The Application of Molecular Techniques to the Study of Hospital Infection

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Context.—Nosocomial infections represent an important cause of morbidity and mortality in hospital settings, resulting in high health care costs. The roles of an epidemic investigation are to recognize that a problem exists, to compare characteristics of affected persons with those of similar but unaffected persons (case-control study), and to identify risk factors. Integrating typing methods as part of conventional epidemiologic surveillance is cost-effective and results in a reduction in rates of nosocomial infections. During the past 10 years, there has been unprecedented progress in molecular biology and in the application of nucleic acid technology to the study of the epidemiology of human infections.

Objectives.—To summarize the available molecular tests for determination of the relatedness of microorganisms causing nosocomial infections, emphasizing the most useful applications of the tests to the study of the epidemiology of hospital-acquired infection; and to discuss the appropriate use of these tests in the prevention and control of hospital-associated infection.

Data Source.—Published English-language literature from 1980 to the present.

Conclusions.—Pulsed-field gel electrophoresis is the method of choice for strain delineation. The newest techniques include polymerase chain reaction and multilocus sequence typing, in which various housekeeping genes that are stable markers of strain identity are sequenced. Molecular techniques are broadly applicable to the study of diverse pathogens. Typing data obtained by DNA analysis should always be considered together with epidemiologic information, because only this combination will enable the most accurate epidemiologic evaluation.

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Nosocomial infections represent an important cause of morbidity and mortality in hospital settings, affecting an estimated 2 million people in the United States annually, or about 5% of hospitalized patients. They result in an estimated 88,000 deaths and $4.5 billion in health care costs annually. Although viruses, fungi, and parasites can cause nosocomial infections, bacterial agents remain the most commonly recognized cause of outbreaks of disease.

Outbreaks of hospital-acquired infection with multidrug-resistant pathogens represent a major and increasing problem in hospitalized patients. Several risk factors have been commonly cited, including the presence of underlying comorbid conditions (diabetes mellitus, renal failure, and malignancies), long hospitalizations, the receipt of prior antimicrobial therapy, and the use of indwelling catheters. The major antimicrobial resistance problems have been in nosocomial gram-positive pathogens and include vancomycin resistance in enterococci, methicillin-resistant *Staphylococcus aureus*, and most recently, glycopeptide-resistant *S aureus*. Among gram-negative bacilli, extended-spectrum beta-lactamase-producing strains of *Escherichia coli* and *Klebsiella pneumoniae*, and fluoroquinolone resistance in *Pseudomonas aeruginosa* and *E coli* have been of most concern.

Strain delineation is essential in establishing the epidemiologic characteristics of nosocomial infections and in designing rational control methods. The role of strain typing methods is to determine whether epidemiologically related isolates are also genetically related, representing the same strain. Historically, the epidemiologic analysis of nosocomial infections has relied on a comparison of phenotypic characteristics such as biotype, serotype, various bacteriophages or bacteriocins, and susceptibility to antimicrobial agents. During the past 2 decades, new technologies based on molecular DNA analysis have been developed in an effort to obtain a more fundamental assessment of strain interrelationships. Establishing clonality of isolates prevents cross-transmission of infection, may disclose the source (environmental or personnel), delineates infecting from noninfecting strains, and distinguishes relapse from reinfection.

INVESTIGATING NOSOCOMIAL INFECTIONS

Endemic infections are defined as sporadic infections that constitute the background rate of infection at an institution. Most nosocomial infections are endemic and are...
the focus of most infection control efforts. Epidemic infections are defined as the occurrence of infection at a rate that is statistically significantly higher than the background rate of such infections. Epidemiologic investigation is usually triggered by an increase in the prevalence of infection due to particular species, by clusters of patients, or by identification of an isolate that has a distinctive susceptibility pattern. The key factors in an epidemic investigation are to recognize that a problem exists, to establish a case-control definition, to confirm cases, and to complete the case finding. The background rate of disease is established, comparing the attack rate with the endemic rate of infection. The clinical entity is verified to exclude the possibility of a pseudoepidemic. The laboratory is the early warning system for epidemiologic surveillance. In a basic investigation, critical data and specimens are collected, empiric control measures are initiated, affected persons are identified, and characteristics of the representative case patients are reviewed in relation to time, person, and place. Characteristics of affected persons are compared with those of a similar but unaffected population by case-control analysis. The outbreak is then characterized, using selective molecular comparison of strains to assist in the clinical epidemiologic assessment. A hypothesis about causation is then generated about epidemiologic characteristics, including mode of spread, reservoirs and vectors, and specific control measures initiated. Ongoing surveillance is conducted to establish the effectiveness of control measures. Strain typing should always be performed in combination with clinical epidemiologic assessment.

Cost-effective Application of Typing Methods

Integrating strain typing with conventional hospital epidemiologic surveillance is cost-effective because of its role in nosocomial infection reduction. The laboratory can make essential contributions through epidemiologic typing by collaborating with the infection control department during outbreak investigations. Molecular techniques for establishing the presence or absence of clonality can be effective in tracking the spread of infections due to genetically related pathogens. The use of molecular tests is essential in some circumstances in establishing the epidemiologic characteristics, information which may be medically and economically beneficial in reducing nosocomial infections.

The importance of molecular testing in epidemic investigation of hospital pathogens is well established. Recent information also suggests that the use of an integrated laboratory assessment of drug-resistant pathogens can have an effect on endemic rates of infection and can provide cost savings. Northwestern Memorial Hospital in Chicago, III, instituted in-house molecular typing to rapidly assess microbial clonality and integrated this typing into its infection control program. Data on nosocomial infections were collected during a 24-month period before implementing and 60 months after implementing the new program. During the intervention period, infections per 1000 patient-days fell by 13%, and the percentage of hospitalized patients with nosocomial infections decreased by 23%. The rate of infection fell to 43% below the national rate. Approximately 50 deaths were avoided during a 5-year period. The typing laboratory costs for the program were $400 000 per year, with a savings of $5 for each dollar spent in relation to nosocomial infection reduction. In that study, and in studies by Moreno et al4 and by Miranda et al4 related to vancomycin resistance in enterococci, methicillin-resistant S. aureus, and multidrug-resistant Klebsiella and Serratia species, cost savings using the molecular tests for endemic nosocomial infections were accomplished by a combination of establishing the clonality of isolates so that early intervention could be accomplished and by determining the unrelatedness of isolates, thereby avoiding unneeded and costly epidemic investigation. Cost reduction was also accomplished by earlier recognition of person-to-person spread of isolates compared with traditional surveillance and by molecular testing to establish the presence of pseudoepidemics, also avoiding further epidemic investigation. During a 1-year study performed by Peterson and Noskin,2 the evaluation of methicillin-resistant S. aureus alone by early molecular typing avoided 5 outbreak investigations (a $26 500 savings) and identified small clusters of skin and soft tissue infection (a $11 000 savings) and pneumonia (a $6000 savings), for a total savings exceeding the cost of the laboratory program. Centers for Disease Control and Prevention investigators5 evaluated the costs and benefits of the PulseNet molecular subtyping-based surveillance system, using as an example investigation by the Colorado state public health laboratories of the 1997 E.coli O157:H7 outbreak in which contaminated frozen hamburger patties were implicated. If 15 cases were averted by the recall of potentially contaminated ground meat, the PulseNet system in Colorado would have recovered all of the start-up costs and the costs for 5 years of operation. These authors point out that the system becomes even more cost-effective if one takes into account the resources that would be wasted to investigate apparent increases in sporadic cases of E.coli O157:H7 infections. Additional opportunities for the use of molecular testing in the early detection of nosocomial drug-resistant pathogens will soon be available. Stosor et al6 demonstrated the capacity for rapid sensitive detection of vancomycin resistance in enterococci contained in rectal swabs from colonized patients. These researchers reported that the cost of rapid detection using polymerase chain reaction (PCR) was equal to the cost of 1 day of isolation precautions. Similar rapid tests for detection of resistant staphylococci by PCR or by fluorescence in situ hybridization and other molecular-based assays are under development and are clinically used in some hospitals. The incorporation of microbial genetic typing is within recommended guidelines for essential activities of infection control in hospitals.7 It is medically useful and is economically justified.

Characteristics of Typing Methods

Typing systems should be standardized, sensitive, specific, based on clear objectives, and critically appraised. All typing systems can be characterized in terms of typeability, reproducibility, discriminatory power, ease of performance, and facility of interpretation. Typeability refers to the ability of a technique to assign an unambiguous result (type) to each isolate. Nontypeable isolates are more common with phenotypic methods, but they have been recognized with all methods. Reproducibility of a method refers to its property to yield the same result on repeat testing of a bacterial strain. Poor reproducibility may reflect technical variation in the method or biologic variation during in vivo or in vitro passage of the organisms to be examined. The discriminatory power of a technique refers...
to its ability to differentiate among epidemiologically unrelated isolates, ideally assigning each to a different type. Phenotypic methods have lower discriminatory power compared with molecular methods. Most molecular methods require costly material and equipment but are easy to learn and are applicable to a variety of species. Phenotypic methods also involve costs in labor and material but are restricted to use with a few species. Ease of interpretation refers to the effort that is necessary to obtain reliable typing information using a particular method.

**PHENOTYPIC METHODS**

Phenotypic methods for determination of the relatedness of microorganisms include evaluation of antimicrobial in vitro susceptibility, serogrouping, biotyping, and bacteriophage or bacteriocin typing. The antibiogram determines the pattern of resistance or the in vitro susceptibility of an organism to a panel of antimicrobial agents. This is routinely performed in most clinical microbiology laboratories using broth automated microdilution or disc diffusion methods. In epidemiologic studies, the antibiogram has limited value, as isolates that are not genetically and epidemiologically related may have the same susceptibility pattern because of acquisition of the same plasmid by multiple species (‘‘plasmid’’ outbreak).

Limitations of serotyping include the limited availability of antisera and problems with standardization of different methods. The advantage of this method is that the serotype appears to be a stable property of a microorganism. Biotyping has poor discriminatory power because variations in gene expression and random mutations may alter biologic properties of microorganisms. An example is 2 colonies of *Klebsiella* species isolated from the same urine sample can differ only in their ability to produce indole from tryptophan. Because the metabolic reaction is the basis of differentiating *Klebsiella oxytoca* (indole positive) from *K pneumoniae* (indole negative), the 2 isolates can be considered different species. However, on detailed examination the isolates can be genotypically identical, suggesting that they are variants from a different clone. Biotyping cannot differentiate among strains in which biochemical diversity is uncommon such as enterococci. This illustrates the limited utility of biotyping in epidemiologic studies. Bacteriophage typing is labor intensive; limitations of the method include poor reproducibility and problematic standardization. Bacteriocin typing may be useful for typing organisms such as *P aeruginosa* and *Candida* species, which are not easily typed by other methods. It is less labor intensive than bacteriophage typing and may be useful in epidemiologic studies when used together with serotyping, antibiograms, or biotyping.

**GENOTYPIC METHODS**

In recent years, molecular techniques have received increased attention as means of analyzing epidemiologic interrelationships. One approach has been to digest chromosomal DNA with restriction enzymes, resulting in a series of various-sized fragments that form different patterns when comparatively analyzed by agarose gel electrophoresis. Differences in these patterns are referred to as restriction fragment length polymorphisms. Enzymes used to cleave DNA recognize numerous sites within the bacterial chromosome, resulting in fragments that are too numerous to be compared accurately after conventional agarose gel electrophoresis. More recently, restriction enzymes have been identified that cleave chromosomal DNA less frequently. The resulting DNA fragments are too large to be separated by conventional agarose gel electrophoresis. Several alternative methods, generally classified as pulsed-field gel electrophoresis (PFGE), are capable of separating these large DNA fragments. Pulsed-field gel electrophoresis is a technique used to separate especially long strands of DNA by length to identify differences among samples. It operates by alternating electric fields to run DNA through a flat gel matrix of agarose.

In conventional agarose gel electrophoresis, DNA molecules that are more than 40 to 50 kilobases (kb) in size exhibit aberrant electrophoretic migration. By changing the duration and the direction of electrical pulses, PFGE allows the separation of DNA molecules across 1000-kb pairs in length (often referred to as megabase-sized DNA). Pulsed-field gel electrophoresis methods differ in the way the pulsed electrophoretic field is delivered to the agarose gel. Two of the most commonly used approaches are contour-clamped homogeneous electric field and field inversion gel electrophoresis. Field inversion gel electrophoresis uses a conventional electrophoresis chamber and periodically inverts the orientation of the positive charge by 180°. Contour-clamped homogeneous electric field uses a more complex electrophoresis chamber with multiple electrodes to achieve a highly uniform electrophoretic field, usually reorienting DNA molecules across a 120° angle. Contour-clamped homogeneous electric field has been used to evaluate the spread of various antimicrobial-resistant bacteria. Isolates that are found to have identical or related restriction endonuclease patterns suggest spread from single strains.

Pulsed-field gel electrophoresis has been applied to at least 38 pathogens or pathogen groups, including *Acinetobacter baumanii*, *Acinetobacter calcoacetus*, *Campylobacter coli*, and *Campylobacter jejuni*. Contour-clamped homogeneous electric field has been used for typing of *Candida* species. All bacterial and fungal species are typeable with PFGE, although the isolation of intact chromosomal DNA may be difficult for some species (e.g., *Clostridium difficile*). In general, PFGE is one of most reproducible and highly discriminatory typing methods available and generally is the method of choice for most epidemiologic evaluations.

**PLASMID ANALYSIS**

Plasmid typing was the first molecular method to be used as a bacterial typing tool. Although not useful as a bacterial typing tool to determine clonal relationships, this technique has been recently applied in clinical situations to determine the evolution and spread of antibiotic resistance within hospitals (e.g., plasmid epidemic among different PFGE isolates or different species of organisms). This technique is not helpful in differentiating between endemic and epidemic strains. Limitations of this method arise from the fact that plasmids can be readily acquired and deleted because they are mobile extrachromosomal DNA fragments; as a consequence, epidemiologically related isolates can exhibit different plasmid profiles. Many plasmids carry antibiotic resistance determinants, contained within mobile genetic elements (transposons) that can be acquired and deleted; as a result, the DNA composition of a plasmid can change rapidly. The selective pressure for nosocomial organisms to express antibiotic...
resistance may cause such plasmids to spread rapidly among strains and among different species.

**TYPING METHODS USING PCR**

Polymerase chain reaction is a biochemical in vitro reaction that permits the synthesis of limitless quantities of a targeted nucleic acid sequence. The procedure requires template DNA or RNA that may be present in the sample in minute quantities. If a specific RNA is sought, a reverse transcriptase step is used initially to convert the RNA into complementary DNA before amplification begins. Once a DNA template is available, 2 oligonucleotide primers, which flank the specific sequence on the template DNA to be amplified (thus defining the starting points for DNA polymerase activity), and a heat-stable DNA polymerase are added. Each amplification lasts approximately 3½ hours and consists of a heat denaturation phase (in which double-stranded DNA is melted into single strands), an annealing phase (in which the primers bind to the target sequences on the single strands), and an extension phase (in which DNA synthesis proceeds from the primers along each strand of the template DNA), therefore generating double-stranded copies of the original template.

**Multiplex PCR**

Multiplex PCR is a method in which more than a primer pair is used in the PCR mixture. The advantages to using this method include the addition of an internal reaction control for the PCR, as well as the addition of primers to detect multiple alleles in a single reaction. The disadvantage is that mixing different primers can cause interference in the amplification process so that optimizing conditions can be difficult, especially if there is a significant increase in the number of different primer pairs used.

**Nested PCR**

Nested PCR involves the sequential use of 2 primer sets. The first set is used to amplify a target sequence; the amplicon obtained is then used as a target sequence for a second amplification using primers internal to those of the first amplicon. This is amplification of a sequence within an amplicon. The advantages are extreme sensitivity and confirmed specificity. The disadvantage is that it requires open manipulation of amplified PCR, leading to increased risk of contamination.

**Arbitrarily Primed PCR**

Arbitrarily primed PCR (AP-PCR), also referred to as the randomly amplified polymorphic DNA assay, is a variation of the PCR technique using a single short primer that is not targeted to amplify any specific bacterial DNA sequence. Rather, at low annealing temperatures the primer will hybridize to multiple random chromosomal locations and initiate DNA synthesis. If one copy of the primer binds to one strand of DNA and another copy of the primer binds to the opposite strand of DNA, but in the proximity of the first primer, a DNA fragment will be synthesized, and amplification of the primer will occur. Although the method is much faster than routine PCR, it is much more susceptible to technical variation than routine PCR using primers directed at known sequences. On independent amplifications of the same strain, the number of copies generated from a particular locus can be appreciably different, yielding wide variations in the intensities of individual fragments. These factors make it difficult to obtain reproducible factors and interpretations with AP-PCR, particularly when attempting to compare isolates tested on different days.

**MULTILOCUS SEQUENCE TYPING**

Multilocus sequence typing (MLST) is a new molecular technique that was developed initially for *S. aureus*. It is based on identifying alleles from DNA sequences of internal fragments of housekeeping genes. Multilocus sequence typing has been successfully used for the study of molecular epidemiology and for the exploration of the population structure and evolution of virulence of various bacterial species.

This technique is highly discriminatory in characterizing bacterial isolates on the basis of allelic profiles generated by determining 450–base pair internal sequence fragments of 7 housekeeping genes. For each gene fragment, the different sequences are designated as distinct alleles, and each isolate is defined by the alleles at each of the 7 housekeeping loci, thus creating the allelic profile or sequence type. Because there are many alleles at each of the 7 loci, it is unlikely to have identical allelic profiles by chance, and isolates with the same allelic profile can be considered members of the same clone. The major advantages of MLST are the ability to compare results obtained in different studies via computerized databases on the Internet and the facility to readily compare sequence data among laboratories.

Multilocus sequence typing has been extended to the identification of hyervirulent lineages of *Nesseria meningitidis* and to the assignment of *Streptococcus pneumoniae* strains to the major hypervirulent clones and to the major penicillin-resistant and multiple antibiotic-resistant clones. In addition, an MLST scheme based on the nucleotide sequences of 7 housekeeping genes has been described for *Enterococcus faecium*.

**INTERPRETATION OF STRAIN TYPING RESULTS**

The goal of strain typing studies is to determine whether epidemiologically related isolates collected during an outbreak of disease are genetically related and represent the same strain. The use of strain typing results in infection control decisions is based on the following 3 assumptions: (1) isolates representing the outbreak are recent progeny of a single common precursor, (2) such isolates will have the same genotype, and (3) epidemiologically unrelated isolates will have different genotypes. By chance, epidemiologically unrelated isolates may have similar or indistinguishable genotypes, particularly if there is limited genetic diversity within a species or subtype.

**Interpretation of PFGE**

To interpret DNA fragment patterns generated by PFGE and to transform these findings into epidemiologically useful information, the microbiologist must understand how PFGE patterns are compared and how random genetic events can alter these patterns. Ideally, the PFGE isolates representing an outbreak strain will be indistinguishable from each other and will be distinctly different from those of epidemiologically unrelated strains. If this occurs, the outbreak is easy to identify. More commonly, random genetic events, including point mutations, insertions, and deletions of DNA, can occur, altering the DNA patterns during the course of an outbreak. The objective of inter-
pretative criteria is to establish a guide for distinguishing true differences in strains from natural genetic variations that occur over time within a given strain.9–10 The interpretative criteria provide consistent objective guidelines for correlating the level of variations observed between an individual isolate and the putative outbreak strain with an estimate of the likelihood that the isolate is, in fact, part of the outbreak strain. This correlation focuses on the number of genetic events required to generate the observed typing variation. Because only a small portion of an organism’s genetic component is undergoing analysis, isolates that give identical results are classified as “indistinguishable,” not as “identical.” A conservative guideline is to consider that isolates differing in 3 or fewer restriction fragment positions could have occurred via 1 genetic event, 4 to 6 restriction fragments via 2 genetic events, and 7 or more restriction fragments via 3 genetic events. Isolates differing in 3 or fewer restriction fragment positions may represent epidemiologically related subtypes of the same strain. Isolates differing in 4 or more restriction fragment positions may represent a more tenuous epidemiologic relationship. Results of several studies using PFGE and other typing methods indicate that single genetic events (ie, random events that may destroy or create a new restriction endonuclease site, or deletions or insertions of new DNA such as plasmids, bacteriophages, or insertion sequences) occur unpredictably even within the time span of a well-defined outbreak (1–3 months).9,10 If 2 genetic events have occurred (based on the finding of 4–6 differences in fragment patterns), the interpretation falls into a gray zone. The results may indicate that these isolates are related, especially if they were collected during a long period (3–6 months), but there is also a possibility that these strains are unrelated and that the similarity was a result of chance. Isolates that differ by the occurrence of 3 genetic events represent genetically different strains and should be considered unrelated.

Analysis of PFGE band patterns can be performed by visual inspection for small numbers of isolates, but for larger numbers of isolates or for comparison of isolates from different institutions, the use of standardized protocols and computerized analysis is preferred. DNA fragments are sized against a well-characterized λ DNA ladder. After electrophoresis, the gels are stained with ethidium bromide, and PFGE patterns are digitalized in a TIFF format (uncompressed TIFF file) using a method such as the GelDoc system (Bio-Rad Laboratories, Hercules, Calif), with analysis of PFGE patterns using BioNumerics software (Applied Maths, Kortrijk, Belgium). These programs have the capability to normalize the patterns and to compare large numbers of strains over time. They are also useful in the generation of a dendrogram, which is helpful in establishing ancestral relationships among isolates. Band patterns are clustered to deduce a dendrogram into a tree by an unweighted pair group method using arithmetic means. Similarities in band patterns between strains are determined using the Dice coefficient.16 In general, strains are considered identical if they show 100% similarity and are considered clonally related if they show greater than 80% similarity (generally comparable to the 3 or fewer fragment difference already noted). The dendrogram results in an interpretable tree at the species and subspecies levels. The use of a dendrogram is not a purely phylogenetic representation of lineages but rather a tool that provides a visual description of genetic similarities and differences between groups. Sample results of PFGE and a dendrogram for community-associated methicillin-resistant Staphylococcus aureus isolates from southeastern Michigan are shown in Figure 1 and Figure 2, respectively.

In a study of the epidemiologic characteristics of vancomycin resistance in enterococci, the glycopeptide resistance element Tn1546 in 124 VanA E faecium clinical isolates from 13 Michigan hospitals was evaluated using PCR fragment length polymorphism analysis.17 This study was conducted because it was observed that there was a considerable amount of heterogeneity by PFGE among vancomycin-resistant E faecium strains even in epidemiologically related isolates. Analysis of plasmids showed diversity; however, the finding of related Tn1546 elements suggested the presence of a transposon epidemic. A combination of PFGE, plasmid analysis, and PCR analysis
Interpretation of AP-PCR

For AP-PCR, interpretation of identical fragment patterns and of patterns with 4 or more fragment differences is straightforward. However, there are no criteria for interpreting a change in the size of a single band or in the intensity of several bands. For strains yielding results such as these, it may be necessary to try alternate primers or to vary the reaction conditions. The reproducibility and discriminatory power of each primer and amplification protocol need to be validated by analyzing sets of isolates that previously have been well defined by epidemiologic data or by independent typing studies. Such analysis is available for few species (S. aureus and C. difficile). Because variation of AP-PCR cannot be correlated with specific genetic events, the principles defined previously for PFGE cannot be applied to typical AP-PCR patterns. Results of a multicenter study demonstrated that, although partici-

pating laboratories obtained different AP-PCR products, the same epidemiologic clusters were identified satisfactorily. Therefore, if variability of fragment sizes in AP-PCR products can be considered epidemiologically unrelated isolates, those showing no differences or changes only in band intensity can be considered epidemiologically related. Changes in 2 bands remain difficult to interpret.

COMPARISON OF TECHNIQUES

When comparing molecular methods such as PFGE versus PCR, it is important to consider what each method is actually assessing. With PFGE, the macrorestriction fragment patterns reflect the distances between rare restriction sites around the chromosome; therefore, the method can detect differences between isolates resulting from chromosomal changes that affect these sites or the distances between them. In this sense, PFGE provides a global chromosomal overview, scanning more than 90% of the chromosome (ie, the sum of the restriction fragment sizes), but it has only moderate sensitivity because minor genetic changes may go undetected. Conversely, PCR-based methods generally survey undefined regions representing less than 10% of the chromosome (the sum of the ampiclon sizes). Because PCR products are usually small (≤5 kb), electrophoretic analysis can detect even small genetic changes affecting their size. It is accepted that some basic level of genetic change could be expected to occur in most nosocomial isolates as they move from patient to patient (ie, a single genetic event). With PFGE, a single detectable chromosomal change would result in a difference of at least 2 band positions. Polymerase chain reaction would minimally detect a difference of 1 position (eg, the addition, loss, or shift in electrophoretic migration of a single band). The greater the genetic difference between isolates, the more likely it is that they are epidemiologically unrelated. However, minor genetic differences may be of epidemiologic significance in more clonal organisms that, by definition, have a low degree of genetic variability. For example, in analyzing methicillin-resistant S. aureus strains, one must consider the endemic nature of the organism and its tendency for clonal uniformity. Therefore, even a small change in chromosomal fingerprints may be epidemiologically significant.

CONCLUSIONS

Evaluating bacteria, mycobacteria, and fungi for molecular relatedness was performed previously by methods that were insensitive, lacked reproducibility, and needed standardization. Because of limitations of traditional phenotyping methods, new molecular techniques have been developed during the past 2 decades. Molecular techniques have contributed to a better understanding of the epidemiologic characteristics of nosocomial infections. By using these techniques, it is possible to compare the molecular relatedness of organisms for which no other reliable typing method exists. Most recently, MLST is proving to be a useful research technique for the study of the global epidemiologic characteristics of S. aureus and other organisms and has the added advantage that sequence data can be stored in a central database. Using a combination of several methods increases the specificity and sensitivity of epidemiologic typing. The strengths and limitations of molecular methods need to be considered when determining the best application to a particular epidemiologic or patient care question. Molecular methods of strain com-

Figure 2. Dendrogram of community-associated methicillin-resistant Staphylococcus aureus isolates.
parison remain an essential tool in our testing armamentarium but should be used only in conjunction with an appropriate epidemiologic investigation.

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