



### Symposium 4.3

#### Microbial Typing – A Weapon Against Resistance

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Microbial typing is used to track the spread of infectious diseases. The need to differentiate closely related organisms is important from the perspectives of public health and individual healthcare facilities. Understanding clonality of an outbreak allows implementation of appropriate control measures and potentially, control of the outbreak (2-4, 10). Typing results are important in evaluating resistance and antimicrobial consumption data; a high frequency of resistant isolates might represent the spread of a single resistant clone independent of antibiotic pressure or selection of many different resistant isolates as a result of high consumption of antimicrobial agents, or a combination of both (1, 15). Control of one resistant bacterium can potentially have the ancillary affect of quelling dissemination of other, coexisting pathogens (5).

The ideal typing method provides easily interpretable results based on objective criteria which are reproducible both within and between laboratories, and allows differentiation of unrelated strains (11). Additionally, the ideal technique should be standardized, technically facile, rapid and cost-effective (11). In this session, commonly used molecular typing strategies will be overviewed.

Pulsed field gel electrophoresis is widely used (4), as a result of its discriminatory capacity between related and unrelated bacterial isolates, and acceptable intralaboratory reproducibility. This technique is particularly helpful at the level of the local microbiology laboratory. It involves embedding organisms in agarose, lysing organisms *in situ*, and digesting chromosomal DNA with restriction enzymes that cleave infrequently, thereby generating large restriction fragments. Slices of agarose containing chromosomal DNA fragments are inserted into wells of an agarose gel. Restriction fragments are resolved into a pattern of discrete bands on the gel by an apparatus that switches the direction of current in a predetermined “pulsed-field” pattern resulting in smaller fragments migrating faster through the gel than larger ones. DNA restriction patterns of the isolates are then compared to one another to determine their relatedness. Pulsed field gel electrophoresis analysis provides a global chromosomal overview, scanning most of the chromosome, but has only moderate sensitivity as minor genetic changes may go undetected. Published interpretative guidelines (12) are widely used, and standardized protocols have been established for some bacterial pathogens, allowing intralaboratory comparison of patterns. This technique can, however, be costly, labor intensive and time-consuming. There can be problems with ambiguous bands, variable signal intensities, background noise of the electrophoretic profile, different mobilities of high and low molecular bands, uncertainty of the genetic identity of two bands of equal size, and gel distortion (6).

Multilocus sequence typing involves sequencing nucleic acid fragments from a limited number of housekeeping genes of the organism being studied (6). It allows the construction of electronically accessible genetic databases and is less labor intensive than pulsed field gel electrophoresis. The method may provide limited discriminatory power as it only analyzes the sequences of highly conserved genes (6). It is therefore ideally suited to the investigation of bacterial phylogeny and evolution of population lineages rather than typing of strains in individual hospitals (13).

Variable-number of tandem repeat analysis (multilocus variant-repeat analysis) detects variations in short sequence repeat motifs of bacterial genomes (6). This approach may not be as discriminatory as pulsed field gel electrophoresis or multilocus sequence typing, however, it is easy to perform, rapid, and inexpensive (8). The DiversiLab System (Bacterial Barcodes, Houston, TX) has standardized repetitive element sequence-based PCR, which uses consensus PCR primers to amplify DNA sequences located between successive repetitive elements, and incorporated automated detection and analysis to ease interpretation and data manipulation (11).

Randomly amplified polymorphic DNA analysis (arbitrarily primed PCR) involves randomly amplifying segments of the target DNA by using arbitrary primers that do not have any predefined homology to the target sequence. Randomly amplified polymorphic DNA analysis surveys relatively undefined regions representing a

small percentage of the chromosome. Amplified DNA is visualized via gel electrophoresis, and patterns are compared to one another. This technique can lack reproducibility between laboratories as a result of nonstringent PCR conditions.

Ribotyping involves detection of genomic restriction fragments containing ribosomal RNA sequences, and has been commercialized as the RiboPrinter® Microbial Characterization System (Dupont Qualicon, Wilmington, DE).

Amplified fragment length polymorphism analysis (AFLP® Microbial Fingerprinting, Applied Bioscience, Foster City, CA) produces a distinctive DNA fingerprint by selective PCR amplification of restriction fragments of the entire microbial genome. The procedure includes the preparation of a template where genomic DNA is digested with two restriction enzymes ("rare cutter" and "frequent cutter"), which produce cohesive fragment ends and cut DNA with different frequencies. Following digestion, genomic restriction fragments are modified by ligation of synthetic, double-stranded oligonucleotide adapters with ends complementary to those of the restriction fragments. Thus, after the ligation step, genomic restriction fragments have termini of known sequences. Such an amplified fragment length polymorphism template is submitted to highly stringent PCR amplification with primers complementary to their targets. Amplified fragments are separated by electrophoresis and visualized by sequencing gel analysis or by the laser detection system of an automated sequencing instrument. This is a reliable and robust technique that provides excellent performance in terms of reproducibility and resolution. Amplification of DNA fragments surrounding rare restriction sites (ADSRRS fingerprinting) is a similar technique based on the digestion of total bacterial DNA with two restriction enzymes differing in cleavage frequency, ligation with two different oligonucleotide adapters, and suppression of PCR (9). Suppression of PCR allows amplification of only a limited subset of DNA fragments, as only those with two different oligonucleotides ligated at the ends of complementary DNA strands are amplified (9). Amplified DNA fragments are compared using conventional gel electrophoresis (9).

Other techniques include multilocus enzyme electrophoresis (a technique that determines relationships based on the presence and electrophoretic characteristics of a variety of enzymes), insertion sequence profiling (6), single nucleotide polymorphism detection, and genus/species-specific approaches such as spa typing (i.e., definition of the composition of repeats at the 3' end of the *Staphylococcus aureus* protein A gene, *spa*), phage typing, and SCCmec typing (7, 14).

## References

1. Bonora, M. G., M. Solbiati, E. Stepan, A. Zorzi, A. Luzzani, M. R. Catania, and R. Fontana. 2006. Emergence of linezolid resistance in the vancomycin-resistant *Enterococcus faecium* multilocus sequence typing C1 epidemic lineage. *J Clin Microbiol* 44:1153-5.
2. Chlebicki, M. P., M. L. Ling, T. H. Koh, L. Y. Hsu, B. H. Tan, K. B. How, L. H. Sng, G. C. Wang, A. Kurup, M. L. Kang, and J. G. Low. 2006. First outbreak of colonization and infection with vancomycin-resistant *Enterococcus faecium* in a tertiary care hospital in Singapore. *Infect Control Hosp Epidemiol* 27:991-3.
3. Christiansen, K. J., P. A. Tibbett, W. Beresford, J. W. Pearman, R. C. Lee, G. W. Coombs, I. D. Kay, F. G. O'Brien, S. Palladino, C. R. Douglas, P. D. Montgomery, T. Orrell, A. M. Peterson, F. P. Kosaras, J. P. Flexman, C. H. Heath, and C. A. McCullough. 2004. Eradication of a large outbreak of a single strain of vanB vancomycin-resistant *Enterococcus faecium* at a major Australian teaching hospital. *Infect Control Hosp Epidemiol* 25:384-90.
4. Chuang, V. W., D. N. Tsang, J. K. Lam, R. K. Lam, and W. H. Ng. 2005. An active surveillance study of vancomycin-resistant enterococcus in Queen Elizabeth Hospital, Hong Kong. *Hong Kong Med J* 11:463-71.
5. Donskey, C. J., A. J. Ray, C. K. Høyen, P. D. Fuldauer, D. C. Aron, A. Salvator, and R. A. Bonomo. 2003. Colonization and infection with multiple nosocomial pathogens among patients colonized with vancomycin-resistant enterococcus. *Infect Control Hosp Epidemiol* 24:242-5.
6. Garaizar, J., A. Rementeria, and S. Porwollik. 2006. DNA microarray technology: a new tool for the epidemiological typing of bacterial pathogens? *FEMS Immunol Med Microbiol* 47:178-89.
7. Ko, K. S., J. Y. Baek, J. Y. Lee, W. S. Oh, K. R. Peck, N. Lee, W. G. Lee, K. Lee, and J. H. Song. 2005. Molecular characterization of vancomycin-resistant *Enterococcus faecium* isolates from Korea. *J Clin Microbiol* 43:2303-6.
8. Koh, T. H., L. Y. Hsu, L. L. Chiu, and R. V. Lin. 2006. Emergence of epidemic clones of vancomycin-

- resistant *Enterococcus faecium* in Singapore. *J Hosp Infect* 63:234-6.
9. Krawczyk, B., K. Lewandowski, M. Bronk, A. Samet, P. Myjak, and J. Kur. 2003. Evaluation of a novel method based on amplification of DNA fragments surrounding rare restriction sites (ADSRRS fingerprinting) for typing strains of vancomycin-resistant *Enterococcus faecium*. *J Microbiol Methods* 52:341-51.
  10. Mascini, E. M., A. Troelstra, M. Beitsma, H. E. Blok, K. P. Jalink, T. E. Hopmans, A. C. Fluit, R. J. Hene, R. J. Willems, J. Verhoef, and M. J. Bonten. 2006. Genotyping and preemptive isolation to control an outbreak of vancomycin-resistant *Enterococcus faecium*. *Clin Infect Dis* 42:739-46.
  11. Pounder, J. I., C. K. Shutt, B. J. Schaecher, and G. L. Woods. 2006. Clinical evaluation of repetitive sequence-based polymerase chain reaction using the Diversi-Lab System for strain typing of vancomycin-resistant enterococci. *Diagn Microbiol Infect Dis* 54:183-7.
  12. Tenover, F. C., R. D. Arbeit, R. V. Goering, P. A. Mickelsen, B. E. Murray, D. H. Persing, and Swaminathan. 1995. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: Criteria for bacterial strain typing. *Journal of Clinical Microbiology* 33:2233-2239.
  13. Willems, R. J., J. Top, M. van Santen, D. A. Robinson, T. M. Coque, F. Baquero, H. Grundmann, and M. J. Bonten. 2005. Global spread of vancomycin-resistant *Enterococcus faecium* from distinct nosocomial genetic complex. *Emerg Infect Dis* 11:821-8.
  14. Zhang, K., J. A. McClure, S. Elsayed, T. Louie, and J. M. Conly. 2005. Novel multiplex PCR assay for characterization and concomitant subtyping of staphylococcal cassette chromosome mec types I to V in methicillin-resistant *Staphylococcus aureus*. *J Clin Microbiol* 43:5026-33.
  15. Zinn, C. S., H. Westh, and V. T. Rosdahl. 2004. An international multicenter study of antimicrobial resistance and typing of hospital *Staphylococcus aureus* isolates from 21 laboratories in 19 countries or states. *Microb Drug Resist* 10:160-8.