



TYPING METHODS:

It is sometimes important to analyse multiple isolates within a given species to determine whether they represent a single strain or multiple strains. If a species of bacteria is isolated and cultivated in the laboratory it is known as a strain. A single isolate with distinctive characteristic[s] may also represent a strain.

Members of the same species that have small differences between them can be distinguished by additional methods. These species is then subdivided into subspecies, subgroups, biotypes, serotypes, variants etc.

The process of differentiating strains based on their phenotypic and genotypic differences is known as 'typing'.

These typing methods are useful in hospital infection control, epidemiological studies, and understanding the pathogenesis of infection.

In hospital settings they may be used to:

- determine whether a set of isolates obtained from one patient represents a single infecting strain or multiple contaminants.
- determine whether a series of isolates obtained over time represents relapse of an infection due to single strain or separate episodes of disease due to different strains.

Criteria for evaluating typing systems:

1. Typeability: It is the ability to obtain unambiguous positive result for each isolate analysed. Non-typeable isolates are those that give either a null or an uninterpretable result.
2. Reproducibility: It is the ability of the technique to yield the same result when same strain is tested repeatedly.
3. Discriminatory Power: It is the ability to differentiate among unrelated strains. Ideally, each unrelated isolate is detected as unique.
4. Ease of performance: To be widely useful, a typing method should be applicable to a broad range of microorganisms as well as inexpensive and technically easy.
5. Ease of interpretation: The typings tests must produce reproducible and unambiguous results that can be interpreted easily.

Types of typing methods:

1. Phenotypic techniques, those that detect characteristics expressed by the microorganism and
2. Genotypic techniques, those that involve direct DNA-based analysis of chromosomal or extrachromosomal genetic elements.

PHENOTYPIC METHODS:

Phenotypic properties are properties like shape, size, staining properties, biochemical properties, antigenic properties that can be measured without reference to the genome.

These phenotypic methods are limited by:

- Capacity of microorganisms to alter the expression of genes, which may occur spontaneously or in response to environmental stimuli. Cells, which are genetically indistinguishable, may have quite distinct phenotypes/ phenotypic properties if growing under different conditions.
- Point mutations that may not bring considerable change in genotype can result in abnormal regulation or function responsible for a particular phenotype.
- Large fractions of stains are untypeable

1. Biotyping

Biotyping makes use of the pattern of metabolic activities expressed by an isolate, colonial morphology and environmental tolerances. Strains are referred to as "biotypes". Biotyping may be performed manually or using automated systems.

Examples of biotyping methods:

- * Sugar fermentation
- * Amino acid decarboxylation/deamination
- * Standard enzymatic tests such as IMViC, Citrate, urease
- * Tolerance to pH, chemicals and dyes
- * Hydrolysis of compounds
- * Haemagglutination
- * Hemolysis

Advantages:

Most strains are typeable. The techniques are reproducible with relatively ease in performance and interpretation.

Disadvantages:

They have poor discriminatory power. Variation in gene expression is the most common reason for isolates that represent single strain to differ in one or more biochemical reactions. Point mutation too contributes to this problem.

2. Phage Typing

Strains can be characterised by their pattern of resistance or susceptibility to a standard set of bacteriophages. This relies on the presence or absence of particular receptors on the bacterial surface that are used by the virus to bind to the bacterial wall. This method is used to type isolates of *Staphylococcus aureus* and *Salmonella* spp. Such stains are referred as 'phage types'.

Advantages:

This technique has fair amount of reproducibility, discriminatory power and ease of interpretation.

Disadvantages:

This technique requires maintenance of biologically active phages and hence is available only at reference centres. Even for the experienced worker, the technique is demanding. Many strains are non-typeable.

3. Bacteriocine Typing

An isolate is assessed for susceptibility to a set of bacterial peptides (bacteriocine) produced by certain bacteria. Bacteriocines produced by a particular strain are usually only active against other strains of the same species. It has been used to type stains of *Pseudomonas aeruginosa*, *E.coli*, *Yersinia pestis* etc.

Advantages:

This technique has fair amount of reproducibility, discriminatory power and ease of interpretation.

Disadvantages:

This technique is available only at reference centres. Even for the experienced worker, the technique is demanding. Many strains are non-typeable.

4. Serotyping

Serotyping is based on fact that strains of same species can differ in the antigenic determinants expressed on the cell surface. Surface structures such as lipopolysaccharides, membrane proteins, capsular polysaccharides, flagella and fimbriae exhibit antigenic variations. Strains differentiated by antigenic differences are known as 'serotypes'. Serotyping is used in several gram negative and gram positive bacteria. Serotyping is performed using several serologic tests such as bacterial agglutination, latex agglutination, co-agglutination, fluorescent and enzyme labelling assays.

Advantages:

Most strains are typeable. They have good reproducibility and ease of interpretation though some have ease of performance.

Disadvantages:

Some autoagglutinable (rough) strains are untypeable. Some methods of serotyping are technically demanding. There is dependency on good quality reagent from commercial sources. In-house preparation of reagents is difficult process. Serotyping has poor discriminatory power due to large number of serotypes, cross-reaction of antigens and untypeable nature of some strains.

5. Antimicrobial Susceptibility Typing (Antibiogram):

This typing technique involves comparison of different isolates to a set of antibiotics. Isolates differing in their susceptibilities are considered as different strains. The identification of new or unusual pattern of antibiotic resistance among isolates cultured from multiple patients is often the first indication of an outbreak.

Advantages:

Almost all strains are typeable. The technique has ease of performance and interpretation with fair amount of reproducibility.

Disadvantages:

As a consequence of various genetic mechanisms, different strains may develop similar resistance pattern thus reducing the discriminating power. The susceptibility pattern of isolates taken over a period of time that represents the same strain may differ for one or more antibiotics due to acquisition of resistance.

6. Protein Typing

Protein typing relies on major or minor differences in the range of proteins made by different strains. Variations in the types and structures of the proteins expressed by bacteria can be detected by several methods. The proteins, glycoproteins or polysaccharides are extracted from a culture of the strain, separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and stained to compare with those of other strains. More-similar organisms display more-similar protein patterns.

In another method termed immunoblotting, the electrophoresed products are transferred to nitrocellulose membrane and then exposed to antisera raised against specific strain. The bound antibodies are then detected by enzyme-labelled anti-immunoglobulins.

These methods are currently employed for epidemiological studies of *Staphylococcus aureus* and *Clostridium difficile*.

Advantages:

Almost all strains are typeable and techniques have good reproducibility and ease of interpretation.

Disadvantages:

Since the patterns detected are very complex, comparisons among multiple strains are difficult and the interpretation becomes difficult. Methods employed are technically demanding and equipments are costly and hence are not available in all laboratories.

7. Multilocus Enzyme Electrophoresis (MLEE)

Here, the isolates are analysed for differences in the electrophoretic mobilities of a set of metabolic enzymes. Cell extracts containing soluble enzymes are electrophoresed in starch gels. Variations in the electrophoretic mobility of an enzyme, referred to as 'electromorph', typically reflect amino acid substitution that alter the charge of the protein.

Advantages:

Almost all strains can be typed. The technique has excellent reproducibility and ease of interpretation.

Disadvantages:

It is only moderately discriminatory for the epidemiological analysis of clinical isolates. It requires techniques and equipments that are not available in most laboratories.

GENOTYPIC METHODS:

These methods involve the study of the microbial DNA, the chromosome and plasmid, their composition, homology and presence or absence of specific genes. Originally performed only in research laboratories have now found their way into diagnostic laboratories. Due to their complexities and cost involved, they are however limited to few laboratories only.

1. Plasmid analysis:

The number and sizes of plasmids carried by an isolate can be determined by preparing a plasmid extract and subjecting it gel electrophoresis.

Advantages:

Most strains are typeable and have good ease of interpretation.

Disadvantages:

Reproducibility of this method suffers due to the existence of plasmid in different molecular forms such as supercoiled, nicked or linear, each of which migrate differently on electrophoresis. Since plasmids can be spontaneously lost or readily acquired, related strains can exhibit different plasmid profiles. Since certain genes are

contained in transposons that can be readily acquired or deleted, the composition of plasmid DNA can change rapidly. Clinical isolates lacking plasmids are untypeable. Those strains with one or two plasmids provide poor discriminatory powers.

2. Restriction Endonuclease Analysis (REA) of Chromosomal DNA

A restriction endonuclease enzymatically cuts DNA at a specific nucleotide recognition sequence. The number and sizes of restriction fragments are influenced by the recognition sequence of enzyme and composition of DNA. Bacterial DNA is digested with endonucleases that have relatively frequent restriction sites, thereby generating hundreds of fragments ranging from ~0.5 to 50 kb in length. Such fragments can be separated by size using agarose gel electrophoresis. The pattern stained by ethidium bromide and examined under UV light. Different strains of the same species have different REA profiles because of variations in their DNA sequences.

Advantages:

All the strains can be typed with good reproducibility.

Disadvantages:

The complex profile consists of hundreds of bands that may be unresolved or overlapping thus making comparison difficult. The pattern may consist of bands generated from digestion of plasmids too. These reduce the ease of interpretation and discriminatory power.

3. PFGE of Chromosomal DNA

This technique overcomes the limitations of REA. It is a variation of agarose gel electrophoresis in which the orientation of the electric field across the gel is changed periodically. This modification enables large fragments to be effectively separated by size.

Advantages:

All the strains can be typed with good reproducibility. Restriction profiles are easily read and interpreted.

Disadvantages:

The process takes 2-4 days and requires costly reagents and equipment besides being labour intensive.

4. Southern blot analysis of RFLPs

In contrast to REA of DNA, southern blot analyses detect only the particular restriction fragment. The DNA is digested by endonuclease, the fragments are separated by gel electrophoresis and the fragments transferred to nitrocellulose membranes. The fragments containing specific sequences are then detected by labelled DNA probes. Variations in the number and sizes of the fragments detected are referred to as restriction fragment length polymorphism (RFLP).

Ribotyping is the blotting of restriction enzyme digestion of 16s rRNA, 25s rRNA and one or more tRNAs. As the 16SrRNA is so highly conserved it is a very useful molecule for comparing relatedness of organisms over the course of evolution. Organisms are classified as separate species if their sequences show less than 98% homology and are classified as different genera if their sequences show less than 93% identity.

Advantages:

All strains carrying loci homologous to probe are typeable. They are reproducible, have good ease of interpretation.

Disadvantages:

The discriminatory power depends on the choice of probes. The process requires costly reagents and equipment besides being labour intensive.

5. Nucleotide Sequence Analysis:

Genotype information at highest precision may be determined as DNA (or RNA) nucleotide-base sequences. RNA's are often sequenced either by converting the RNAs into DNA or by sequencing the DNA gene that gives rise to the RNA. By using Polymerase Chain Reaction (PCR) to amplify a known DNA segment and automated techniques to sequence the amplified product, it is possible to compare multiple isolates.

Advantages:

This technique can apply on all strains; results are reproducible with ease in interpretation.

Disadvantages:

The process requires costly reagents and equipment besides being labour intensive.

Last edited on June 2006