INTRODUCTION

In nature there is a theoretical gene pool from which microorganisms can acquire genetic determinants that increase their chances of survival in hostile environments. This gene pool consists of genomic DNA, plasmids, phages, insertion sequences, and transposable elements, all of which exist in a dynamic equilibrium. Among the genes that can be obtained by bacteria are those encoding antimicrobial resistance determinants. The development of resistance determinants and R plasmids was first reviewed by Watanabe in 1971 (45); the review was expanded by Kopecko and colleagues in 1976 to include the role of transposons (16), and there were subsequent reviews by Cohen et al. (5), Davies and Smith (8), Davies and Gray (7), and others (2, 15). According to Davies and Gray, many antimicrobial resistance genes could have originated in antibiotic-producing strains of soil bacilli. These resistance determinants, which protected the host from the action of an antibiotic, could have been excised from their chromosomal location by insertion elements or transposons and disseminated by these genetic elements to other bacteria (7). The integration of these determinants into the complement of genetic material of a bacterium is often a function of appropriate selective pressure. Indeed, the gene pool of resistance determinants available to bacteria seems vast, especially when judged by the number of infections caused by multiply resistant organisms seen in hospitals and medical centers today.

Using techniques borrowed from the molecular biology laboratory, clinical and medical microbiologists have begun to seek out and characterize this resistance determinant gene pool and to study the movement of genetic elements, particularly among pathogenic bacteria (2, 19, 31, 41, 46). The most useful technique in this regard is the gene-specific DNA probe. Herein, I review the application of DNA probe technology to the assessment of the size and extent of the resistance determinant gene pool and the use of DNA probes as epidemiologic tools for the tracing of multiresistant bacteria.

NEED FOR SENSITIVE EPIDEMIOLOGIC TOOLS

During the early 1970s, plasmid fingerprinting and antibiogram analysis were widely used to monitor the dissemination of multiresistant bacteria causing nosocomial infections and, in some cases, to monitor the specific plasmids that carried those resistance determinants (25, 33, 43). This, however, was a rather insensitive way to monitor specific genes. Furthermore, the utility of plasmid screens and antibiograms decreased as the duration of an epidemic lengthened, primarily owing to the introduction of transposons into resident plasmids and chromosomes of endemic strains, which changed their plasmid profile and antibiogram (5, 30, 31, 34). Transposons were clearly responsible for the evolution of the R factors noted during several of the outbreaks described in this period (30, 31).

Because of their high specificity and sensitivity, DNA probes are well suited to the tracing of genes within bacteria regardless of whether the genes are located on plasmids or on chromosomes. I will review several of the DNA probes that have contributed to our understanding of various resistance determinants.

TRIMETHOPRIM RESISTANCE

The first DNA probes to antimicrobial resistance genes were those developed by Fling and co-workers to characterize trimethoprim resistance genes (9). These investigators examined 42 trimethoprim-resistant clinical isolates of bacteria using probes specific for the type I and II dihydrofolate reductase (DHFR) genes. Seventeen of the plasmid-containing isolates carried the DHFR type I gene, and 11 of the isolates examined harbored a plasmid-encoded DHFR type II gene. Eight additional isolates apparently harbored DHFR type I genes on their chromosomes. Five plasmids showed homology to both type I and II probes; the type I reactions probably were false-positives owing to the presence of streptomycin resistance gene segments on the type I probe. One isolate did not react with either DHFR gene probe and was assumed to represent a third class of trimethoprim resistance genes. Further studies on the DHFR enzyme from this isolate showed that it differed from that of the others in its molecular weight, inhibition profile, and level of resistance, confirming its unique nature.

Rudy and Murray used these same probes to characterize the DHFR genes present in trimethoprim-resistant strains of Escherichia coli isolated from U.S. students studying in Mexico who had taken prophylactic trimethoprim or trimethoprim-sulfamethoxazole (32). After first showing that 41 of 100 fecal isolates could transfer trimethoprim resistance to recipient strains of E. coli during conjugal matings, these investigators demonstrated that 12 of the transconjugants contained plasmids that were identical by restriction endonuclease analysis. Each of these plasmids was shown by probe analysis to carry a DHFR type I gene. The availability of probes to the DHFR genes allowed this determination to be made simply and rapidly.

Pulkkinen et al. (27) studied the prevalence of Tn7 (a trimethoprim resistance transposon) in members of the family Enterobacteriaceae in trimethoprim-resistant isolates from the Turku City Hospital in Finland. These studies revealed that of 199 trimethoprim-resistant enterobacteria, 47.2% contained Tn7.

Thus, DNA probes have proven to be useful for rapidly defining the prevalence of a specific resistance gene in
AMINOGLYCOSIDE RESISTANCE GENES

The first probe to an aminoglycoside resistance gene was described by Tenover et al. (40) in 1984. The probe was developed to monitor the dissemination of the 2'-O-adenylation transferase [ANT(2')] gene during an ongoing epidemic of multiresistant nosocomial infections in Seattle, Wash. (Adenylation, phosphorylation, and acetylation of the aminoglycoside-aminocyclitol substrate are the most common mechanisms of bacterial resistance to this class of antimicrobial agents.) These investigators used a 310-base-pair DNA probe derived from the epidemic R factor pLS1000 (43), which proved to be highly specific for the ANT(2') gene and exhibited greater sensitivity than the traditional phosphocellulose paper binding assay. When a contiguous (nonoverlapping) 280-base-pair 4x4I fragment from within the open reading frame of the ANT(2') gene was used as a probe instead of the 310-base-pair fragment, hybridization was noted with plasmids containing either the ANT(3') structural gene or the ANT(2') gene. DNA sequence data has shown that the N terminus of the ANT(2') gene, which is the area containing the 28-base-pair fragment, shares significant DNA homology with the published sequence of the ANT(3') gene (12; F. C. Tenover, unpublished observations). Such a relationship was predicted by Yagisawa and Davies in 1979 (47).

Although the phosphocellulose paper binding assay is more sensitive for the detection of aminoglycoside-phosphorylating enzymes than adenylylating enzymes, studies reported by Young et al. showed that the assay cannot reliably differentiate the 3'-aminoglycoside phosphotransferase type I [APH(3')-I] and APH(3')-II enzymes (48). This problem was overcome by using DNA probes directed against the structural genes of these two enzymes. Using a spot hybridization method, Young et al. showed that the DNA probes could differentiate these two genes rapidly and accurately (48). Contrary to previous reports, the APH(3')-I and APH(3')-II genes were never found together in any of the strains examined. These investigators also confirmed the presence of another class of APHs in strains of both E. coli and Pseudomonas aeruginosa that had been described earlier by Matsuhashi and colleagues (20, 21).

The ANT(2'), APH(3')-I, and APH(3')-II probes have been used in conjunction with a modified colony hybridization technique (10) to track aminoglycoside-resistant organisms from patients at the Seattle Veterans Administration Medical Center (41). In these studies, patients predisposed to chronic urinary tract infections, often owing to previous spinal cord injuries, were found to harbor a variety of multiresistant organisms. Although the bacterial genera and plasmids changed at frequent intervals, virtually all the organisms isolated from urine carried either the ANT(2') or APH(3')-1 determinant and often both (41). Whether this represents repeated infection from an exogenous source contaminated with multiresistant organisms or colonization of the bladder remains to be shown.

Groot Obbink et al. (11) also developed a probe to the AAD(2') [same as ANT(2')] structural gene. These investigators used their probe to examine a large variety of gentamicin-resistant strains. Hybridization of the probe to strains devoid of plasmids and strains containing nontransmissible plasmids led these investigators to speculate that the AAD(2') gene is located on a transposon.

Recently, Lambert and co-workers, using phosphotrans-
ferase probes from staphylococci, streptococci, and gram-negative bacteria, showed the presence of a streptococcal phosphotransferase gene in the gram-negative organism Campylobacter coli. This was perhaps the first documentation of in vivo transfer of a gram-positive gene to a gram-negative organism (17). The relationship between the apha3 genes of streptococci and campylobacter were confirmed by DNA sequence analysis (44).

Thus, probes to the structural genes of aminoglycoside-modifying enzymes have played a major role in delineating the number of genes involved in epidemics, helped define their modes of transmission, and suggested that patients themselves may serve as the natural reservoir of multiresistant determinants within a hospital setting.

BETA-LACTAMASE GENES

Cooksey et al. (6) developed a probe to the TEM-1 beta-lactamase gene found in gram-negative enteric organisms. Using this probe, obtained from a cloning vector pBR322, they showed that homology exists between the TEM type beta-lactamases (TEM-1 and TEM-2) and the OXA-2 gene from the plasmid R46. Studies on the epidemiology of the various beta-lactamase genes using these probes are under way.

A similar probe derived from pBR322 was used by Murphy-Corb et al. to demonstrate the integration of ampicillin resistance genes into the chromosome of Haemophilus influenzae (23).

Recently, Perine and co-workers used a beta-lactamase probe to detect penicillin-producing Neisseria gonorrhoeae directly in urethral exudates from males. The probe assay was 91% sensitive and 96% specific when compared with the chromogenic cefalosporin test performed on isolated colonies of N. gonorrhoeae (26). More studies of this type (i.e., parallel identification and susceptibility testing with probes) would be of value for determining the feasibility of probes as rapid diagnostic aids for physicians offices, outpatient clinics, and Third World countries. Caution must be invoked here, however, because the presence of a resistance gene in a bacterium does not necessarily mean that a resistance phenotype will be manifested.

TETRACYCLINE RESISTANCE

Mendez and co-workers developed three DNA probes to differentiate the tetracycline resistance determinants encoded on plasmids from gram-negative bacteria. Using the probes, four classes were defined: A, B, C, and D. Classes A and B were further subdivided based on the level of resistance to minocycline and chlortetracyclin demonstrated by the host organism (22). Class D was represented by a single plasmid, RA1, which did not hybridize to the other probes. These studies were instrumental in documenting the heterogeneity of tetracycline resistance determinants in gram-negative bacilli. Marshall et al. (19), using the above tetracycline class-specific DNA probes, plus a fourth probe developed from plasmid RA1 for class D determinants, examined 225 tetracycline-resistant, lactose-fermenting coliforms collected from human and animal feces. They found the class D determinant (Tn10 type) to be present in 73.3% of the resistant isolates examined. The type A gene (as present on the plasmid RPl) was present in 21.7% of the isolates, and type C (from pSc101) was present in 8% of the isolates. Multiple tetracycline resistance determinants were found in 5.5% of the isolates tested. No class D determinants were found; one strain did not hybridize to any of the four probes and probably represents yet another class of tetracy-
The increase of the types of tetracycline resistance determinants has been demonstrated in several bacteria, including Haemophilus influenzae. In each case, the tetracycline resistance determinant was of class B (Tn10 type) (18).

Marshall and co-workers used these same four probes to analyze tetracycline-resistant strains of H. influenzae. In each case, the tetracycline resistance determinant was of class B (Tn10 type) (18).

Using a DNA probe developed from the mercury resistance determinant on the plasmid R100, Barkay et al. noted homology with Thiothrix ferroxidans BA-4 and several plasmid-containing mercury-resistance strains of E. coli (1). No homology was noted, however, between the probe and crude lysates of mercury-resistant strains of Staphylococcus aureus, Bacillus cereus, and Mycobacterium scrofulaceum. Studies on the role of mercury resistance genes in promoting the development of new phenotypes in bacterial communities in polluted environments are under way.

OTHER STUDIES USING DNA PROBES

Taylor and Brose used a battery of DNA probes to specific transposons to aid in constructing the genetic maps of R plasmids from Salmonella typhi (38). This concept of using batteries of probes to study resistance determinants could be of value in two other arenas: first, the study of the evolution of multiply resistant Salmonella species and the impact of antibiotic feed supplements on their development, and, second, the global dissemination of antimicrobial resistance genes. Reports concerning the former are prominent in the recent literature (13, 14, 37), and the discussion concerning the role of antibiotics in animal feed is far from over (36).

Probes to specific resistance determinants could be used to study the development of resistance to antimicrobial agents among several animal populations with and without feed supplements in a manner similar to the studies of Christie and Dunny (4). This would, perhaps, give us a broader knowledge of the resistance determinant gene pool. Second, considering the number of probes now available and the ability to rapidly screen many organisms by spot hybridization methods, the stage is set for a major study of the global dissemination of antimicrobial resistance genes. The work of O’Brien et al. demonstrated that there is, in fact, global dissemination of specific R factors (24), and a report by Shimizu and co-workers showed that large numbers of isolates from around the world have already been collected (35). Thus, the impact of DNA probes on our understanding of the epidemiology of resistance genes is now limited only by our ability to design appropriate experiments.

CONCLUSIONS

Studies of resistant bacteria using nucleic acid probes have confirmed the emergence of new antibiotic resistance genes and made possible large studies of the movement of specific resistance determinants during outbreaks of nosocomial and community-acquired infections. They have illustrated the fact that the theoretical gene pool of antimicrobial resistance determinants is not only in the intestines of humans and animals but in the environment as well. The use of probes for the rapid determination of the antimicrobial susceptibility of organisms directly in patient specimens is intriguing and merits further study. Probes to other classes of antimicrobial agents, including antifungal and antipsychotic agents, should be considered to expand our knowledge of the evolution and dissemination of such genes.

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LITERATURE CITED


