

Molecular Epidemiologic Typing of Microbial Pathogens

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The laboratory characterization of microbial pathogens to provide evidence for their biologic and genetic relatedness is frequently useful to epidemiologists as an aid in the investigation of infections. In many situations, species identification and antimicrobial susceptibility testing can determine whether the isolates are epidemiologically related. If a cluster of infections is caused by an organism such as *E. coli*, *S. epidermidis*, or *P. aeruginosa*, which is a frequent or universal member of the normal flora or environment, additional tests might be required to determine whether the isolates are related. Sub-species delineation or strain or subtype determination is done to determine whether different isolates give the same or different results to one or more tests. If isolates from different patients give the same result or "fingerprint," the isolates probably originated from a single clone and were transmitted from patient-to-patient from a common source or by a common mechanism [1-4]. Similarly, if the same strain or subtype of an organism is repeatedly isolated from a single patient, the organism most likely is infecting or colonizing the patient and it is unlikely to be a contaminant.

Epidemiologic typing methods include both phenotypic (traditional and protein-based) and genotypic (DNA-based) methods. Phenotypic methods such as antimicrobial susceptibility profiles, biochemical profiles, bacteriophage susceptibility patterns, multilocus enzyme electrophoresis (MLEE) profiles, and immunoblot fingerprinting have allowed investigators to describe the epidemiology of some nosocomial infections. However, the phenotypic methods discriminate poorly among strains, they frequently require labor intensive, long procedures, and they often produce variable results [1-3, 5-7]. Consequently, newer genotypic or DNA-based typing methods, which have eliminated most of these limitations, have become the preferred techniques for epidemiologic typing [1-4].

Investigators have used a variety of DNA-based methods to genotype nosocomial pathogens. All of these methods use electric fields to separate DNA--either restriction endonuclease digestion fragments, amplified DNA fragments, or whole chromosomes or plasmids--into unique patterns or "fingerprints" that are visualized by staining the DNA with ethidium bromide or by nucleic acid probe hybridization. Epidemiologically related isolates share the same DNA profile or

“fingerprint” pattern, whereas, epidemiologically unrelated isolates have distinctly different patterns.

In addition to identifying gross fingerprints, molecular methods can be adapted to identify specific genes. For example, researchers have used probe hybridization or DNA amplification techniques to detect several different genes that encode antibiotic resistance, thus, providing an antibiotic resistance genotype [8-10]. Microbiologists can use molecular detection of antibiotic resistance to calibrate conventional susceptibility tests [11]. These methods might also allow clinicians to choose antibiotic therapy that would be least likely to select resistant organisms given the isolates genetic background. Furthermore, these methods enhance the ability of the microbiologist and the infection control personnel to track the spread of specific resistance genes within and among health care facilities and communities. Antibiotic resistance genotyping alone is not highly discriminatory [12]. However, the combination of antibiotic resistance genotyping with other genotyping methods is a very powerful means of characterizing the epidemiology of antimicrobial resistance among nosocomial pathogens

DNA-based typing methods have enabled investigators to study the relationship between colonizing and infecting isolates in individual patients [13-15], distinguish contaminating from infecting strains [16], document cross-infection among hospitalized patients [17-20], and evaluate reinfection versus relapse in patients being treated for an infection [21-24]. Many DNA-based typing methods can be used to study nosocomial infections, but certain methods may be more useful or are more easily applied to some organisms than others [2,3]. Several excellent and comprehensive reviews provide more detailed information on each technique, and discuss the practical applications, strengths and weaknesses of each test [1-4].

All laboratory tests have limitations, and the genotypic typing methods are no exception to this rule. Microbiologists and infection control staff who want to use these techniques as tools for epidemiologic investigations must understand these limitations so that they do not misinterpret the test results. The DNA patterns generated by these techniques are often highly complex and quite difficult to analyze. Thus, investigators must become well versed in the basic principles of molecular biology and epidemiology and must learn the art of reading the patterns. Computer-assisted systems can help investigators compare complex banding patterns [25]; however, these systems still require the user to do considerable editing. Problems with DNA extraction and

digestion or differences in the conditions of amplification (PCR-based methods) or electrophoresis can cause variations in the final profile, further complicating the analysis of DNA banding patterns. Consequently, two or more isolates can only be compared if they were typed under identical conditions. The laboratory ideally should use one test run to assess all isolates that must be compared. Finally, the methodology, nomenclature, and reference strains have not been standardized which can impede the interpretation and comparison of results obtained by different methods or different laboratories [26]. Flexible and sophisticated computer-based analysis systems such as Dendron[®] (Solltech, Iowa City, IA) and fully automated molecular typing systems such as the RiboPrinter[®] (Qualicon, Wilmington, DE) are major steps towards standardization and quantitative analysis of molecular typing results [23, 25, 27]. In addition, groups of investigators have begun developing standards and guidelines for the use of DNA-based typing methods, but more work must be done in this area [26-28].

Molecular epidemiologic typing methods allow microbiologists and infection control staff to identify specific strains within a given species, which in turn allows the team to study the epidemiology of nosocomial pathogens and then develop effective measures to prevent their spread within hospitals. When the infection control team considers using molecular typing in an epidemiologic investigation, the members must understand that there is not one best method. Pulsed-field gel electrophoresis and certain PCR-based typing methods perform well for a wide array of nosocomial pathogens and are the molecular typing methods most frequently used to investigate nosocomial infections. Despite theoretical or actual limitations, many typing methods work quite well when used in the context of a careful epidemiologic investigation [2, 4]. In contrast, if investigators use the most powerful and sophisticated typing methods indiscriminately in the absence of sound epidemiologic data, these techniques may provide conflicting and confusing information.

REFERENCES

1. Arbeit RD. Laboratory procedures for the epidemiologic analysis of microorganisms. In: Murray PR, Baron EJ, Pfaller MA, Tenover FC, Tenover FC, eds. *Manual of Clinical Microbiology*, 6th ed. Washington, DC: American Society for Microbiology, 1995; 190-208.
2. Maslow J, Mulligan ME. Epidemiologic typing systems. *Infection Cont Hosp Epidemiol* 1996; 17:595-604.
3. Sader HS, Hollis RJ, Pfaller MA. The use of molecular techniques in the epidemiology and control of infectious diseases. *Clin Lab Med* 1995; 15:407-431.
4. Weber S, Pfaller MA, Herwaldt LA. Role of molecular epidemiology in infection control. *Infect Dis Clin N Am* 1997; 25:858-870.
5. Falkiner FR. Epidemiological typing: A user's view. *J Hosp Infect* 1988; 11(suppl A):303-309.
6. Farmer JJ. Conventional typing methods. *J Hosp Infect* 1988; 11(suppl A):309-314.
7. Herwaldt LA, Boyken LD, Pfaller MA. Biotyping of coagulase-negative staphylococci: 108 isolates from nosocomial blood stream infections. *Diagn Microbiol Infect Dis* 1990; 13:461-466.
8. Arlet G, Philippon A. PCR-based approaches for the detection of bacterial resistance. In: Erlich HA, ed. *PCR Technology: Principles and Applications for DNA Amplification*. New York: WH Freeman & Co., 1992; 665-687.
9. Persing DH, Relman DA, Tenover FC. Genotypic detection of antimicrobial resistance. In: Persing DH, ed. *PCR Protocols for Emerging Infectious Diseases*. Washington DC: American Society for Microbiology 1996; 33-57.
10. Tenover FC, Popovic T, Olsvik O. Genetic methods for detecting antibacterial resistance genes. In: Murray PR, Baron EJ, Pfaller MA, Tenover FC, Tenover FC, eds. *Manual of Clinical Microbiology*, 6th ed. Washington DC: American Society for Microbiology 1995; 1368-1378.
11. Cormican MG, Wilke WW, Barrett MS, Pfaller MA, Jones RN. Phenotypic detection of *mec A*-positive staphylococcal blood stream isolates: High accuracy of simple disk diffusion tests. *Diagn Microbiol Infect Dis* 1996; 25:107-112.
12. Tenover FC, Arbeit R, Archer G, et al. Comparison of traditional and molecular typing methods for *Staphylococcus aureus* isolates. *J Clin Microbiol* 1994; 32:407-415.

13. Swaminathan B, Matar GM. Molecular typing methods: Definitions, applications, and advantages. In: Persing DH, Smith TF, Tenover FC, White TJ, eds. *Diagnostic Molecular Microbiology: Principles and Applications*. Washington, DC: American Society for Microbiology, 1993; 26-50.
14. Reagan DR, Pfaller MA, Hollis RJ, Wenzel RP. Nosocomial candidemia: Characterization of the sequence of colonization and infection using DNA fingerprinting and a DNA probe. *J Clin Microbiol* 1990; 28:2733-2738.
15. Voss A, Hollis RJ, Pfaller MA, Wenzel RP, Doebbeling BN. Investigation of the sequence of colonization and candidemia in non-neutropenic patients. *J Clin Microbiol* 1994; 32:975-980.
16. Tenover FC. Plasmid fingerprinting: A tool for bacterial strain identification and surveillance for nosocomial and community acquired infections. *Clin Lab Med* 1985; 5:413-436.
17. Back NA, Linnemann CC, Pfaller MA, Staneck JL, Morthland V. Recurrent epidemics caused by a single strain of erythromycin-resistant Staphylococcus aureus: The importance of molecular epidemiology. *J Am Med Assoc* 1993; 270:1329-1333.
18. Boyce JM, Potter-Bynoe G, Opal SM, et al. A common-source outbreak of Staphylococcus epidermidis infections among patients undergoing cardiac surgery. *J Infect Dis* 1990; 161:493-499.
19. Bingen EH, Weber M, Dorelle J, et al. Arbitrarily primed polymerase chain reaction as a rapid method to differentiate crossed from independent Pseudomonas cepacia infections in cystic fibrosis patients. *J Clin Microbiol* 1993; 31:2589-2593.
20. Sader HS, Pignatari AC, Leme I, et al. Epidemiologic typing of multiply drug-resistant Pseudomonas aeruginosa isolated from an outbreak in an intensive care unit. *Diagn Microbiol Infect Dis* 1993; 17:13-18.
21. Arbeit RD, Slutsky A, Barber TW, et al. Genetic diversity among strains of Mycobacterium avium causing monoclonal and polyclonal bacteremia in patients with AIDS. *J Infect Dis* 1993; 167:1384-1390.
22. Maslow JN, Whittam T, Wilson RA, et al. Clonal relationship among blood stream isolates of Escherichia coli. *Infect Immun* 1995; 63:2409-2417.
23. Pfaller MA, Wendt C, Hollis RJ, et al. Comparative evaluation of an automated ribotyping system versus pulsed-field gel electrophoresis for epidemiological typing of clinical isolates of Escherichia coli and Pseudomonas aeruginosa from patients with recurrent gram-negative bacteremia. *Diagn Microbiol Infect Dis* 1996; 25:1-8.
24. Maslow JN, Mulligan ME, Arbeit RD. Molecular epidemiology: The application of contemporary techniques to typing bacteria. *Clin Infect Dis* 1993; 17:153-164.

25. Schmid J, Voss E, Soll DR. Computer-assisted methods for assessing strain relatedness in Candida albicans by fingerprinting with the moderately repetitive sequence Ca3. J Clin Microbiol 1990; 28:1236-1243.
26. Arbeit RD, Goering RV, Tenover FC, et al. How to select and interpret molecular strain typing methods for epidemiologic studies of bacterial infections: A review for health care epidemiologists. Infect Cont Hosp Epidemiol 1997; 18_426-439.
27. Struelens MJ and the Members of the European Study Group on Epidemiological Markers of the European Society for Clinical Microbiology and Infectious Diseases. Consensus guidelines for appropriate use and evaluation of microbial epidemiologic typing systems. Clin Microbiol Infect 1996; 2:2-11.
28. Tenover FC, Arbeit RD, Goering RV, et al. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: Criteria for bacterial strain typing. J Clin Microbiol 1995; 33:2233-2239.