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MINIREVIEW

Preventing Antibiotic Resistance through Rapid Genotypic Identification of Bacteria and of Their Antibiotic Resistance Genes in the Clinical Microbiology Laboratory

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INTRODUCTION

The emergence of drug resistance in microorganisms is a serious problem, and several strategies have been proposed to try to tackle it. Prevention should be the ultimate solution, and vaccines have been suggested as a strategy that can be used to slow down the emergence of drug resistance by decreasing the infection rate and hence antibiotic usage (15). Unfortunately, we are far from this ideal. Broad surveillance programs (22) and education of clinicians, pharmacists, veterinarians, drug company representatives, and the public about the spread of antimicrobial resistance and the consequences of antibiotic misuse should also have a significant impact (21). Restrictive use of newer and broad-spectrum antibiotics has also been applied and advocated. Strict application of therapeutic guidelines might also be useful.

Another strategy is to increase our understanding of the biochemical basis of antimicrobial resistance mechanisms which should suggest preventive and therapeutic strategies for overcoming resistance (17). The understanding of resistance mechanisms is also necessary for the development of the tools necessary to detect resistance by methods other than phenotypic testing, which now includes disc diffusion or dilution tests (MIC tests) to evaluate the susceptibilities of microbes to antibiotics. We advocate that rapid ($\leq$1 h) identification of microorganisms should literally modify the habits of the prescribers and contribute to a reduction of the dissemination of antimicrobial resistance. While most DNA-based tests are presently used to identify viruses or bacteria whose identification is tedious, like *Mycobacterium tuberculosis* or chlamydia, we are suggesting that the time is ripe for the use of these tests to identify bacteria causing common and deadly bacterial diseases. We believe that the simultaneous rapid genotypic identification of bacteria and their antibiotic resistance genes will have a major impact on the treatment of infectious diseases while contributing to a better control of antimicrobial resistance (14).

Speed is the essence when one deals with bacterial infections. Although the Gram stain can sometimes be helpful, presently, diagnosis in the clinical microbiology laboratory is only confirmatory because a clinical decision has been made long before (usually 48 h) the identity of the organism responsible for the infection and its susceptibility to antibiotics become available. With the actual state-of-the-art technology, which dates back to the last century, we cannot even tell accurately before 18 to 24 h whether a clinical sample has bacteria or not. This is of importance because no bacteria can be grown out of more than 80% of all normally sterile clinical samples sent to clinical microbiology laboratories (4). The lack of a timely response by the laboratory has consequences on antibiotic usage and prescription. Patients must be treated empirically. When severe or nosocomial infections are suspected, they are often treated with broad-spectrum antibiotics. The increased use of broad-spectrum antibiotics is not restricted to hospitalized patients in intensive care units or patients seen in emergency rooms, however. Indeed, a recent American survey has indicated that toxic and expensive broad-spectrum antibiotics are prescribed more frequently for the treatment of common infections by office-based physicians (14). Clearly, with 80% of normally sterile specimens received in the microbiology laboratory not growing any microorganism, several patients are receiving antibiotics even if they do not have a bacterial infection because there are no accurate ways of determining before the next day whether the clinical sample harbors bacteria. In line with this latter argument, a recent study in Spain has indicated that on any particular day, the number of antibiotic prescriptions exceeded by three times the number of bacterial infections diagnosed (3). Moreover, microbiologic results are available so slowly that physicians rarely consult them unless the patient is not responding to the given antibiotic. If physicians could have in hand the identity of the microorganism and its resistance profile from the microbiology laboratory at the same time that they have the biochemistry and hematology results, antibiotic prescription rates could go down dramatically, and when antibiotics are needed, more targeted and inexpensive antibiotics could be used. On the other hand, whether you are using phenotypic or genotypic identification systems, the presence of bacteria or even the absence of bacteria in the clinical specimens does not necessarily mean the presence or the absence of infection because clinical judgment should always prevail.

RAPID IDENTIFICATION OF MICROORGANISMS AS A MEANS OF DECREASING THE EMERGENCE OF ANTIMICROBIAL RESISTANCE

The advances in sample preparation, DNA-based amplification techniques, and product detection have evolved to the extent that it is now possible to identify microorganisms directly from clinical specimens in 1 h (13). Moreover, as these

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DNA-based tests evolve, their sensitivity will allow the detection of a single copy of the genome of a microorganism. If the precise identification of the microbial agents responsible for infections were available within 1 h when the results of other laboratory tests are available to the physician, it would have a major impact on the management and treatment of patients. The use of universal probes based either on the rRNA gene (11) or on some other conserved region of microorganism genomes should indicate whether or not the patient is infected with a bacterium. Because more than 80% of normally sterile clinical specimens (blood, cerebrospinal fluid, joint fluid, etc.) sent to the microbiology laboratory are not "infected" or do not harbor bacteria (4), the use of universal primers should permit determination in 1 h of whether or not the patient suffers from a bacterial infection. Obviously, universal probes would not be useful for sputum or surgical wound specimens or specimens from other nonsterile clinical sites. Provided that appropriate controls are included and relevant sensitivity is reached, the absence of amplification products would suggest the absence of bacterial infections and the use of antibiotics could be avoided. In contrast, the detection of an amplification product with universal primers would indicate that a bacterium is present. However, it would not provide information on the nature of the bacterium and hence on the antibiotic to be used. Therefore, universal primers are useful for screening negative samples but are of limited value for orienting the choice of antibiotics in the case of a positive reaction.

There are now specific DNA probes or amplification primers for almost every relevant pathogenic organisms (8, 26), and these primers can be used to identify the bacteria present in clinical specimens. Because multiple bacteria can be isolated from different sites, it would be advisable to carry out reactions under multiplex conditions, i.e., with more than one pair of primers per reaction. It would be possible to discriminate the amplicon either by size on agarose gel electrophoresis or with a different fluorochrome if fluorescence was to be used as the detection method. It should also be possible to decrease substantially the number of primers by generating genus-specific or even group-specific PCR primers. This approach has the benefit on the one hand of decreasing the complexity of the amplification reactions and on the other of increasing the proportion of bacteria detected. With group-, genus-, and species-specific amplification primers it should be possible to detect most microorganisms responsible for any type of infection. Nevertheless, there will always be the rare uncommon pathogen that is responsible for an infection but that may not be detected with the available primers. Because the universal primers would have detected the presence of an infection but none of the genus- or species-specific primers would have produced an amplification product, it would indicate that the infection is due to an uncommon pathogen. In those rare instances, culture may be requested if species determination was thought to be useful, but with time, most microorganisms could be identified by DNA-based tests. Rapid bacterial identification would be of major benefit to the clinician, but because the antibiotic susceptibility profile is an important parameter in the management of infections, we believe that a rapid identification system will fully blossom only when both bacterial identification and the resistance profile are provided simultaneously.

FROM PHENOTYPIC TO GENOTYPIC TESTING OF RESISTANCE

Presently, susceptibility (in contrast to resistance) is the parameter provided to clinicians. Although the phenotypic technique of susceptibility testing is relatively simple, it requires bacterial isolation, and hence, the result is not available until 2 days after a treatment is started. The phenotypic approach also has some shortcomings; since different bacterial species have different susceptibilities to the same antibiotics, breakpoints of different values must be tested. There is also no international agreement for the interpretation of breakpoints in antibiotic susceptibility tests. Finally, several of the presently performed susceptibility tests are highly dependent on experimental conditions, and often, more than one method would need to be performed to obtain an accurate susceptibility profile. If we take only β-lactam antibiotic testing as an example, special precautions must be taken for testing for penicillin resistance in *Streptococcus pneumoniae*, for methicillin resistance in *Staphylococcus aureus*, and for the presence of extended-spectrum β-lactamases in members of the family *Enterobacteriaceae*.

To increase the rapidity and accuracy of resistance testing, the use of a genotypic approach has recently been advocated (6, 23), and numerous DNA-based assays for the detection of bacterial resistance have been developed (1, 19). This novel approach is a true revolution because it relies on a completely different concept; testing for resistance instead of testing for susceptibility. Several clinical studies will be required, however, to validate the genotypic approach. Indeed, is the presence of a resistance gene always indicative of a resistant bacterium? If a gene coding for a resistance to a drug is not detected, does it mean that the bacterium is susceptible to that drug? One stumbling block in using DNA-based assays for resistance testing is the formidable complexity of resistance mechanisms. Drug resistance may arise because drug uptake may be thwarted by loss of the uptake system or alteration of the membrane composition; once the drug is inside the cell it may be inactivated or excreted (modified or not), or if drug activation is required, activation mechanisms may be suppressed. Drug-microbial target interactions may be less effective because the target is modified or alternative pathways may bypass the blocked target. Resistance to the same drug can be due to several different mechanisms. For example, resistance to β-lactam antibiotics can be due to decreased uptake, increased efflux, inactivation enzymes, or modified target. Furthermore, there are several different enzymes that can confer resistance by the same biochemical pathway. For instance, several dozen plasmid-mediated β-lactamases confer resistance by inactivating β-lactam antibiotics. Nevertheless, as our understanding of drug resistance mechanisms increases, we should be able to generate the appropriate tools to detect resistance. In addition, new resistance genes will undoubtedly arise in bacteria in the future. To prevent the possibility that a clinician could unknowingly use an antibiotic to which the organism is resistant, continuously updated epidemiological studies would help in the selection of the right set of primers for the detection of relevant new types of resistance in particular organisms. Finally, technological innovations in DNA-based diagnostics should also allow the detection of multiple alleles or genes at once.

Although the multiplicity of resistance mechanisms will complicate the detection of the resistance genotype for certain specimens, there are clear cases in which detection of resistance could easily be implemented and could have an immediate impact on the treatment of infectious diseases. Molecular diagnostic methods have already found a niche in the clinical microbiology laboratories where they are used.

The detection of antibiotic resistance genes in gram-positive bacteria should also be relatively easy. Indeed, resistance to key antibiotics such as methicillin and vancomycin is due to few genes. This small heterogeneity of resistance determinants is in

contrast to the situation prevailing in several gram-negative bacteria. Methicillin resistance in *S. aureus* and coagulase-negative staphylococci is due to the synthesis of a novel penicillin-binding protein encoded by the *mecA* gene. Resistance to methicillin has important implications, often necessitating patient isolation and the use of vancomycin. However, despite numerous guidelines for optimization of the phenotypic detection of methicillin resistance, it is becoming increasingly clear that *mecA* detection is becoming the "gold standard" for the detection of methicillin resistance (25). All the studies reported to date indicate an excellent correlation between the presence of the *mecA* gene and methicillin resistance (19). In *mecA*-positive staphylococci, the use of vancomycin is warranted. When *mecA* is absent, cells could exhibit intermediate levels of methicillin resistance due to overexpression of a β-lactamase. In these cases, however, β-lactam antibiotics or β-lactam–β-lactamase inhibitor combinations would likely be more effective and appropriate than vancomycin. Several multiplex PCR assays that permit both *S. aureus* identification and *mecA* detection have been developed, and some reports have described the utility of those tests directly with positive blood culture vials (5, 24). The rapid identification of *mecA* should restrict the use of vancomycin and hence the emergence of resistance to this drug of last resort.

Indeed, resistance to vancomycin is now widespread in enterococci (10), and there are now enterococci that seem to have become resistant to all currently available antibiotics. There is (justified) concern that the vancomycin resistance genes could transfer from enterococci to staphylococci. Transfer has already been shown to occur under laboratory conditions (16), and one of the vancomycin resistance genes was recently observed in streptococci (20). Rapid detection of vancomycin-resistant enterococci (VRE) would permit prevention measures including the isolation of infected patients to reduce the possibility of transmission of VRE to other hospitalized patients. Resistance to vancomycin is due to *van* genes, whose products are similar to D-alanine:D-alanine ligases, enzymes involved in cell wall biosynthesis. Three genes, *vanA*, *vanB*, and *vanC*, contribute to vancomycin resistance (10). Amplification assays for the detection of *van* genes have been developed (7, 18), and the introduction of these techniques into the routine clinical laboratory could have major implications on patient management. The Centers for Disease Control and Prevention has recommended that patients infected with VRE be isolated. The rapid simultaneous identification of enterococci and their antibiotic resistance genes would be a useful epidemiological tool. Because the *vanC* gene is not transferable and the *vanA* and *vanB* genes are transferable, these new genotypic tools could be useful for tracing the spread of resistance between microbes.

As observed in many gram-negative bacteria, when more than one gene can cause resistance to a class of drug, it is possible to use multiplex reactions to decrease the complexity of the test. Some of the genes are sufficiently closely related that a set of amplification primers can be used to amplify several members of the same family of resistance genes (2), and this should be another strategy for decreasing the complexity of the amplification reactions. Clearly, an improved method of detecting the amplification products, such as tests with matrix hybridization chips (9, 12), should permit the detection of multiple mutations in a single reaction. Taken together, all these arguments make us believe that genotypic detection of resistance is possible.

The development of rapid diagnostic identification methods and genotypic resistance testing at a competitive price should greatly reduce the emergence of drug resistance. This will be achieved by prescribing antibiotics only to the patients who require them. Because the bacteria will be identified rapidly, targeted antibiotics will be used and broad-spectrum antibiotics will be used only when dealing with resistant organisms. These tests would appropriately and rapidly identify the patients who should be isolated to prevent the disastrous spread of multidrug-resistant organisms within institutions. It should thus prevent the unnecessary isolation of patients suspected of carrying VRE, methicillin-resistant *S. aureus*, or other resistant pathogens. On occasion, emergency rooms and hospitals had to be closed in Canada pending the phenotypic identification of these pathogens and the results of susceptibility tests. The continuing implementation of molecular tests in the routine microbiology laboratory will contribute to a definitive diagnosis and should have a major impact on the clinical management of infectious diseases. It would also reduce global health care costs and, it is hoped, save lives while contributing to a major reduction in the spread of antibiotic resistance.

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