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MINIREVIEW

Genotypic Approach to the Study of Bacterial Resistance to Antibiotics†

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The genotypic approach to the study of prokaryotic resistance to antibiotics, i.e., detection and characterization of resistance at the genetic level rather than at the phenotypic level, is a recent concept. This concept was made possible by (i) the development of certain techniques of molecular biology (e.g., gene cloning and DNA hybridization, sequencing, and synthesis), (ii) the elucidation of the biochemical mechanism of the vast majority of resistance traits, a prerequisite to the understanding of cross-resistance, (iii) the study of the regulation and heterologous expression of resistance genes, and (iv) detailed examination of the phenotype resulting from the presence of a given resistance mechanism using pairs of isogenic bacteria (8). The possibilities offered by this approach are considered here, with particular reference to the identification and study of the distribution of genes conferring in human pathogens resistance to antibiotics used in the clinical environment. For the sake of simplicity, this review focuses on a few clinically relevant examples.

METHODS

Two major techniques can be used to detect antibiotic resistance genes: DNA hybridization (26) and the polymerase chain reaction (PCR) (42). Probes based on fragments of cloned genes must imperatively be intragenic to be specific, a necessary but not always sufficient requirement (27). They are convenient to reveal, because of cross-hybridization, genes closely related in structure (19). By contrast, oligonucleotides can be designed to be specific or universal. Specific oligonucleotides allow detection of a single point mutation in both eukaryotic (10) and prokaryotic (26) genomes, whereas universal oligonucleotides are intended to detect genes that encode izozymes and that have resulted from divergent or convergent evolution (2). The latter goal, however, is better achieved by PCR using primers that are complementary to conserved regions of the resistance genes (3). Gene heterogeneity and nonspecific hybridization are two of the major limiting factors in detection of antibiotic resistance genes. Because it requires two specific nucleotide sequences located, in a certain distance range, on the complementary strands of DNA, PCR can easily overcome these limitations (3).

Non-isotopically labeled probes are nearly as sensitive as their radioactive counterparts (41), and PCR does not require any radioactivity. However, routine use of these two techniques in medical microbiology will depend upon automation, in particular to lower the cost, of the corresponding reaction. Since bacterial identification and detection of resistance are more easily performed on samples with a single infecting organism, these techniques will most certainly be first applied to normally sterile biological fluids, such as blood, spinal fluid, and urine.

RATIONALE

As opposed to the classical phenotypic approach, detection of antibiotic resistance at the gene level implies that a given mechanism confers clinical resistance independently of the bacterium (prokaryotic host), of the patient (eukaryotic host), and of the detection system used.

Prokaryotic host. Study of a large number of clinical isolates of gram-positive cocci indicated that aminoglycoside (not including streptomycin) resistance in these microorganisms was due to acquisition of highly conserved genes encoding three distinct modifying enzymes (27). Staphylococci are exquisitely susceptible to aminoglycosides, whereas enterococci and streptococci are naturally resistant to low levels of the drugs (20). Therefore, although in both cases clinical resistance is achieved, synthesis of a modifying enzyme confers on staphylococci resistance to moderate levels of aminoglycosides and on enterococci and streptococci resistance to high levels of the drugs (27). This difference led to the establishment, for the two groups of bacteria, of separate lists of breakpoints with very different values for each antibiotic that has to be tested separately. By contrast, a set of three probes only allows detection of aminoglycoside resistance in *Enterococcus*, *Streptococcus*, and *Staphylococcus* spp. and does so more efficiently than any manual or automated system based on phenotype analysis (13, 27).

Although, as is discussed below, detection of antibiotic resistance can be easily coupled with refined bacterial identification, this example shows that knowledge of the biochemistry and genetics of a resistance system can greatly facilitate its detection.

Eukaryotic host. There is no international agreement on breakpoints for interpretation of in vitro antibiotic susceptibility tests (45). This is rather surprising in view of the fact that, although their relative incidences may vary depending upon the ecosystem considered, resistance genes and their bacterial hosts are similar worldwide. In addition, the same antibiotic families are used with analogous dosages, routes, and indications in the various countries. The net result of this lack of consensus is the multiplicity of critical values which define clinical categories (1, 5, 15, 25, 39, 44) that, sometimes, do not even overlap. Although subjective inter-

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pretation of the results is possible and under certain circumstances even advisable (see below), genotypic susceptibility relies on a unique experimental criterion: the presence or absence of a resistance determinant. This imposed technical fact should help in diminishing, and ideally suppressing, differences due to geographical origin of the clinical isolates.

Detection system. Comparative analysis, with the same collection of selected strains, of systems for in vitro testing of bacterial susceptibility to antibiotics indicated a large disparity in the results obtained, although the breakpoints for clinical categorization were kept uniform (13). This variability results from technical differences, mainly culture medium (composition solid, semisolid, or liquid), inoculum (size and physicological state), end points and optical system for reading, and drugs tested; certain antibiotics, often displaying an intrinsic activity lower than that of the other members of the family, are more likely to show resistance. An example of the influence of the antibiotics tested is provided by mutation *nalC* in members of the family *Enterobacteriaceae*. This event confers low-level resistance to quinolones but supersusceptibility to molecules containing a piperazine moiety (35). Since it is not possible to test all the members of the rapidly growing family, generally only a few quinolones are included in antibiotic susceptibility tests and the result obtained is considered to be valid for the majority of the other drugs in the group. In these conditions, cinoxacin, flumequin, nalidixic acid, oxolinic acid, piromidic acid, and rosoxacin detect *nalC*, whereas ciprofloxacin, norfloxacin, oxfloxacin, and pipemidic acid do not. These various error factors do not exist with molecular biology techniques that can be more easily standardized, in particular when automated. In addition, the sensitivity of PCR is such that, in the near future, this technique will detect a single bacterium as starting material (4), allowing analysis of samples in the absence of any need to culture them.

IMPLICATIONS

Genotypic versus phenotypic resistance. Genotypic resistance results from mutations or acquisition of foreign genetic information; it is defined relative to the parental strain considered as susceptible. Phenotypic resistance relates to arbitrarily chosen breakpoints and depends upon the experimental conditions. For example, susceptible enterobacteria grown aerobically are susceptible to aminoglycosides but, like anaerobes (6), are resistant when grown under anaerobic conditions. Genotypic resistance, in particular when due to the acquisition of exogenous DNA, most often correlates with clinical resistance (14).

Intermediate category. As mentioned above, the genotypic approach gives only yes (presence of a resistance gene) or no (absence of a resistance gene) answers and results therefore in the disappearance of the intermediate category. In phenotypic studies, this clinical category is largely due to technical variability and knowledge uncertainty. This class can be recreated in the case of a newly detected resistance mechanism or a recently launched antibiotic belonging to a new family for which no feedback from the results of therapy is yet available. A clinical category of this sort must not be confused with interpretation of the results to account for relative resistance, which can be overcome by increasing drug dosage or differential expression of resistance depending upon the bacterial host or upon the site of infection. For instance, penicillin resistance following mutational alteration in *Neisseria* spp. can be easily overcome by increased dosages in *N. gonorrhoeae* but not *N. meningitidis* (28).

Detection is independent of regulation of gene expression. Antibiotic resistance can be expressed constitutively (e.g., aminoglycoside resistance in gram-positive and gram-negative bacteria) or be inducible by subinhibitory concentrations of drugs (e.g., resistance to macrolide-lincosamide-streptogramin B-type [MLS] antibiotics by production of an rRNA methylase, resistance to glycopeptides in enterococci). In the latter situation, testing with an antibiotic which is a poor inducer or with too high concentrations of a good inducer can lead to failure to detect resistance. Probing for the presence of structural genes for resistance avoids this type of major discrepancy.

LIMITATIONS

Intrinsic resistance. The design of oligonucleotide probes for hybridization or of primers for PCR requires detailed knowledge of the resistance mechanism to be detected and of the corresponding genes. Unfortunately, bacterial intrinsic resistance to antibiotics is often poorly understood or has not been studied, although it can be of clinical importance (e.g., *Leuconostoc* species and glycopeptides). It cannot therefore be screened for by DNA molecular techniques. Alternatively, this type of resistance can be undetectable because of lack of a specific target as in the case of impermeability to the drugs (e.g., low-level MLS resistance in members of the family *Enterobacteriaceae*). However, since this resistance is by definition genus specific, it can be inferred from bacterial identification. For example, all *Pseudomonas aeruginosa* strains are intrinsically resistant to narrow-spectrum cephalosporins and all *Campylobacter jejuni* and *C. coli* strains are intrinsically resistant to trimethoprim.

Mutations. Although, as mentioned above, oligonucleotides can easily detect point mutations in total-cell DNA (26), the existence of multiple loci leading to resistance by mutation in a housekeeping structural (e.g., encoding DNA gyrase, transcriptase, or porins) or regulatory (e.g., *ampR*) gene does not favor a molecular approach for detection of this pathway to resistance. The PCR technique is also inadequate to detect such events unless, by chance, the mutation causes a mismatch at the 3′-hydroxyl end of one of the amplification primers. Alternatively, mutations within the amplified gene can be detected by denaturing gradient gel electrophoresis (34). This limitation in detection of single-base changes applies primarily to bacterial genera (e.g., *Mycobacterium* spp.) or antibiotic families (ansamycins, polypeptides, quinolones) in or against which acquired plasmid-mediated antibiotic resistance has not yet been detected. However, information at the gene level on these resistance systems is scarce, and it may well turn out that, as in the case of extended-spectrum β-lactamases (22, 37), a few genetic loci only are responsible for resistance. The fact that amino acid substitutions in subunits A and B of *Escherichia coli* DNA gyrase responsible for high-level and low-level quinolone resistance are located within a small region of each subunit and that half of the *gyrA* mutations occur at the same locus is compatible with this notion (46). Most interestingly, in the organisms studied so far, *E. coli*, *Staphylococcus aureus*, and *Bacillus subtilis*, the substituted residues are located in regions of extended peptide homology and four of them are conserved in the three species (17). It therefore appears that a set of oligonucleotides should allow detection of high- and low-level quinolone resistance in both gram-positive and -negative bacteria.

Unknown genes. Despite extensive study of bacterial re-

sistance to antibiotics, certain resistance determinants in human pathogens must have escaped detection. However, study of the prevalence of resistance by nucleic acid hybridization should rapidly reduce the number of unknown genes since it allows detection of genetic information that escapes the current detection techniques (e.g., the bla_{tem-13} gene encoding penicillinase TEM-13 in members of the family *Enterobacteriaceae* [22]). Also, and most importantly, the ability of PCR to amplify genes belonging to different hybridization classes and encoding isozymes allows detection of resistance determinants not yet characterized (3, 21).

Silent genes and pseudogenes. The existence of genes that are not phenotypically expressed (24) can lead to false-positive results (27). Because molecular biology techniques have been applied only recently to the epidemiology of resistance, the incidence in nature of such silent or remnant genetic structures is not known.

ADVANTAGES

Clonal analysis. As mentioned above, PCR can provide simultaneous identification and antibiotic susceptibility results on a single cell that does not even need to be viable, a likely circumstance after antibiotic therapy. This ultimate sensitivity allows exploration of the genome of a clinical isolate exactly as it was in the biological sample rather than that of its progeny, often following numerous generations. This direct approach avoids variation in resistance, acquisition by mutation or loss by plasmid curing, of the human pathogen studied during subculture. This phenomenon is not rare and can in fact lead to interesting observations (12).

As for breakpoints, there is no international agreement on a culture medium for in vitro antibiotic susceptibility testing. Although Mueller-Hinton medium is the most widely used, it is not entirely defined and differences in composition and variability in production constitute an important source of result fluctuation in classical techniques (13). Growth steps can also constitute a source of error in the evaluation of the activity of certain families of antibiotics (e.g., trimethoprim and sulfonamide, because of the presence of thymidine in the medium). Bacterial species causing infections that would most benefit from lack of growth in detection of antibiotic resistance genes include the slowly growing bacteria *Mycobacterium* spp.; the fastidious bacteria anaerobes, *Borrelia burgdorferi*, *Campylobacter* spp., capnophilic gram-negative bacilli (*Haemophilus* spp., *Cardiobacterium hominis*, *Actinobacillus actinomycetemcomitans*, *Eikenella corrodens*, and *Kingella kingae*), *Chlamydia* spp., *Helicobacter pylori*, *Leptospira* spp., *Mycoplasma* spp., *Neisseria* spp., *Streptococcus pneumoniae*, and *Ureaplasma urealyticum*; the in vivo cultivable bacteria *Rickettsia* and *Treponema* spp.; and the "dangerous" bacteria *Brucella* spp., *Francisella tularensis*, and *Mycobacterium tuberculosis*.

Adjustable sensitivity. In the PCR technique, the level of detection can be controlled by adjusting the reaction conditions and the number of cycles during which the reaction is allowed to proceed. This feature can be of particular interest with certain samples (e.g., urine) with which a minimum bacterial count is required before the sample is considered infected. Conversely, PCR has been made quantitative (43), and it will be possible to quantitate the number of cells present in a clinical sample, which may be useful to monitor certain infections.

Rapidity. In particular, because of lack of bacterial growth, the period of time required for genotypic detection of resistance genes can be as short as 4 h. This characteristic is important in case of therapeutic emergency with antibacterial agents (i.e., meningitis in a patient allergic to β-lactams, neonatal meningitis, septic shock, septicemia due to gram-negative bacteria, and staphylococcal infections). It may also turn out to be useful for one-time treatment of urethritis and urinary tract infections.

Bypass of gene expression level. It has recently been demonstrated that, as for penicillinases (9), the level of resistance conferred by extended-spectrum β-lactamases of the TEM type depends upon the promoter strength of the structural gene for the enzyme (36). When a weak promoter is present, the use of certain cephalosporins does not allow the detection of phenotypic resistance (36) whereas the host is resistant to virtually all members of the antibiotic family (18). As in the case of inducible resistance, detection of the resistance gene ignores this type of difficulty. Difference in the promoter strength for TEM-type enzymes is due to a single base-pair change (9) or to insertion of an IS-like element in the promoter consensus sequence (16). Therefore, a TEM-universal probe or PCR using appropriate amplification primers combined with oligonucleotides centered on the mutation or internal to the IS-like element can not only detect the resistance mechanism but also provide information on the quantity of enzyme produced. Alternatively, a gene dosage effect due to copy number of a gene or a replicon can be detected by quantitative PCR. In addition, a set of oligonucleotide probes can identify the enzyme variant present, even when two isozymes reside within the same cell (22).

Mechanisms difficult to detect. Methicillin resistance in *Staphylococcus* spp. and, more recently, low-level glycopeptide resistance in *Enterococcus* spp. (32), for example, are difficult to detect by classical methods. The mere multiplicity of techniques for detection of the former mechanism (14) indicates that none of them is satisfactory. Easy detection of *mecA*, the gene for the inducible additional penicillin-binding protein present in methicillin-resistant strains, allows rapid and unambiguous characterization of one of the most clinically important resistance mechanisms.

Pitfalls in phenotypic detection. In staphylococci and enterococci, with the exception of streptomycin, modifying enzymes account for aminoglycoside resistance and the structural genes for the enzymes are highly conserved (27). When tested by assays evaluating the bacteriostatic activity of antibiotics, the presence of an enzyme confers on the host in vitro resistance to the aminoglycosides that are modified, but with two major exceptions: amikacin and netilmicin (20). Modification of the two drugs by the cells apparently does not affect their bacteriostatic activity, whereas their bactericidal activity and the bactericidal synergy they usually display when combined with β-lactams are abolished (20). Therefore, in vitro determination of the bacteriostatic activities of amikacin and netilmicin is misleading and should not be performed. By contrast, probes appear to be specific and there is an excellent agreement (equal to or greater than 99.8%) between the enzyme contents of the strains and the hybridization results (27).

Masked resistance determinants. In gram-positive cocci, certain aminoglycoside-modifying enzymes, e.g., APH(3′) or ANT(4′), cannot be detected phenotypically when they coexist in the same bacterium with activities having larger substrate ranges such as the bifunctional enzyme APH(2″)-AAC(6′) (27). DNA probes and PCR circumvent this difficulty and allow more accurate studies on the distribution of resistance mechanisms (27).

EXAMPLES OF APPLICATIONS

Genes successful in certain ecosystems. The gene *tet(M)*, responsible for cross-resistance to tetracycline and minocycline (7), has been detected in numerous bacterial species responsible for sexually transmitted diseases and urinary tract infections, such as *Gardnerella vaginalis*, *Haemophilus ducreyi*, *Mycoplasma hominis*, *N. gonorrhoeae*, *Streptococcus* spp. and *Ureaplasma urealyticum* (31). In the two latter species, *tet(M)* is the only gene responsible for resistance to tetracycline, an antibiotic considered the therapy of choice in genital infections. Detection of tetracycline resistance in the responsible, fastidious bacteria would thus certainly benefit from the genotypic approach. However, clinical samples in this type of infection always contain a heterogeneous mixture of bacteria. Since *tet(M)* is also widely disseminated in other bacterial species (e.g., *Clostridium difficile*, *Fusobacterium nucleatum*, *Peptococcus* spp., *Veillonella parvula*, *Eikenella corrodens*, and *Kingella denitrificans*, it is necessary to ensure that the resistance gene is harbored by the microorganism responsible for infection. As mentioned above, this can be achieved simultaneously by the same approach applied to bacterial identification that can be conveniently performed at various levels of specificity: gram, genus, or species. The link between both types of information, detection of resistance gene and host identification, could be established on the similarity of the absolute quantitative results in the two reactions performed. Detection of taxonomic and resistance genes in the same clinical sample by DNA-DNA hybridization has been reported (33). More simply, coamplification of the two types of genes using only two amplification primers could solve the problem of mixed cultures by demonstrating a physical link (presence on the same DNA fragment) of the two genes.

The genes *tet(M)* and *tet(O)*, originally detected in *Campylobacter* spp. and later in *Enterococcus* and *Streptococcus* spp., including group B streptococci, display 76% sequence similarity (38). A probe specific for *tet(M)* can therefore also detect *tet(O)* under moderately stringent conditions (23). However, this technical adjustment is not advisable since, under these conditions, interpretation of the results becomes subjective (40). The design of oligonucleotides that prime the amplification of the two resistance determinants would be more judicious.

Specific probes outside resistance genes. In an attempt to elucidate the sudden acquisition in 1977 and subsequent spread of multiple antibiotic resistance in *Streptococcus pneumoniae* in the absence of plasmids, we detected the transposon Tn*1545* in the chromosome of a clinical isolate (11). This element confers resistance to kanamycin and structurally related aminoglycosides by synthesis of an APH(3'), to MLS antibiotics by production of an rRNA methylase, and to tetracycline-minocycline because of the presence of a *tet(M)* gene. Tn*1545* is a member of a related family of elements that are self-transferable by conjugation to a variety of gram-positive bacteria in which they transpose. We recently demonstrated that integration of the conjugative tranposons requires a protein designated Int-Tn (29). Using probes intragenic to *tet(M)* and *int*, we have screened 47 strains of *S. pneumoniae* resistant to multiple antibiotics, including tetracycline, from various geographical origins. All the strains hybridized to *tet(M)* and all but one hybridized to the *int* probe (30). These results indicate that Tn*1545*-like transposons are responsible for dissemination of antibiotic resistance genes in *S. pneumoniae* and that a probe internal to a gene physically linked to resistance determinants and required for their dissemination can be used as a probe for detection of acquired resistance in a bacterial species.

CONCLUSION: RETURN TO THE SOURCE

Study of the genetics and biochemistry of resistance to antibiotics led to major discoveries in cell physiology in the early days of molecular biology. It is therefore only simple justice that, in turn, the most recent techniques of molecular biology, including the PCR, are applied to the detection and characterization of resistance to antibiotics in prokaryotes. One of the most interesting features of the genotypic approach to resistance is that it takes into account the knowledge accumulated, mainly during the last 15 years, on the mode of action and the mechanism of resistance to antibiotics. This method clearly does not constitute a universal answer to the problems of antibiotic therapy, in particular since the screening is oriented toward bacterial resistance whereas the basis for decisionmaking is susceptibility. However, and at a minimum, it should allow the ruling out of inappropriate therapy. This Cartesian method should lead to (i) international standardization of criteria for resistance and susceptibility, a prerequisite to valuable exchange of scientific information among medical microbiologists, (ii) improvement of classical techniques by allowing adjustment of breakpoints (27) until the disappearance of these arbitrarily chosen values, (iii) discovery of yet-undetected mechanisms of resistance (22), and (iv) better evaluation of new antibacterial agents (8).

By placing emphasis on resistance genes rather than on the host or the drug tested and by its ability to be general or exquisitely specific in terms of resistance mechanism, the genotypic approach leads to new concepts in medical microbiology. The most important, due to sensitivity of the techniques involved, is to consider bacteria not as massive populations of progeny but as individuals.

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REFERENCES

1. **Acar, J. F., E. Bergogne-Bérézin, Y. Chabbert, R. Cluzel, A. Courtieu, P. Courvalin, H. Dabernat, H. Drugeon, J. Duval, J. P. Flandrois, J. Fleurette, F. Goldstein, M. Meyran, C. Morel, A. Philippon, J. Sirot, C. J. Soussy, A. Thabaut, and M. Véron.** 1990. Communiqué 1989 du Comité de l'Antibiogramme de la Société Française de Microbiologie, p. 323–327. *In* P. Courvalin, H. Drugeon, J. P. Flandrois, and F. Goldstein (ed.), Bactéricidie. Maloine, Paris.
2. **Arthur, M., A. Brisson-Noël, and P. Courvalin.** 1987. Origin and evolution of genes specifying resistance to macrolide, lincosamide and streptogramin antibiotics: data and hypothesis. J. Antimicrob. Chemother. **20**:783–802.
3. **Arthur, M., C. Molinas, C. Mabilat, and P. Courvalin.** 1990. Detection of erythromycin resistance by the polymerase chain reaction using primers in conserved regions of *erm* rRNA methylases genes. Antimicrob. Agents Chemother. **34**:2024–2026.
4. **Atlas, R. M., and A. K. Bej.** 1990. Detecting bacterial pathogens in environmental water samples by using PCR and gene probes, p. 399–406. *In* M. A. Innis, D. H. Gelfand, J. J. Sninsky, and T. J. White (ed.), PCR protocols: a guide to methods and applications. Academic Press, San Diego.
5. **British Society for Antimicrobial Chemotherapy.** 1988. Breakpoints in in-vitro antibiotic sensitivity testing. Report by a working party of the British Society for Antimicrobial Chemo-

therapy. J. Antimicrob. Chemother. 21:701–710.
6. **Bryan, L. E., S. K. Kowand, and H. M. van den Elsen.** 1979. Mechanism of aminoglycoside antibiotic resistance in anaerobic bacteria: *Clostridium perfringens* and *Bacteroides fragilis*. Antimicrob. Agents Chemother. 15:7–13.
7. **Burdett, V., J. Inamine, and S. Rajogopalan.** 1982. Heterogeneity of tetracycline resistance determinants in *Streptococcus*. J. Bacteriol. 149:995–1004.
8. **Chabbert, Y. A., and A. Jaffe.** 1982. Sch 29482: activity against susceptible and β-lactam resistant variants of *Enterobacteriaceae*. J. Antimicrob. Chemother. 9(Suppl. C):203–212.
9. **Chen, S. T., and R. C. Clowes.** 1987. Variations between the nucleotide sequence of Tn*1*, Tn*2*, and Tn*3* and expression of β-lactamase in *Pseudomonas aeruginosa* and *Escherichia coli*. J. Bacteriol. 169:913–916.
10. **Conner, B. J., A. A. Reyes, C. Morin, K. Itakura, R. L. Teplitz, and R. B. Wallace.** 1983. Detection of sickle cell β^s-globin allele by hybridization with synthetic oligonucleotides. Proc. Natl. Acad. Sci. USA 80:278–282.
11. **Courvalin, P., and C. Carlier.** 1987. Tn*1545*: a conjugative shuttle transposon. Mol. Gen. Genet. 206:259–264.
12. **Courvalin, P., C. Carlier, and Y. A. Chabbert.** 1972. Plasmid-linked tetracycline and erythromycin resistance in group D "*Streptococcus*." Ann. Inst. Pasteur 123:755–759.
13. **Courvalin, P., J. P. Flandrois, F. Goldstein, A. Philippon, C. Quentin, and J. Sirot (ed.).** 1988. L'Antibiogramme automatisé. MPC/Vigot, Paris.
14. **Courvalin, P., F. Goldstein, A. Philippon, and J. Sirot (ed.).** 1985. L'Antibiogramme. MPC-Videom, Paris.
15. **Deutches Institut fur Normung.** 1984. Methoden zur empfindlichkeitsprufung von bakteriellen krankheitserregern (ausser Mykobakterien) gegen chemotherapia. DIN 58940. Deutches Institut fur Normung.
16. **Goussard, S., W. Sougakoff, C. Mabilat, A. Bauernfeind, and P. Courvalin.** Submitted for publication.
17. **Hopewell, R., M. Oram, R. Briesewitz, and L. M. Fisher.** 1990. DNA cloning and organization of the *Staphylococcus aureus gyrA* and *gyrB* genes: close homology among gyrase proteins and implications for 4-quinolone action and resistance. J. Bacteriol. 172:3481–3484.
18. **Jacoby, G. A., and I. Carreras.** 1990. Activities of β-lactam antibiotics against *Escherichia coli* strains producing extended-spectrum β-lactamases. Antimicrob. Agents Chemother. 34: 858–862.
19. **Leclercq, R., A. Brisson-Noël, J. Duval, and P. Courvalin.** 1987. Phenotypic expression and genetic heterogeneity of lincosamide inactivation in *Staphylococcus* spp. Antimicrob. Agents Chemother. 31:1887–1891.
20. **Leclercq, R., S. Dutka-Malen, A. Brisson-Noël, C. Molinas, E. Derlot, M. Arthur, and P. Courvalin.** Resistance of enterococci to aminoglycosides and glycopeptides. Rev. Infect. Dis., in press.
21. **Mabilat, C., and P. Courvalin.** 1989. Gene heterogeneity for resistance to macrolides, lincosamides and streptogramins in *Enterobacteriaceae*. Ann. Microbiol. (Institut Pasteur) 139:677–681.
22. **Mabilat, C., and P. Courvalin.** 1990. Development of "oligotyping" for characterization and molecular epidemiology of TEM β-lactamases in *Enterobacteriaceae*. Antimicrob. Agents Chemother. 34:2210–2216.
23. **Martin, P., P. Trieu-Cuot, and P. Courvalin.** 1986. Nucleotide sequence of the *tetM* tetracycline resistance determinant of the streptococcal conjugative shuttle transposon Tn*1545*. Nucleic Acids Res. 14:7047–7058.
24. **Mazodier, P., E. Giraud, and F. Gasser.** 1982. Tn*5* dependent streptomycin resistance in *Methylobacterium organophilum*. FEMS Microbiol. Lett. 13:27–30.
25. **National Committee for Clinical Laboratory Standards.** 1984. Performance standards for antimicrobial disk susceptibility tests. M2-A3. National Committee for Clinical Laboratory Standards, Villanova, Pa.
26. **Ouellette, M., J. J. Rossi, R. Bazin, and P. H. Roy.** 1986. Oligonucleotide probes for the detection of TEM-1 and TEM-2 β-lactamase genes and their transposons. Can. J. Microbiol. 33:205–211.
27. **Ounissi, H., E. Derlot, C. Carlier, and P. Courvalin.** 1990. Gene homogeneity for aminoglycoside-modifying enzymes in gram-positive cocci. Antimicrob. Agents Chemother. 34:2164–2168.
28. **Pérez-Trallero, E., L. Aldamiz-Echevarria, and E. G. Pérez-Yarza.** 1990. Meningococci with increased resistance to penicillin. Lancet 335:1096.
29. **Poyart-Salmeron, C., P. Trieu-Cuot, C. Carlier, and P. Courvalin.** 1989. Molecular characterization of two proteins involved in the excision of the pneumococcal transposon Tn*1545*: homologies with other site-specific recombinases. EMBO J. 8:2425–2433.
30. **Poyart-Salmeron, C., P.Trieu-Cuot, C. Carlier, and P. Courvalin.** Submitted for publication.
31. **Roberts, M. C.** 1990. Characterization of the TetM determinants in urogenital and respiratory bacteria. Antimicrob. Agents Chemother. 34:476–478.
32. **Sahm, D. F., J. Kissinger, M. S. Gilmore, P. R. Murray, R. Mulder, J. Solliday, and B. Clarke.** 1989. In vitro susceptibility studies of vancomycin-resistant *Enterococcus faecalis*. Antimicrob. Agents Chemother. 33:1588–1591.
33. **Sanchez-Pescador, R., M. S. Stempien, and M. S. Urdea.** 1988. Rapid chemiluminescent nucleic acid assays for detection of TEM-1 β-lactamase-mediated penicillin resistance in *Neisseria gonorrhoeae* and other bacteria. J. Clin. Microbiol. 26:1934–1938.
34. **Sheffield, V. C., D. R. Cox, L. R. Lerman, and R. M. Myers.** 1989. Attachment of a 40-base-pair G+C-rich sequence (GC-clamp) to genomic DNA fragments by the polymerase chain reaction results in improved detection of single-base changes. Proc. Natl. Acad. Sci. USA 86:232–236.
35. **Smith, J. T.** 1984. Mutational resistance to 4-quinolone antibacterial agents. Eur. J. Clin. Microbiol. 3:347–350.
36. **Sougakoff, W., S. Goussard, and P. Courvalin.** 1988. The TEM-3 β-lactamase, which hydrolyzes broad-spectrum cephalosporins, is derived from the TEM-2 penicillinase by two amino acid substitutions. FEMS Microbiol. Lett. 56:343–348.
37. **Sougakoff, W., S. Goussard, G. Gerbaud, and P. Courvalin.** 1988. Plasmid-mediated resistance to third-generation cephalosporins caused by point-mutations in TEM-type penicillinase genes. Rev. Infect. Dis. 10:879–884.
38. **Sougakoff, W., B. Papdopoulou, P. Nordmann, and P. Courvalin.** 1987. Nucleotide sequence and distribution of gene *tetO* encoding tetracycline resistance in *Campylobacter coli*. FEMS Microbiol. Lett. 44:153–159.
39. **Swedish Reference Group for Antibiotics.** 1981. A revised system for antibiotic sensitivity testing. Scand. J. Infect. Dis. 13:148–152.
40. **Taylor, D. E.** 1986. Plasmid-mediated tetracycline resistance in *Campylobacter jejuni*: expression in *Escherichia coli* and identification of homology with streptococcal class M determinant. J. Bacteriol. 165:564–569.
41. **Tham, T. N., C. Mabilat, P. Courvalin, and J. L. Guesdon.** 1990. Biotinylated oligonucleotide probes for the detection and the characterization of TEM-type extended broad spectrum β-lactamases in *Enterobacteriaceae*. FEMS Microbiol. Lett. 69:109–116.
42. **Vliegenthart, J. S., P. A. G. Ketelaar-van Gaalen, and J. A. M. van de Klundert.** 1990. Identification of three genes coding for aminoglycoside-modifying enzymes by means of the polymerase chain reaction. J. Antimicrob. Chemother. 25:759–765.
43. **Wang, A. M., M. V. Doyle, and D. F. Mark.** 1989. Quantitation of mRNA by the polymerase chain reaction. Proc. Natl. Acad. Sci. USA 86:9717–9721.
44. **Werkgroep Richtlijnen Gevoeligheidsbepalingen.** 1981. Report: standaardisatie van gevoeligheidsbepalingen. Werkgroep, Richtlijnen Gevoeligheidsbepalingen, Bilthoven.
45. **World Health Organization.** 1982. WHO expert committee for biological standardization. Technical report series no. 673, p. 156–192.
46. **Yoshida, H., M. Bogaki, N. Nakamura, and S. Nakamura.** 1990. Quinolone resistance-determining region in the DNA gyrase *gyrA* gene of *Escherichia coli*. Antimicrob. Agents Chemother. 34:1271–1272.