficult. Also, typing may be costly and some of the more sophisticated methods require skills or equipment that is not present in all (reference) laboratories. Even a general typing method such as PFGE may not be in use in some laboratories, or it may be too costly to apply to a large number of isolates. Faced with an international foodborne outbreak, it should therefore always be considered if it may be more rational to send isolates (or for some methods just DNA-preparations) from one country to another, rather than relying on each country performing typing on its own.

The typing methods to use will vary by organism and the exact circumstances of the outbreak, but there will often be a logical hierarchy in the methods which means that the typing efforts can be taken to different levels. As a general principle typing should only be taken as far as it is necessary for the outbreak investigation. Depending on the outbreak settings, different levels of detail in the typing may be necessary to show that different isolates are likely to be identical. It is therefore important that epidemiologists and typing experts communicate with one another and understand the limitations of the typing methods as well as the epidemiological methods in use.

Main points to consider in international outbreaks

- Agree on typing method and protocol to use, PFGE will often be the first choice for bacteria
- Use of different typing methods in different countries may not be a problem if a subset of outbreaks strains are compared using all the different methods and found to be in agreement
- The level of typing required is that which give a sufficient level of discrimination for confirmation and management of the outbreak investigation
- Discuss the potential need for sending isolates between countries to speed up the investigation
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Typing methods of choice

An overview of commonly used typing methods that may be of relevance in national or international FWD outbreak settings is shown by organism in the accompanying table.

Though the choice of typing methods may be guided by a variety of factors and will depend on the circumstances, recommendations can be given. PFGE will often be the first choice high-discriminatory typing method for bacterial agents (for salmonella see below) for the following reasons:

- PFGE may be used for most foodborne bacterial organisms, including all salmonella serotypes (although with varying efficiency).
- There is generally international agreement about the exact protocols to use.
- The method is well-established and in current use in most member state reference laboratories.
- There is an established international network of cooperation (PulseNet International) and in some countries large collections of reference profiles have been generated over the past decade.

PFGE protocols may be downloaded from the webpage of PulseNet International (pulsenetinternational.com).

Typing methods for salmonella

For salmonella, the disease agent that is most frequently the cause of international foodborne outbreaks, additional typing methods may often be used. Classical serotyping is, however, (still) a prerequisite for epidemiological investigations.

- For rare serotypes, serotyping may be sufficient to recognise an outbreak at the national level, but additional PFGE typing will often be needed (at least for a subset of case-isolates) to link outbreaks occurring in different countries.
- For the two serotypes *S. Enteritidis* and *S. Typhimurium*, additional typing methods are required, because these serotypes occur so frequently that clusters can not be recognised based on serotyping alone. Phage typing is a phenotypic typing method that has been used for both these serotypes for several decades and in a number of outbreaks has proven to be an efficient tool. However, this method also has several drawbacks, among which are that it is not in use in many countries (including the US), that several different phage typing systems exist and that it is technically difficult. Results are dependent on subjective interpretation of patterns of phage lysis; there is generally no permanent record of the pattern interpreted.
 - For *S. Typhimurium*, MLVA typing has been used successfully for several international outbreaks in recent years. It is more discriminatory than PFGE. The *S. Typhimurium* MLVA protocol which should be followed is the one by Lindstedt et al which has gained widespread acceptance in Europe.
 - For *S. Enteritidis*, PFGE typing does not add much discrimination within the serotype and therefore phage typing may in many settings be the most valuable typing method. Although several MLVA methods have been published for *S. Enteritidis*, there is currently not one validated and generally accepted MLVA protocol or other highly-discriminatory method for this serotype.

Finally, it should be said that antibiograms, which are often available as they are produced for clinical reasons, may also provide good typing information for epidemiologists, particularly if the strain in question happens to possess an unusual resistance profile.

References

- van Belkum et al.: Guidelines for the validation and application of typing methods for use in bacterial epidemiology. Clin Microbiol Infect. 2007, Suppl 3:1-46.
- Lindstedt et al.: Multiple-locus variable-number tandem-repeats analysis of Salmonella enterica subsp. enterica serovar Typhimurium using PCR multiplexing and multicolor capillary electrophoresis. J Microbiol Methods, 2004, 59:163-72.