The Garrod Lecture

Evasion of antibiotic action by bacteria

P. Courvalin*

Department of Biology, University of California San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0116, USA

Antibiotics have reduced the mortality from infectious diseases but not the prevalence of these diseases. Use, and often abuse, of antimicrobial agents encourages the evolution of bacteria toward resistance, often resulting in therapeutic failure. This evolution is due to the emergence of 'new' resistance mechanisms and to the spread of well-characterized mechanisms of resistance to the majority of bacterial species. Bacterial resistance can be intrinsic or acquired. Intrinsic resistance is species or genus specific and delineates the spectrum of activity of the antibiotic. Acquired resistance is present in only certain strains of a species or of a genus. The latter results from mutation in a gene located in the host chromosome or a plasmid or from acquisition of new genetic information by a bacterium, mainly by conjugation or transformation. In this review, recent developments in the understanding of biochemical mechanisms and the genetics of resistance is considered for the clinically important antibiotic families.

Introduction

I would like to thank the British Society for Antimicrobial Chemotherapy for the invitation to contribute to the series of lectures in honour of Professor Lawrence Garrod. I would also like to dedicate this lecture to Professor Yves-Achille Chabbert for introducing me to the study of antibiotic resistance. Over the years bacteria have developed numerous, and often elegant, ways to escape the action of antimicrobial agents. These consist of (i) mutational alteration of 'house keeping' genes not previously involved in resistance or of genes that already conferred resistance to certain antibiotics, (ii) modulation of expression of genetic information at the transcriptional or at the translational level, (iii) facilitation of horizontal gene transfer between phylogenetically remote genera and (iv) the design of smart and often integrated protein systems. In this review, certain of these mechanisms recently elucidated at the molecular level in human pathogens will be considered.

Better to be resistant than virulent

Intrinsic resistance or, more accurately, insensitivity, refers to a trait that is present in all the members of a given bacterial genus or species. This notion, however, like many others in biology, is relative. For example, Gram-negative bacteria, in particular

*Present address: Unité des Agents Antibactériens, CNRS EP J0058, Institut Pasteur, 28, rue du Dr. Roux, 75724 Paris Cedex 15, France.
Tel: +33-(1) 45 68 83 20; fax: +33-(1) 45 68 83 19.
Enterobacteriaceae, are considered as naturally resistant to macrolides (MICs of erythromycin from 2 to 256 mg/L). However, higher local antibiotic concentrations (0.5–6 mg/g of faeces) are obtained in the lumen of the intestinal tract after oral administration of recommended doses (Andremont & Tancrède, 1981). These bacteria can thus be considered as susceptible, in particular in the setting of selective decontamination of the gut or of prevention of travellers’ diarrhoea (Andremont & Tancrède, 1981; Andremont Sancho-Garnier & Tancrède, 1986). Similarly, despite the natural resistance of streptococci and enterococci to low levels of aminoglycosides (MICs from 32 to 128 mg/L), systemic infections due to these microorganisms can be successfully treated with combinations of aminoglycosides and β-lactams (Leclercq et al., 1992). However, intrinsic resistance, in particular when multiple, can account for the epidemiological success of certain ‘new’ pathogens. Implantation and dissemination of Acinetobacter spp. and of Pseudomonas spp. in ecosystems subjected to high antibiotic selective pressure (e.g. intensive care and burn units) and the progressive emergence in clinical settings of Leuconostoc mesenteroides, Enterococcus gallinarum, Enterococcus casseliflavus-Enterococcus flavescens, Pedicoccus pentosaceus and of certain species of Lactobacillus that are naturally insensitive to glycopeptides, certainly reflects the ‘tuning’ of these microorganisms to ‘antibiotic polluted’ ecosystems, despite their low degree of pathogenicity. This observation suggests that antibiotic resistance is more important than virulence factors for successful spread in an increasing number of ecological niches where elderly and immuno-compromised patients prevail. Interestingly, the notion of intrinsic resistance has recently evolved following the study of Gram-negative bacterial species. Certain resistances are not due to ‘passive’ impermeability but rather to ‘active’ efflux of the antibiotics (Nikaido, 1994; Poole, 1994).

Intrinsic resistance can be due to the presence of chromosomal, species-specific, genes that mediate antibiotic resistance, even if their raison d’être is clearly distinct. For example, in the Acinetobacter genus, structural genes for 6'-aminoglycoside acetyltransferases have been detected in every species studied so far (Rudant et al., 1994). It has been proposed that the primary substrate for these enzymes is the peptidoglycan that has a three dimensional structure similar to that of aminoglycosides (Rather et al., 1993). These genes are often present at a single copy per genome, expressed at low levels and thus confer resistance to low concentrations of antibiotics only (Ploy et al., 1994). This situation represents a pitfall in detection of resistance in clinical isolates. However, these genes are convenient not only for detection of the resistance they specify but also for identification of the host bacterium at the species level (Ploy et al., 1994), a feature particularly useful during epidemics. In motile enterococci, low-level vancomycin resistance is due to production of VanC ligases which catalyse synthesis of D-alanine-D-serine (Billot-Klein et al., 1994; Reynolds et al., 1994). This dipeptide is subsequently incorporated into peptidoglycan precursors that have less affinity for vancomycin than those ending in D-alanine-D-alanine. The structural genes for these ligases, as well as those for the D-alanine-D-alanine ligases, are of great help for detection of low-level vancomycin resistance of the VanC type as well as for speciation of clinically relevant enterococci (Dutka-Malen, Evers & Courvalin, 1995).

**Acquired resistance**

*Indigenous*

*Mutations.* The major advantage, in terms of dissemination of resistance, of mutations is that they are stably inherited by the progeny. Certain of their apparent
limitations can be easily overcome by bacteria: rarity, since bacterial populations are often very large and actively growing which favours the occurrence of errors in DNA replication; independence, since they often confer cross-resistance, albeit at various levels, to all the members of a given antibiotic family (e.g., quinolones after mutation in the gyrA gene or cephalosporins, except cephamycins, following a point mutation in a \( \text{bla}_{\text{TEM}} \) or \( \text{bla}_{\text{SHV}} \) gene) or can occur sequentially, as exemplified by multidrug resistant \textit{Mycobacterium tuberculosis}; and instability, since they can be deleterious for the host and are often only transiently required. However, dominance of the mutated trait (versus the wild-type allele) is, as for resistance due to acquisition of foreign DNA, strictly required. This explains why qualitative alterations mainly occur in the primary sequence of structural genes that direct the production of proteins that are strongly preferred targets for certain antibiotics (e.g., transcriptase for rifampicin, DNA gyrase for quinolones, ribosomal protein S12 for streptomycin). Mutations in chromosomal genes clearly represent the mechanism of resistance to antibiotics in species, such as \textit{Mycobacterium} spp, that are not known to exchange DNA under natural conditions.

Prokaryotic drug addicts. Mutants dependent for growth upon the presence of an antibiotic in the culture medium have been known for a long time. However, if after more than 30 years of vancomycin use, emergence of resistance to this antibiotic in enterococci came as a surprise, the appearance of vancomycin-dependent mutants was even more surprising. These variants can be easily selected in the laboratory (Gutmann et al., 1992) and are isolated with increasing frequency in clinical settings (Green et al., 1995; Rosato et al., 1995). They are likely to be missed since they grow only in the presence of the antibiotic. They presumably arise from a mutation in the chromosomal gene for the D-alanine-D-alanine ligase and therefore rely for survival on induction, by vancomycin, of production of an additional Van-type D-alanine-D-lactate ligase.

Exogenous

Bacteria can acquire foreign DNA by three mechanisms: transduction, transformation and conjugation (Mazodier & Davies, 1991). The role in nature of transduction may be limited to closely related species by the high degree of specificity of the adsorption step in bacteriophage invasion. Similarly, transformation may be confined to intrageneric transfer. In this process, short, usually intragenic, DNA fragments are transferred and are stabilized in the genome of the naturally transformable transcipient by homologous recombination. The latter step can occur efficiently between sequences that display less than 25% primary sequence divergence (Spratt et al., 1992). This mechanism accounts for the build up of mosaic genes responsible for penicillin resistance by production of hybrid penicillin binding proteins in \textit{Streptococcus pneumoniae}, \textit{Neisseria gonorrhoeae} and, more recently, \textit{Neisseria meningitidis} (Spratt et al., 1992).

Two types of genetic elements are self-transferable by conjugation: plasmids and transposons. Conjugative plasmids can efficiently transfer among Gram-positive or Gram-negative bacteria belonging to different genera but not between the two groups of microorganisms (Courvalin, 1994). This is due to the fact that their host range for replication is more narrow than that for transfer. They can, indeed, conjugate between the two groups of bacteria but cannot establish themselves stably by replication into the new host. Certain non-conjugative plasmids from Gram-positive bacteria that have
a σ type of replication can replicate in *Escherichia coli* (Goze & Ehrlich, 1980) whereas certain non-self-transferable plasmids from Gram-negative bacteria can replicate in Gram-positive microorganisms (Gormley & Davies, 1991). Combined together these two observations suggest that the trans-Gram gene transfer observed in nature (Lambert *et al.*, 1985) could conceivably occur by plasmid mobilization (Figure 1) or plasmid conduction (Figure 2). Consistent with this notion, plasmid transfer from Gram-positive to Gram-negative bacteria by mobilization has been obtained in an animal model, even in the absence of antibiotic selective pressure (Doucet-Populaire *et al.*, 1992).
Conjugative transposons of Gram-positive cocci represent an efficient mode of transfer of antibiotic resistance genes between phylogenetically distant bacterial genera. These compact elements have a very broad host range of transfer that include many Gram-positive species (Clewell & Flannagan, 1993) but also Gram-negative bacilli (Poyart-Salmeron, Celli & Trieu-Cuot, 1995), in the genome of which they integrate at very high frequencies. They thus combine the ‘selfishness’ of mutations, that are hereditary, and the ‘altruism’ of plasmids that are self-transferable by conjugation. Elements of this type can confer resistance to up to three families of antibiotics (Courvalin & Carlier, 1986) and, in the case of ‘jumbo transposons’, can possess a modular structure in which a ‘classical’ (most often composite) transposable element is integrated into a conjugative transposon (Le Bouguéneç, de Csepédés & Horaud, 1988).

Another type of genetic element, that could be tentatively designated ‘integrative plasmid’, is also present in Gram-positive bacteria. These structures, like conjugative transposons (Poyart-Salmeron et al., 1989), excise and integrate by a mechanism analogous to that of the temperate bacteriophages of Enterobacteriaceae but, unlike transposons, possess an origin of vegetative replication (Pernodet, Simonet & Guérineau, 1984). These episomes can, therefore, exist under two physical states: integrated, where they are part of the genome of the host, or free, as autonomously replicating plasmids.

Illegitimacy is the rule. The movements of the majority of the mobile elements can be considered to proceed via illegitimate recombination. This also holds true for conventional transposons, whether composite (flanked by insertion sequences) or members of the Tn3 family (Berg & Howe, 1989). This recombination mechanism allows integration of these structures in replicons that have a base composition notably distinct from that of the donor molecule. Since the guanine + cytosine content of genes tends to be species specific (Syvanen, 1994; Muto and Osawa, 1987), a feature that prevents their spread by transformation as already discussed, illegitimate recombination facilitates migration of DNA between evolutionary distant microbes (Courvalin, 1994). In addition, and as will be discussed below, by allowing integration at multiple sites of the target replicon, illegitimate recombination modulates expression of the jumping genes.

The ultimate level of organization for migrant genetic information is provided by integrons. In this system, isolated gene ‘cassettes’ can be integrated by site-specific recombination in a genetic unit that, in addition to the recombination system, also provides a promoter for expression of the incoming DNA (Hall & Stokes, 1993; Hall & Collis, 1995). Integrons lead to the construction of resistance operons that are often part of transposons themselves located on self-transferable plasmids (Mabilat et al., 1992).

Dissemination of antibiotic resistance under natural conditions is often due to a combination of gene transfer systems acting in parallel or in series. For example, the apparently recent emergence of antibiotic resistance in *Listeria* spp. is due to acquisition of self-transferable plasmids (Poyart-Salmeron et al., 1990) or conjugative transposons (Poyart-Salmeron et al., 1992), both types of elements originating in enterococci-streptococci. Dramatically efficient dissemination among enterococci of high-level resistance to glycopeptides is due to transposition of elements containing the *vanA* operon on self-transferable plasmids followed by intra- and inter-specific conjugation (Arthur et al., 1993).
Modulation of resistance gene expression

After having examined the qualitative changes (mutations or acquisition of foreign DNA) that take place in the genome of a prokaryote and can lead to resistance to antibacterial agents, I will consider the quantitative changes that result in expression or enhanced expression of resistance genes.

Modulation at the transcriptional level

Integration of an insertion sequence can lead to decoding of genetic information i.e., expression of a previously silent gene or of a gene cluster. This can occur with an indigenous gene, as in the case of carabapenem resistance by production of a β-lactamases in Bacteroides fragilis (Podglajen, Brevil & Collatz, 1994), or following acquisition of a foreign gene as in the case of high-level erythromycin resistance in E. coli by a modification of the drug target (Arthur & Courvalin, 1993). In both instances the movable element provides the gene with a functional promoter.

Two component regulatory systems that are common in the prokaryotic world (Hoch & Silhavy, 1995) represent an elegant solution to inducibility of resistance. Such transcriptional switches have recently been found to be responsible for induction of glycopeptide resistance in enterococci by both vancomycin and teicoplanin in VanA-type strains (Arthur, Molinas & Courvalin, 1992) and by vancomycin only in VanB-type isolates (Evers & Courvalin, 1996). In the latter strains, constitutive mutants resistant to both glycopeptides can be isolated readily, either in vivo in patients, even in the absence of selective pressure, (Hayden et al., 1993) or in the laboratory (Gutmann et al., 1992). Molecular studies of these mutants may shed some light on the precise mechanism of induction as well as on the induction signal, which remains unknown.

The genomic environment also plays some role in resistance gene expression. This is particularly relevant for genes that are part of transposable elements. Phenotypic expression of these genes relies heavily, not only on the nature of the primary sequence (presence of transcriptional start and stop signals), but also on the topology of the flanking target DNA.

Amplification of genes, that are flanked by directly repeated sequences, can also increase the level of resistance due to gene dosage effect (Labigne-Roussel, Briaux-Gerbaud & Courvalin, 1983). However, the structures generated by this phenomenon are unstable (they can be deleted by homologous recombination) and this mechanism is thus better adapted to cope with stress conditions, such as profound but transient environmental changes, associated with selective pressure.

Gene fusion also provides a mean for coordinated gene expression. When it fuses the 5′ portion of a gene with an intact open reading frame (ORF) it allows the ORF to borrow the promoter for that gene. Alternatively, the in-vivo fusion of two genes can result in an extended spectrum of antibiotic resistance. This is particularly striking in the case of the APH(2")-AAC(6′) bifunctional enzyme of Gram-positive cocci (Ferrette, Gilmore & Courvalin, 1986). Fusion of the genes specifying a 2"-O-phosphotransferase and a 6′-N-acetyltransferase leads to resistance by inactivation of all commercially available aminoglycosides.
Translation attenuation

Post-transcriptional regulation is a mechanism of modulation of genetic expression that is very common in nature. In Gram-positive bacteria, it accounts for inducibility of resistance to macrolides, lincosamides and streptogramin B-type antibiotics (drugs that are not related in structure), the so-called MLSB phenotype (Leclercq & Courvalin, 1991a), and for chloramphenicol resistance by production of an acetyltransferase (Lovett, 1994).

Independence of regulatory and structural regions

Gene regulatory regions and ORFs have to be combined in a precise manner to give rise to expression of genetic information. They represent, however, different functional entities that have a certain degree of freedom. This is obvious, as already discussed, in the case of insertion sequences that contain a promoter in their 3' portion or of integrons. This also holds true for gene fusions, an observation that led to the success of ‘reporter genes’ in molecular biology (Arthur et al., 1993). Similar genetic engineering in nature leads to induction of chloramphenicol resistance by macrolide antibiotics (Rogers, Ambulos & Lovett, 1990). This surprising combination is achieved by the fusion of a leader peptide for induction of the MLSB-type of resistance with an ORF specifying a chloramphenicol acetyltransferase.

Contribution of the level of gene expression to bacterial resistance to antibiotics is exemplified by the emergence of extended spectrum β-lactamases in Gram-negative bacteria. These enzymes, which confer resistance to penicillins and cephalosporins (except cephemycins), are point-mutant derivatives of penicillinases belonging to the TEM or SHV family (Sougakoff et al., 1988). In the TEM lineage, two penicillinases (TEM-1 and TEM-2), that differ by a single amino acid substitution that has no consequence on enzyme activity, are the progenitors of the numerous variants recently detected in clinical isolates (Mabilat & Courvalin, 1990). Although in nature TEM-1 is, by far, more common, the majority of extended spectrum β-lactamases derive from TEM-2 (Davies, 1994). This is consistent with the fact that the TEM-2 promoter is stronger than that for TEM-1 and with the observation that the level of resistance achieved by β-lactamases correlates with the amount of enzymes produced by the host cells (Sougakoff et al., 1988).

An example of combination of qualitative (in the primary sequence of the enzyme) and of quantitative (in the level of enzyme production) changes in genetic information to achieve high-level resistance to a large variety of β-lactams is provided by TEM-6 (Goussard et al., 1991). The structural gene for this β-lactamase exhibits, relative to that of TEM-1, two mutations resulting in amino acid changes that considerably enlarge the enzymic substrate range. In addition, insertion upstream from the gene, of an IS-1-like element generates a strong, nearly consensus, promoter. The net result of these genetic events is resistance of the host to high levels of all β-lactams, except cephamycins and carbapenems.

Combinatorial gene transfer

Successful dissemination of genes in nature is often the result of a combination of transfer mechanisms. It has been proposed that the flux of resistance determinants
observed between Gram-positive cocci and Gram-negative bacteria results from transfer by conjugation of broad-host range plasmids followed by transposition in the recipient and stabilisation of the incoming DNA (Courvalin, 1994). Similarly, dissemination of the vanA gene cluster responsible for high-level resistance to glycopeptides in enterococci is probably due to the same two mechanisms but in the opposite relative order: transposition of Tn1546-like transposons on self-transferable plasmids followed by spread by conjugation (Arthur et al., 1993). Interestingly, detailed structural analysis of Tn1546 suggests a modular construction of the element by recruitment of genes from various sources (Arthur et al., 1993). This process, which allows the build up of very compact structures able to accomplish distinct functions (transposition, antibiotic resistance by redirected synthesis of peptidoglycan, induction of resistance) has already been proposed for the genesis of other elements (Rowland & Dyke, 1990).

Bacterial rendezvous
Transfer of genetic information, not only by conjugation but also by transformation, implies a close contact between donor and recipient bacteria. This prerequisite indicates that bacteria should share certain ecosystems, such as the gut of mammals, for horizontal DNA exchange to occur. This is not a limiting factor for bacteria belonging to a given species or genus that have the same habitat. It can be, however, of importance for phylogenetically distant bacteria and adds to other barriers for genetic exchange such as restriction endonucleases, limitation in heterologous gene expression, low DNA homology that prevents recombination, narrow host range of plasmid transfer or replication, etc. The ecology of bacteria may explain why plasmid-mediated vancomycin resistance, although detected in enterococci 10 years ago (Leclercq et al., 1988), has not yet emerged in staphylococci despite the impressive selective pressure exerted in hospital wards. There is no mechanistic limitation to such a transfer, which has been obtained under laboratory conditions (Noble, Virani & Cree, 1992). However, enterococci, that are usually present in the digestive tract, and staphylococci, that are skin commensals, lack a meeting point. Thus, it may well be such transfer of genetic information, which is predictable since resistance determinants in enterococci and staphylococci are very similar (Ounissi et al., 1990), will take place in the perianal region of patients receiving therapy with oral vancomycin (or other antibiotics active against Gram-positive bacteria that are subject to resistance by plasmids that also confer glycopeptide resistance). For similar reasons, recent acquisition of resistance genes by Listeria spp. may have occurred from enterococci in the vagina (Quentin et al., 1990).

Contribution of antibiotics to gene spread
Antibiotics are responsible for dissemination of resistance determinants by mechanisms more sophisticated than simple direct selective pressure. The first example of this is the enhancement by erythromycin of the intracellular movements of Tn917 (Tomich, An & Clewell, 1980) which confers resistance to erythromycin and related antibiotics. Even more dramatic is the facilitation of gene transfer among bacteria by antibiotics. Tetracyclines have been implicated in induction of transfer of their own resistance in anaerobes (Privitera, Sebald & Fayolle, 1979) and antibiotics active at the cell wall facilitate gene transfer between Gram-negative and Gram-positive bacteria (Trieu-Cuot, Derlot & Courvalin, 1993).
A recent and stimulating hypothesis is that commercially available antibiotic preparations promote resistance to the drug they contain because they are contaminated with microorganism DNA (Webb & Davies, 1993). Antibiotic producing organisms, in order to protect themselves from being killed by the secondary metabolites they produce, often encode modifying enzymes similar to those responsible for resistance in human pathogens. The finding, in antibiotic preparations, of DNA pieces that encode resistance mechanisms led to the suggestion that prescription of antibiotics results in direct dissemination of resistance determinants in humans (Webb & Davies, 1993).

Co-selection

For emergence of resistance. It needs to be kept in mind that treating a patient with an antibiotic results in a strong selective pressure on the entire bacterial flora of the patient and not only on the (putative) pathogen responsible for the disease. In addition, the microbial antagonisms observed in the intestinal tracts of humans and experimental animals can be affected, to various extents, by antimicrobial agents (Andremont, Raibaud & Tancrède, 1983).

For transfer of resistance. As already mentioned, plasmid-mediated resistance to glycopeptides was successfully conjugated in the laboratory from enterococci to S. aureus (Noble et al., 1992). However, in these experiments, transfer was obtained when transconjugants were selected by erythromycin but not by vancomycin. The conjugative plasmid confers resistance to both macrolides (constitutively) and glycopeptides and it appears that vancomycin, probably because of the inducible character of glycopeptide resistance, is a much poorer selective agent than erythromycin.

Recruitment of chromosomal genes

In Gram-negative bacilli, genes encoding class C β-lactamases are chromosomally located and often species specific (Bush, 1989). However, in recent years, transferable resistance to cephalosporins due to the presence of such genes on plasmids has been reported (Bauerfeind, Chong & Schweighart, 1989; Bauerfeind et al., 1990; Papanicolaou, Medeiros & Jacoby, 1990). This observation stresses that the distinction between indispensable (chromosomal) and dispensable (plasmid) genes is somewhat oversimplistic.

Subversion of chromosomal genes

Among the various MLS resistance determinants in staphylococci, msrA (macrolide streptogramin resistance) confers resistance by energy-dependent efflux of the antibiotics (Ross et al., 1990). The gene product, MsrA, is an ATP-binding protein member of the ATP-binding cassette (ABC) transporter/channel superfamily (Higgins, 1995). The MsrA protein does not contain the membrane-spanning domains that are typical of the other members of the family. Since, however, transfer of the cloned msrA gene alone is able to confer full phenotypic resistance to an heterologous staphylococcal host, it has been proposed that MsrA can divert, to its own benefit, a protein encoded by the chromosome of the new host (Ross et al., 1995).
Acquired glycopeptide resistance in enterococci is due to synthesis of peptidoglycan precursors ending in D-alanine-D-lactate, instead of D-alanine-D-alanine in susceptible strains (Arthur & Courvalin, 1993). Two genes only, which encode a D-dehydrogenase and a ligase of broad-substrate specificity, are required to redirect synthesis of a peptidoglycan that has no affinity for glycopeptides (Arthur & Courvalin, 1993). This parsimony, in terms of acquisition of foreign genetic information, is possible only because the isosteric new substrate, D-alanine-D-lactate, can be efficiently processed by chromosomally encoded proteins such as the adding enzyme, the transpeptidases and the carboxypeptidases.

**Gene decoding by transfer**

As discussed above, expression of genes previously silent can be achieved in an indigenous fashion, i.e., following a genetic event in the bacterial host. However, decoding can also result from heterologous expression following inter-generic transfer (Tietze & Brevet, 1991). This is exemplified by tetQ-mediated tetracycline resistance by efflux of the antibiotic in *E. coli*, whereas the same gene does not confer resistance to the anaerobe *B. fragilis* in which it was originally discovered (Speer & Salyers, 1989). Similarly, transposon Tn5, first found in *E. coli*, confers to this species resistance to kanamycin only, whereas, after transplantation into *Rhizobium meliloti*, it confers in addition resistance to streptomycin by directing synthesis of a 6-aminoglycoside nucleotidyltransferase (Mazodier, Giraud & Gasser, 1983). These observations raise the question of the authentic origin of these genes (Zilhao, Papadopoulou & Courvalin, 1988). They may well have been engineered in genera distinct from those where they were first detected and in which they are not expressed.

**Overlapping phenotypic resistance**

Studies at the genetic level of overlapping phenotypic resistance, indicated the frequent co-existence, within the same bacterium, of various determinants conferring resistance to the same antibiotic family. For example, staphylococci and enterococci often encode multiple aminoglycoside modifying enzymes with overlapping substrate ranges (Ounissi et al., 1990). This is also the case for acquired tetracycline resistance in streptococci-enterococci and in staphylococci. In the latter genus the tet genes act co-operatively to confer high-level resistance (Bismuth et al., 1990). This is in contrast with the situation in *Streptococcus-Enterococcus* spp. where similar genes do not contribute in an additive fashion to tetracycline resistance (Charpentier & Courvalin, 1995). This difference in level of resistance depending upon the bacterial host is not understood. Similarly, genes encoding an erythromycin esterase and a ribosomal methylase in *E. coli* act synergically to confer very high levels of resistance to the drug (Arthur & Courvalin, 1986). Interestingly, co-existence in the same strain of the two resistance mechanisms is due to occurrence of two, individually rare, genetic events, i.e., acquisition by Gram-negative bacteria of genetic information originating in Gram-positive cocci (Arthur & Courvalin, 1986; Brisson-Noël, Arthur & Courvalin, 1988).

Association of genes can also fight synergic combinations of antibiotics. The streptogramins, pristinamycin and virginiamycin (also designated synergistins), are composed of two structurally distinct molecules belonging to types A and B, that act
synergically. Curiously, the commonest genes in staphylococci encoding the enzymes, streptogramin A-acetyltransferase and streptogramin B-hydrolase, that confer resistance to these antibiotic mixtures are often found adjacent on the same plasmid (Allignet Aubert, Morvan & El Solh, unpublished data). This is particularly interesting since no single mechanism confers cross-resistance to the two groups of molecules (Leclercq & Courvalin, 1991a and b).

A high percentage of Enterobacteriaceae specify two variants of the TEM-family of β-lactamases. In the majority of the strains a TEM-1 or TEM-2 penicillinase co-exists with a mutant derivative exhibiting an enlarged substrate profile (Mabilat & Courvalin, 1990). This combination is particularly useful since the mutant enzyme confers resistance to a large variety of β-lactams, including the most recently developed cephalosporins but is super-susceptible to β-lactamase inhibitors, whereas the penicillinase, in particular when overproduced, confers resistance to β-lactamase inhibitors.

**Dramatic evolutions**

Recent years have experienced a dramatic increase in the prevalence and in the variety of antibiotic resistance traits (Neu, 1992) but also in the understanding of biochemical mechanisms of resistance (Davies, 1994) and of genetic means for dissemination of this resistance. The former evolution is clearly associated with use, misuse, and abuse of antibiotics but also with the discovery of new antibiotics, whereas the latter is largely due to the recent, impressive evolution in the techniques of molecular biology that one can apply to the study of prokaryotes. If enhanced resistance of bacteria has caused numerous and obvious problems it has also provided us with interesting findings, e.g. in genetics, transposons (conjugative or not), insertion sequences and integrons and, in biochemistry, post-transcriptional control. Despite their multiplicity, there is a certain degree of specificity in the distribution of resistance mechanisms: e.g. the success of mutations in *Mycobacteria* spp. However, there is unity in terms of energy saving: acquisition of DNA is minimized by ‘hijacking’ chromosomal genes (Arthur & Courvalin, 1993) and compact gene clusters are tightly regulated either at the level of transcription (Hall & Stokes, 1993) or of translation (Rogers et al., 1990).

**Acknowledgement**

I thank M. H. Saier Jr. for laboratory hospitality.

**References**

Allignet J., Aubert, S., Morvan, A. & El Solh, N. (1996). Distribution of the genes encoding resistance to streptogramin A among staphylococci resistant to this antibiotic. Submitted for publication.


P. Courvalin


(Received 18 October 1995; accepted 22 January 1996)