INTERNATL MICROBIOL (1998) 1:279–284

REVIEW ARTICLE

Rafael Gómez-Lus

Department of Microbiology, Hospital Clínico Universitario, Zaragoza, Spain

Received 25 June 1998
Accepted 25 July 1998

Correspondence to:
Department of Microbiology.
Hospital Clínico Universitario.
Domingo Miral, s/n.
50009 Zaragoza. Spain.
Tel.: +34-976761759. Fax: +34-976761693.
E-mail: gomezlus@posta.unizar.es

Evolution of bacterial resistance to antibiotics during the last three decades

Summary Bacterial resistance to antibiotics is often plasmid-mediated and the associated genes encoded by transposable elements. These elements play a central role in evolution by providing mechanisms for the generation of diversity and, in conjunction with DNA transfer systems, for the dissemination of resistances to other bacteria. At the University Hospital of Zaragoza, extensive efforts have been made to define both the dissemination and evolution of antibiotic resistance by studying the transferable R plasmids and transposable elements. Here we describe the research on bacterial resistance to antibiotics in which many authors listed in the references have participated. The aspects of bacterial resistance dealt with are: (i) transferable resistance mediated by R plasmids in Gram-negative bacteria, (ii) R plasmid-mediated resistance to apramycin and hygromycin in clinical strains, (iii) the transposon Tn*1696* and the integron In*4*, (iv) expression of *Escherichia coli* resistance genes in *Haemophilus influenzae*, (v) aminoglycoside-modifying-enzymes in the genus *Mycobacterium* with no relation to resistance, and (vi) macrolide-resistance and new mechanisms developed by Gram-positive bacteria

Introduction

Bacterial resistance to antibiotics in Gram-negative bacilli is most commonly mediated by R plasmids and by genes carried by transposons (Tn) and integrons (In). In Gram-positive cocci the conjugative chromosomal transposons are fundamental for antibiotic resistance. They are characterized by their related features to R plasmids, bacteriophages and the classic transposons (for instance, Tn*5* and Tn*10*). All these genetic elements play a central role in bacterial evolution, providing mechanisms to generate diversity and having DNA transfer systems able to disseminate sequences among other bacteria. Good examples of those phenomena are the participation of transposons in the rapid spread of antibiotic resistance among bacterial populations—above all in those causing nosocomial infections—and the role of integrons in the maintenance and spread of the *sul1* gene in enterobacteria.

Transferable resistance mediated by R plasmids in Gram-negative bacteria

At the University Hospital of Zaragoza and other hospital centres in Zaragoza, research has been carried out regarding the evolution and spread of antibiotic resistance over the last 30 years. We have studied conjugative R plasmids (tra^+), transposable elements and the biochemical mechanisms of resistance. In 1974 we characterized the plasmid pUZ1 (initially R1033) from a strain of *Pseudomonas aeruginosa* coming from the Regional Center for Traumatology, near the University Hospital of Zaragoza. The plasmid had a size of 68 kb. Its incompatibility group was P (IncP) and it was transferable to *Escherichia coli* J62 by conjugation. The plasmid coded for resistance to ampicillin, tetracycline, gentamicin, kanamycin, streptomycin, chloramphenicol, sulfamides and mercuric chloride [19]. The donor as well as the recipient strain coded for the enzymes TEM-1, AAC(3)-I, APH(3')-I, and ANT(3'') [9]. Plasmid pUZ1 contained two transposons, the already known Tn*3*, which carries the bla_{TEM-1} gene, and a new transposon, Tn*1696* [17], which bears the genes *aacC1*, *aadA1*, *sul1* and *mer*, which will be discussed later.

Four years later, we isolated different strains of enterobacteria and of *P. aeruginosa* (Table 1), which contained also plasmids of the IncP group, of the same size, with identical restriction patterns and enzymes as pUZ1 [6, 15]. These findings supported the hypothesis that R plasmids spread to proximal areas, with the advantage of being able to propagate between the Enterobacteriaceae and Pseudomonadaceae, which is characteristic of the IncP group.

In 1976 we detected a 73 kb plasmid which belonged to the group IncM and, thus, only transferable to enterobacteria. The plasmid conferred resistance to ampicillin, tetracycline, gentamicin, tobramycin and dibekacin and produced the enzymes TEM-1 and AAC(3)-V [14]. Following this, numerous strains of enterobacteria were isolated which also carried plasmids from the IncM group, with identical properties, genotypes and phenotypes (Table 2).

In one of the strains of *Proteus vulgaris* (strain 18182) two conjugative plasmids were detected. One was from the IncM group (pUZ3a) and the other from the IncP group (pUZ3b). The pattern of resistance transferred corresponds to that described in Tables 2 and 1, respectively. In addition, as a result of the epidemiological monitoring of the strains carrying R plasmids, we found that certain plasmids which were initially transferable (tra^+) later lost this capacity (tra^-). We also found plasmid loss or recombination of plasmid genes with the chromosome, which suggested mechanisms of transposition.

A case to point out was a transconjugant of *E. coli* (strain 3644) which had lost the plasmid it was carrying but had conserved its pattern of resistance. The alternative strategy was to use the plasmid pUZ8, lacking transposable elements, and introduce it into the aforementioned transconjugant of *E. coli* J62 (F^- *his lac nal*^{R} *pro trp*). Through conjugation with *E. coli* J53 (F^- *met pro rif*^{R}), a plasmid (pUZ3644) was obtained which contained a 27 kb fragment, carrying the bla_{TEM-1} and *aacC5* genes, flanked by two copies of an insertion sequence, IS*140*. In this segment, the composite transposon Tn*2922* was characterized. Tn*2922* is a co-integrate capable of transposing as a unit and formed by the fusion of the genes *tnpR*, bla_{TEM-1} and *aacC5* [10]. These two genes make up a transcription unit initiated by the bla_{TEM-1} gene promoter, which is responsible for the mobilization of one of the IS*140*.

The capacity for transposition between replicons containing the *bla* genes is noteworthy, and it is reflected in the numerous plasmid incompatibility groups, in which the *bla* genes have been detected: 18 for β-lactamase TEM-1, 7 for TEM-2, 3 for OXA-1, 4 for OXA-2, and 2 for OXA-3. We have already referred to part of our experiments involving the bla_{TEM-1} genes isolated from plasmids in the groups IncP and IncM. In France, the gene bla_{TEM-3} has also been characterized in R plasmids from the IncM group. This gene codes for plasmid β-lactamases of extended spectrum. Previously, in the same country, an IncC plasmid had been described which carried a bla_{TEM-4} gene coding for an extended β-lactamase spectrum, named TEM-4.

R plasmid-mediated resistance to apramycin and hygromycin in clinical strains

In scientific literature there are examples of antibiotic resistance genes that have been transported to man by bacteria of animal

Table 1 Incompatibility group P plasmids isolated in the Hospital Clínico Universitario, Zaragoza, from 1974 until 1978. Resistance pattern transferred to *Escherichia coli* K-12: Ap, Tc, Gm, Km, Cm, Sm, Su*

Donor strain	Date isolation‡	Origin	Hospital department	Plasmid ¶
P. aeruginosa CRT	2/74	Exudating wound	Traumatology	pUZ1
S. marcescens 965	5/76	Exudation	Surgery	pUZ7
K. pneumoniae 21938	7/76	Exudation	Surgery	pUZ9
K. pneumoniae 19932	5/76	Urine	Urology	pUZ14
K. pneumoniae 19990	6/76	Urine	Urology	pUZ15
S. marcescens 785	4/77	CSF**	Neurosurgery	pUZ270
S. marcescens 1018	7/77	Urine	Urology	pUZ350
S. marcescens 1706	3/78	Gastric juice	Pediatrics	pUZ613

* Ap, Tc, Gm, Km, Cm, Sm, Su: (ampicillin, tetracycline, gentamicin, kanamycin, chloramphenicol, streptomycin, sulfonamide).
‡ Month/year. ¶ Size of all plasmids: ca. 68 kb. ** Cerebrospinal fluid.

Table 2 Incompability group M plasmids isolated in the Hospital Clínico Universitario, Zaragoza, from 1976 until 1983. Resistance pattern transferred to *Escherichia coli* K-12: Ap, Tc, Gm, Tm, Dkb, Nt*

Donor strain	Date isolation‡	Origin	Hospital department	Plasmid¶
E. coli 15159	4/76	Exudation	Surgery	pUZ2
C. freundii 19162	5/76	Urine	Surgery	pUZ4
K. pneumoniae 17434	4/76	Urine	Urology	pUZ5
E. coli 20092	6/76	Urine	Urology	pUZ6
P. mirabilis 23861	9/76	Urine	Internal medicine	pUZ21
K. pneumoniae 22829	9/76	Urine	Urology	pUZ24
E. coli 11607	9/76	Urine	Urology	pUZ25
E. coli 28487	10/76	Urine	Pediatrics	pUZ27
E. cloacae 3674	8/80	Urine	Internal medicine	pUZ1338
K. pneumoniae 3684	8/80	Urine	Pediatrics	pUZ1321
S. marcescens 4945	2/82	Urine	Surgical ICU**	pUZ1863
E. cloacae 5051	3/82	Bronchial aspirate	Internal medicine	pUZ1894
A. calcoaceticus 5726	2/83	Bronchial aspirate	Medical ICU**	pUZ2145

* Ap, Tc, Gm, Tm, Dkb, Nt: (ampicillin, tetracycline, gentamicin, tobramycin, dibekacin, netilmicin).
‡ Month/year. ¶ Size of all plasmids: ca. 73 kb. ** Intensive care units.

origin. It is well known that the correct use of antibiotics in veterinary medicine is of great concern, as it was reported in the Swann Report (1969) (Report of Joint Comittee on the Use of Antibiotics in Animal Husbandry and Veterinary Medicine, Her Majesty's Stationery Office, London [1969]). Such a transfer happened with the use of the aminoglycosides apramycin and hygromycin in cattle and pigs respectively. In fact, through their application, resistant strains emerged, which were isolated from animals which had undergone treatment. The resistance was plasmid mediated, and genes were detected for apramycin-acetyltransferase [AAC(3)-IV] and hygromycin-phosphotransferase [APH-(4)-I] enzymes, which inactivated each of the respective antibiotics.

In 1989 we were able to confirm the isolation of two clinical strains (*Escherichia coli* and *Klebsiella pneumoniae*) resistant to the abovementioned aminoglycosides, with R plasmids of 110 kb which carried the *aacC4* and *aph4* genes. Subsequently, four strains of enterobacteria with similar properties were isolated. The analysis of these gene organizations showed that they were adjacent and grouped in the same orientation as those which had been isolated from animal origin. The two genes for resistance to apramycin and hygromycin form an operon and are associated to IS*140* sequences, which implies a transposable structure [18]. Given that the enzyme AAC(3)-IV inactivates gentamicin and tobramycin, in addition to apramycin, it constitutes a potential risk for selecting resistances in clinically used aminoglycosides. Furthermore, the six strains isolated, carrying the plasmids pUZ6734, pUZ6743, pUZ6776, pUZ7852 and pUZ7874, also showed transferable resistance to ampicillin (β-lactamase TEM-1) and streptomycin [phosphotransferase APH(3'')].

The transposon Tn1696 and the integron In4

While trying to characterize the mycobacterial version of the *aacC3* gene, the *sul3* gene was found, as well as the determinants *int* and *tnpA*. These discoveries allowed us to re-examine the organization of Tn*1696*. This 16 kb transposon carries the *aacC1* gene and the determinants of resistance to sulfamides (*sul1*), chloramphenicol (*cmlA*), streptomycin-spectinomycin (*aadA1*), and mercuric chloride (*mer*). These determinants are located in the integron In*4*, which has a site-specific recombination system that permits the capture of various cassettes of resistance genes [3]. Figure 1 shows the structure of several integrons, among them In*4*, including the cassettes of genes inserted in the variable region [9]. Near the recombination site (*res*), the *int* gene was found. It belongs to the family of integrases, enzymes essential for recombination. In addition, the *cmlA* gene, the determinant for non-enzymatic resistance to chloramphenicol, had a different feature: it has its own promoter. This does not occur in other integrons of Gram-negative bacteria.

Other genes have used this system, among them the most prominent are: *aadA2*, *aadB*, *aacA4*, *dhfrI*, *dhfrIIb*, *dhfrIIc*, *dhfrV*, *oxa1*, *oxa2*, *pse2*, etc. The majority of *dhfr* genes for resistance to trimethoprim are found near integrons that also carry the gene *sul1*, reflecting the joint clinical use of trimethoprim and sulfamethoxazol. In Tn*1696*, the close union of the *aadA* and *aacC1* genes stands out, which implies cross-resistance on the one hand to streptomycin and spectinomycin and on the other to gentamicin, sisomicin and fortimicin. The stable position of the resistance genes in an integron, present in an R plasmid, assures their dissemination and the capture of new genes.

The mechanism of integron mobility is not well known, but there exist at least three possibilities: (i) self mobility, which is deduced from the different genetic locations; (ii) by means of transposons of the Tn*21* family, in which case they are integrated; and (iii), horizontal transfer of integrons by means of R plasmids such as the IncP (pUZ1) and IncW (pSa and R388).

Expression of *Escherichia coli* resistance genes in *Haemophilus influenzae*

The efficiency of genetic mechanism of transference is reflected in the spread of multiple resistance to sulfonamides, β-lactam antibiotics, chloramphenicol, aminoglycosides, etc. The selection can occur simply through the use of one of the antimicrobial agents which affects all the genetic determinants. An interesting example is the resistance of *Haemophilus influenzae* to chloramphenicol in our geographical region (Aragón) (12%), despite limited clinical use of the drug. In contrast, 35% were resistant to ampicillin (Ap^R). And of the chloramphenicol resistant strains, all except one were also resistant to ampicillin. This resistance is mediated by β-lactamases TEM-1. We have not detected any β-lactamase of type ROB-1, supporting the previously suggested hypothesis of a different ROB-1 reservoir for β-lactam resistance such as *Actinobacillus pneumoniae*. The resistance to chloramphenicol is due to the synthesis of an acetyltransferase (CAT) which shares biochemical properties with *E. coli* CAT II and III. Some of these strains hybridized with a probe for *cmlA* (a gene present in the integron In*4* which is located in Tn*1696*), demonstrating permeability changes equivalent to those in *E. coli* and *P. aeruginosa* caused by the protein CmlA. In our laboratory, we found in *H. influenzae* the genes which code for the HI1254 protein, related to the polypeptide CmlA mentioned above, and for the HI1716 protein, which could participate in altering the permeability (Vergara Y, 1996, Ph.D. Thesis, University of Zaragoza). Both of these genes have been characterized in the genome of *H. influenzae* Rd. On the other hand, there is a low level of homology between the *cmlA* gene and the HI1254 protein, which is reflected in weak hybridization with the *cmlA*

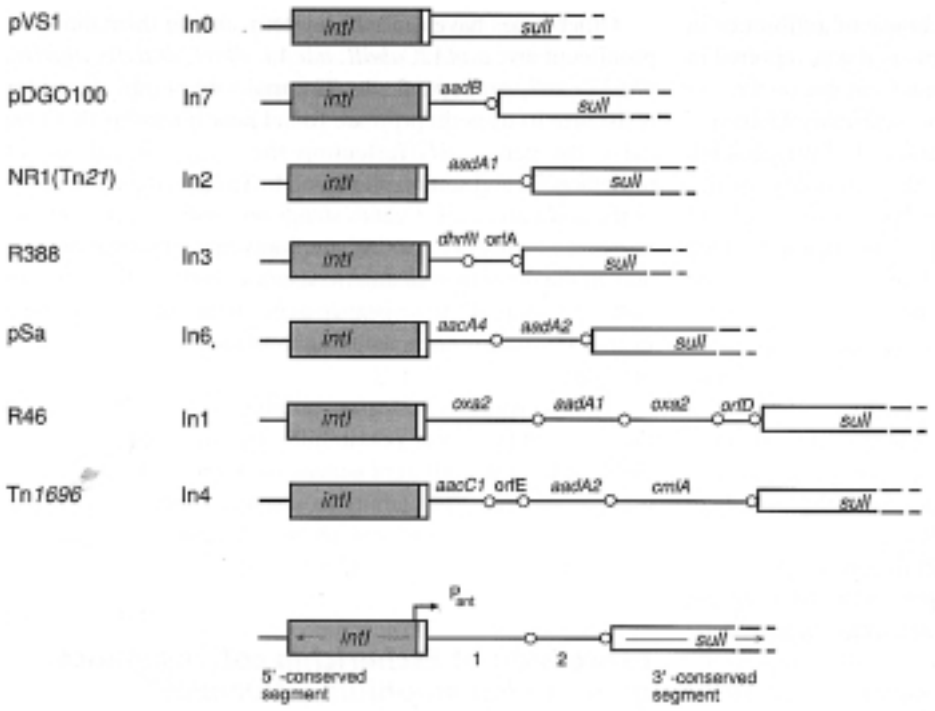

Fig. 1 Structural features of various naturally occurring integrons. Modified from reference [9]. *intI*: integrase gene situated in the 5' conserved segment; *sul1*: sulfonamide resistance gene situated in the 3' conserved segment; gene cassettes: *aadB*, *aadA1*, etc. with circles (O), which represent recombinaton sites of 59 bp

probe. 37.5% of the *H. influenzae* strains Ap^R and Cm^R were also resistant to kanamycin, neomycin and lividomycin through the production of a phosphotransferase APH(3')-I. Transfer of resistance was achieved by electroporation of a competent strain (*E. coli* DH5α) with the total DNA of *H. influenzae*, followed by selection with ampicillin and kanamycin. Using electroporation, 180 colonies of *E. coli* resistant to ampicillin and kanamycin were obtained. DNA was extracted from 9 of the colonies and in each a 7 kb plasmid was found. Restriction enzimes (*Eco*RI and *Pst*I) analysis demonstrated that the plasmids were identical. Crude enzyme extracts from the strains showed β-lactamase TEM-1 (isoelectric point, pI = 5.4) activity and 3'-O-phosphotransferase-[APH(3')-I] activity, which modified kanamycin, neomycin and lividomycin [8].

The purified phosphotransferase had a molecular weight of 26,000 Da and a pI = 5.3, with an aminoacid sequence at the aminoterminal extreme similar to other phosphotransferases in enterobacteria. Hybridizations with the intragenic probe *aph(3')-Ia*, demonstrated that the gene in *H. influenzae* is not located in the transposon Tn*903*, which is a feature that differentiates it from the situation in enterobacteria. In spite of this, it was obvious that the determinants of resistance to ampicillin, chloramphenicol and kanamycin came mostly from *E. coli*, with peculiarities in genetic reorganization and phenotypic expression that reflect the change in bacterial host. In the enterobacteria, the *catI*, *catII* and *catIII* genes are found mainly in IncM plasmids, whereas *cmlA* and related genes are in IncP plasmids. The coexistance of both types of genes in the genus *Haemophilus*, situated in plasmids and/or in the chromosome, shows the heterogeneity of genetic exchange with enterobacteria, and it seems probable that the species *H. parainfluenzae* acted as an intermediate.

Aminoglycoside-modifying-enzymes in the genus *Mycobacterium* with no relation to resistance

The discovery of strains of *Mycobacterium fortuitum* producing aminoglycoside modifying enzymes [AAC(6'), AAC(3) and APH(3')] and in particular acetylases subclass AAC(3)-III, has not been related to resistance nor to the presence of plasmids. A new enzymatic activity that phosphorylated only streptomycin (APH(6)) was demonstrated in strains of *M. fortuitum* and *M. chelonae*. Hybridizations with a probe from *Streptomyces griseus* (*aph-6* gene) were positive not only in the new strains that produced 6-O-phosphotransferase activity but also in 20 strains that did not possess such phosphorylating activity. This suggested that the *aph-6* gene was localized on the chromosome, that could be phenotypically expressed or not, and that did not have any relation to streptomycin resistance. The investigation was extended to other fast growing species (*M. smegmatis* and *M. phlei*) and to slow growing species (*M. avium*, *M. scrofulaceum*, *M. xenopi* and *M. terrae*), verifying in all a positive hybridization, although to a varying degree. Besides, studying a strain of *M. aurum*, the synthesis of an acetylase AAC(2') with a characteristic profile was detected. The substrates were gentamicin C1 and C1a, netilmicin, 6'-ethyl-netilmicin, tobramycin and kanamycin B, without modifying the 2'-deamino-kanamycins—kanamycin A and amikacin—nor the 2'-ethyl-netilmicin (Sánchez-Yangüela E, 1990, M.D. Thesis, University of Zaragoza). However, the

genes encoding acetylase AAC(3) subclass III or the related enzyme were not identified, although the enzyme was present in all the strains of *M. fortuitum* that were studied and in the majority of *M. chelonae*.

In order to detect and characterize the candidate gene *aac3*, probes derived from genes and transposons coding for antibiotic resistance were applied to samples of *M. fortuitum* chromosomal DNA. A positive hybridization was obtained with a probe containing the *aacC1* gene, localized in the previously mentioned transposon Tn*1696*. On determining the nucleotide sequence of the hybridizing region, similarities were detected at the 5' region of the *aacC1* gene. This region comprises the *int* gene, which encodes an integrase, a determinant for site specific recombination. The mycobacterial sequence also contained the *sul3* gene, a homologue to the *sul1* gene present in Tn*1696* and in other members of the Tn*21* family. The region occupied by the *sul3* gene was defined after verifying that it was flanked by two 880 bp sequences, which were called IS*6100*, and that it encoded a transposase. These mycobacterial insertion sequences contained inverted repetitions of 14 bp, which differed by only one nucleotide from IS*15*. The sequence was similar to IS*6*, and it is closely related to elements isolated from Gram-positive and Gram-negative bacteria. The *int* and *sul3* genes, marked by two IS*600* sequences, make up the transposon Tn*610* [11]. The codons of the IS*6100* transposase gene proved to be similar to those of a gene which encoded the main antigenic protein of *M. tuberculosis*. This suggests that this 65,000 Da protein originated in mycobacteria.

The presence of the *sul3* gene seemed obvious, since sulfonamides have been used since 1935 and were applied in the treatment of tuberculosis in the 1950's. Therefore, finding the gene in the *M. fortuitum* FC1 strain suggested a broad diffusion between this species and others from the genus *Mycobacterium*. To test whether this hypothesis was valid, hybridizations were carried out with a probe from the *int* + *sul* genes (1.4 kb, plasmid pIPC1::Tn*610*). All the hybridizations done on 30 strains of *M. fortuitum*, 21 strains of *M. chelonae* and 125 strains of *M. tuberculosis* were negative. It seemed obvious that we had been lucky in choosing *M. fortuitum* FC1. In fact, initially this strain was selected for its intense acetylating activity AAC(3), and it turned out to house the *int*, *sul3*, *aph-6* and *blaF* genes (Timm J, 1994, Ph.D. Thesis, University of Paris VII), and also, as we will describe, the *aac-2'-Ib* gene.

A gene encoding an AAC(2') was cloned from *M. fortuitum*. DNA sequencing results identified an ORF(*aac(2')-Ib*) coding for a putative protein with a predicted molecular mass of 28,400 Da [1]. The deduced AAC(2')-Ib protein showed homology to the AAC(2')-Ia from *Providencia stuartii*. The presence of the *aac(2')-Ib* in all 34 *M. fortuitum* strains was not correlated with any aminoglycoside-resistance phenotype. This suggested that the product of this gene could be implicated in functions other than antibiotic-modification, such as the synthesis of intermediate metabolites, ribosomal proteins, and cell wall components. The insertional activation of *aac(2')-Ib* in *M. smegmatis* mc^2 155 allowed the presence of the gene to be correlated with the resistance to lysozyme. Considering this, it is remarkable that the enzyme AAC(2')-Ia has been shown to contribute to the acetylation of peptidoglycan (PG) in *P. stuartii*. Presumably, aminoglycosides are not the true substrate of AAC(2')-Ia but are acetylated due to their structural similarity with PG [13]. As far as we know, the genes coding for aminoglycoside-modifying enzymes are not strictly speaking resistance genes in mycobacteria. Paradoxically, if these enzymes are implicated in the biosynthesis of the mycobacterial cell wall, they could act as targets for new antibiotics.

Macrolide resistance and new mechanisms developed by Gram-positive bacteria

The resistance of *Streptococcus pneumoniae* to penicillin is a serious problem in Spain. It has become more complicated since the 1980s with an increase in erythromycin resistance, which rose from 0.9% in 1979 to 10% in 1989. Although *Streptococcus pyogenes* remains sensitive to penicillin, in the last few years more and more strains resistant to macrolides have been identified. For this reason, epidemiological monitoring has become essential.

Of the mechanisms that these streptococci have developed for macrolide resistance, the two fundamental ones are the production of both methyltransferases, encoded by the *erm* (*A*, *B*, *C*, *TR*) genes [2], and the efflux systems, which are the phenotypic expressions of the *mefA* (*S. pyogenes*) and *mefB* genes (*S. pneumoniae*), and of the *msrA* (*Staphylococcus epidermidis* and *Staphylococcus aureus*) and *msrB* genes (*Staphylococcus xylosus*). The methylases encoded by the *erm* genes cause ribosomal modification at the level of 23S RNA subunit 50S, giving rise to the MLS_B phenotype (macrolides, lincosamides, streptogramin B), either inducible or constitutive. In our medium, the genes detected in Gram-positive cocci belonged to different classes depending on the genus: *Staphylococcus* (*ermA*, *ermC*, *ermA* + *ermC*), *Enterococcus* (*erm*B) and *Streptococcus* (*ermB*), the expression of which can be inducible or constitutive, irrespective of the class of *erm* gene [7].

The first description of an efflux mechanism against erythromycin was made in 1989, proving the existence of the MS phenotype (14 and 15 membered macrolides, and streptogramine B) as the expression of the *msr*A gene in *S. epidermidis*. One year later the gene was characterized and sequenced, demonstrating that it formed a complex with two other determinants, *smp* and *stp* [16]. In 1992 the *msr*B gene was cloned and sequenced from a plasmid from *S. xylosus*, which expressed the same phenotype [12]. Using initiators M1 and M2 in one strain of group A streptococcus, a 0.4 kb amplification product was obtained, a size compatible with the *msr*A and *msr*B genes. A gene which showed 98% homology to the genes characterized in *Staphylococcus* was cloned and sequenced from the DNA band (Adrián FJ, 1997,

Ph.D. Thesis, University of Zaragoza).

A new phenotype M has been described in *Streptococcus pneumoniae* and *S. pyogenes*, which produces resistance exclusively to 14 and 15 membered macrolides. Investigating this mechanism, a new class of genes was characterized called *mef* (for macrolide efflux): *mef*A in *S. pyogenes* and *mef*E in *S. pneumoniae* [4, 20]. In 1997 a new efflux gene *mre*A (for macrolide resistance efflux) was described in *Streptococcus agalactiae* [5].

In our laboratory, using primers specific for the *mefA* and *mefE* genes we have confirmed the efflux mechanism in the majority of M phenotype strains of the species *S. pyogenes*, *S. pneumoniae* and different species of the viridans group. The differentiating feature is that the *ermB* gene is much more prevalent in the *S. pneumoniae* population with MLS_B phenotype. Whereas in *S. pyogenes* the M phenotype expression of the *mefA* gene is dominant. The M phenotype is infrequent in pneumococci in our geographical region, in contrast to what has been described in other geographical regions. In almost all strains the gene *mefE* was detected.

Other resistance mechanisms such as enzymatic modification (acetylation, phosphorylation, glycosylation and nucleotidation) and hydrolysis (esterases), have minor practical significance in Gram-positive bacteria.

The transfer of macrolide resistance genes in *S. pneumoniae* is mediated by conjugative chromosomal transposons with a broad host spectrum: the genera *Streptococcus*, *Staphylococcus* and *Enterococcus*. Given that resistance to chloramphenicol, tetracyclines and aminoglycosides (high level) is also transmitted by conjugative transposons, these antibiotics can indirectly co-select erythromycin, as is the case with Tn*1545*, which carries the *erm*, *tet*, and *aph-3'* genes. In contrast, the mutation of the *pbp* genes and transformation by *pbp* genes from other species, constitutes the well-documented mechanism of penicillin resistance in pneumococci. *S. pyogenes* exchanges resistance genes by means of conjugative transposons, for R plasmids and for bacteriophages (transduction). Nevertheless, studies on resistance gene transfer made in hospitals tend to be retrospective, hence we must try to examine the process while it is happening, and track the in vivo traffic of R plasmids, transposons and integrons.

References

1. Aínsa JA, Martín C, Gicquel B, Gómez-Lus R (1996) Characterization of the chromosomal aminoglycoside 2'-N-acetyltransferase gene from *Mycobacterium fortuitum*. Antimicrob Agents Chemother 40:2350–2355
2. Arthur M, Molinas C, Mabilat C, Courvalin P (1990) Detection of erythromycin resistance by the polymerase chain reaction using primers in conserved regions of *erm* rRNA methylase genes. Antimicrob Agents Chemother 34:2024–2026
3. Bissonnette L, Champetier C, Buisson JP, Roy PH (1991) Characterization of the non-enzymatic chloramphenicol resistance (*cmlA*) gene of the In*4* integron of Tn*1696*: similarity of the product to transmembrane transport proteins. J Bacteriol 173:4493–4502
4. Clancy J, Petitpas JW, Dib-Hajj F, Bergeron J, Yuan W, Cronan M, Kamath AV, Retsema JA (1996) Molecular cloning and functional analysis of a novel macrolide-resistant determinant, *mefA*, from *Streptococcus pyogenes*. Molec Microbiol 22:867–879
5. Clancy J, Dib-Hajj F, Petitpas JW, Yuan W (1997) Cloning and characterization of a novel macrolide efflux gene, *mreA*, from *Streptococcus agalactiae*. Antimicrob Agents Chemother 41:2719–2723
6. Gómez-Lus R, Larrad L, Rubio-Calvo MC, Navarro M, Lasierra MP (1980) AAC(3) and AAC(6') enzymes coded by R plasmid isolated in a general hospital. In: Mitsuhashi S, Rosival L, Krcmery V (eds) Antibiotic resistance. Heidelberg: Springer-Verlag, pp 295–303
7. Gómez-Lus S, Gómez-Lus R (1993) Detection of new macrolide-lincosamide-streptogramin resistance phenotypes in gram-positive cocci and its genetic basis. In: Einhorn J, Nord CE, Ragnar Norrby S (eds) Recent Advances in Chemother. Washington, DC: American Society for Microbiology, pp 204–205
8. Gómez-Lus R, Vergara Y (1995) Aminoglycoside resistance in *Haemophilus influenzae*. J Chemother 40:97–99
9. Hall RM, Collis CM (1995) Mobile cassettes and integrons: capture and spread of genes by site-specific recombination. Molec Microbiol 15:593–600
10. Martín C, Gómez-Lus R, Ortiz JM, García-Lobo JM (1987) Structure and mobilization of an ampicillin and gentamicin resistance determinant. Antimicrob Agents Chemother 31:1266–1270
11. Martín C, Timm J, Rauzier J, Gómez-Lus R, Davies J, Gicquel B (1990) Transposition of an antibiotic resistance element in mycobacteria. Nature 345:239–243
12. Milton ID, Hewitt CL, Harwood CR (1992) Cloning and sequencing of a plasmid-mediated erythromycin resistance determinant from *Staphylococcus xylosus*. FEMS Microbiol Lett 97:141–148
13. Payie KG, Rather PN, Clarke AJ (1995) Contribution of gentamicin 2'-N-acetyltransferase to the O acetylation of peptidoglycan in *Providencia stuartii*. J Bacteriol 177:4303–4310
14. Rivera MJ, Vitoria MA, Navarro M, Robledano L, Chocarro P, Gómez-Lus R (1984) Hospital dissemination among Gram-negative bacillus strains of an IncM plasmid encoding an AAC(3) and a TEM-1 β-lactamase. Drugs Exptl Clin Res 11:789–795
15. Rivera MJ, Martín C, Gómez-Lus ML, Robledano L, Otal I, Gómez-Lus R (1988) Characterization of a hospital disseminated plasmid encoding resistance to gentamicin and other antimicrobial agents. Microbiología SEM 4:55–59
16. Ross JI, Eady EA, Cove JH, Cunliffe WJ, Baumberg S, Wooton JC (1990) Inducible erythromycin resistance in staphylococci is encoded by a member of the ATP-binding transport super-gene family. Molec Microbiol 4:1207–1214.
17. Rubens CE, McNeill WF, Farrar WE (1979) Transposable plasmid deoxyribonucleic acid sequence in *Pseudomonas aeruginosa* which mediates resistance to gentamicin and four other antimicrobial agents. J Bacteriol 139:877–888
18. Salauze D, Otal I, Gómez-Lus R, Davies J (1990) Aminoglycoside acetyltransferase 3-IV (*aaC4*) and hygromycin B 4-I phosphotransferase (*hphB*) in bacteria isolated from human and animal sources. Antimicrob Agents Chemother 34:1915–1920
19. Smith DI, Gómez-Lus R, Rubio-Calvo MC, Datta N, Jacob A, Hedges RW (1975) Third type of plasmid conferring gentamicin resistance in *Pseudomonas aeruginosa*. Antimicrob Agents Chemother 8:227–230
20. Sutcliffe J, Tait-Kamradt A, Wondrack L (1996) *Streptococcus pneumoniae* and *Streptococcus pyogenes* resistant to macrolides but sensitive to clindamycin: a common resistance pattern mediated by an efflux system. Antimicrob Agents Chemother 40:1817–1824