BACTERIAL RESISTANCE TO AMINOGLYCOSIDES AND BETA-LACTAMS: THE Tn*1331* TRANSPOSON PARADIGM

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1. ABSTRACT

Aminoglycosides (Ags) are a group of antibiotics that exert their bactericidal activity primarily by inhibition of protein synthesis. Aminoglycoside (Ag) molecules bind to the bacterial 30S ribosomal subunit rendering the ribosomes unavailable for translation, which results in cell death. Although these antibiotics are and have been very useful to treat a variety of bacterial infections, in recent years the number of Ag resistant and multiresistant isolates has seriously increased. Mechanisms of resistance to Ag include enzymatic inactivation by acetyltransferases, nucleotidyltransferases (adenylyltransferases), and phosphotransferases, ribosomal alterations, and reduced permeability. Of all Ags, amikacin (Ak) is the most resistant to the action of Ag-modifying enzymes. However, AAC(6')-I type enzymes (a group of 6'-N-acetyltransferases) can utilize Ak as substrate and confer resistance to this antibiotic in addition to other Ags. The gene *aac(6')-Ib* was found in various bacterial species and various research groups performed mutagenesis studies on this or related enzymes. In one case, *aac(6')-Ib* was identified in a transposable element, Tn*1331*, included in pJHCMW1, a plasmid isolated from a clinical *K. pneumoniae* strain. Tn*1331* includes genes encoding two Ag-modifying enzymes (*aac(6')-Ib* and *ant(3'')-Ia*) and two beta-lactamases (bla_{TEM} and bla_{OXA-9}). Characterization of other functions of the pJHCMW1 plasmid showed the presence of an RNA-regulated replication origin and a functional *oriT*. Stability by multimer resolution is achieved by the Tn*1331* resolvase.

2. AMINOGLYCOSIDE ANTIBIOTICS AND RESISTANCE

The Ags are a family of molecules containing a molecular nucleus, an aminocyclitol ring that can be streptidine or 2-deoxystrepamine and two or more aminosugars linked by glycosidic bonds to the nucleus. The first Ag, streptomycin, was introduced in 1944 and was isolated from *Streptomyces* spp. Other Ags were later derived from the same source such as neomycin, kanamycin (Km), tobramycin (Tm), and paromomycin (sufix "-mycin"). Another source for isolation of Ag antibiotics like gentamicin or sisomicin was *Micromonospora spp.* (sufix "-micin"). Ags of semisynthetic origin such as amikacin (Ak) (1) and netilmicin (2, 3) were also obtained by chemical modification of the natural occurring kanamycin A and sisomicin antibiotics.

The bactericidal activity of Ags is primarily exerted by inhibition of protein synthesis. Ag molecules bind to the bacterial 30S ribosomal subunit rendering the ribosomes unavailable for translation which results in cell death (4, 5, 6, 7). The importance of the interaction of the antibiotic molecule with the ribosome has been shown using mutants or chemical modification of ribosomal components. Those derivatives that had a reduced affinity for the Ag molecule exhibited reduced levels of susceptibility (8). Furthermore, Fourmy et al. recently determined the structure of the highly conserved site A of the *E. coli* 16S ribosomal RNA when it is complexed to the Ag paromomycin (9). This study revealed how Ags bind the ribosome. Other major metabolic perturbations caused by Ags, which could be secondary effects, include mistranslation, membrane damage (altered membrane composition and permeability), altered cellular ionic concentrations, and disturbances in synthesis of DNA and RNA (5, 10, 11).

Ags are active primarily against aerobic gram negative bacilli as well as gram positive cocci (12). However, it is well known that bacteria develop resistance to antibiotics and in recent years the number of resistant and multiresistant isolates has seriously increased (8, 13, 14, 15, 16). Mechanisms of resistance to Ags include enzymatic inactivation by acetyltransferases, nucleotidyltransferases (adenylyltransferases), and phosphotransferases, ribosomal alterations, and loss of permeability (14, 17, 18, 19, 20).

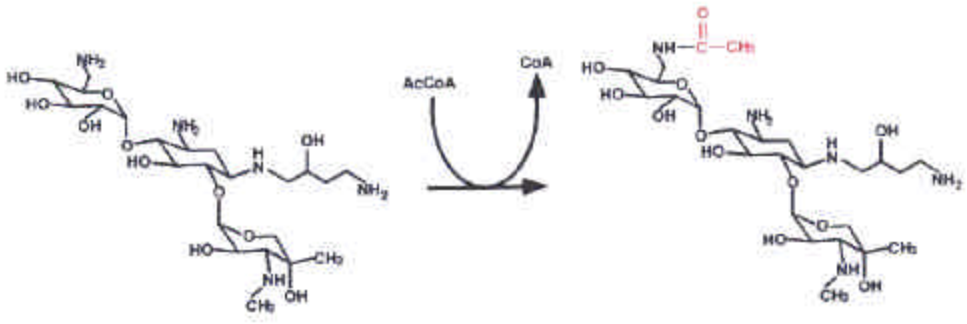

Figure 1. AAC(6')-Ib-mediated acetylation of Ak.

A large number of Ag-modifying enzymes has been described (20) (see also the site "Aminoglycoside Modifying Enzymes" http://www.warn.cas.cz/Topics/miller/amg_root.html by the Schering-Plough group and the Antibiotic Resistance Study Groups). Although detailed studies on several Ag-modifying enzymes have been limited, some mechanistic and mutational studies have been carried out (21, 22, 23, 24, 25, 26, 27) and the crystal structures of two acetyltransferases, a nucleotidyltransferase and a phosphotransferase have been reported (28, 29, 30, 31). Genes encoding Ag-modifying enzymes are often located in plasmids which permits cell to cell dissemination of the Ag resistance trait. Furthermore, several of these genes are also included in transposons (32) and integrons (33) which results in their rapid dissemination at the molecular level. In the next section a transposon, Tn*1331*, which includes genes encoding resistance to Ags and beta-lactams as well as a structure resembling the variable portion of integrons is described in some detail.

3. THE MULTIRESISTANCE TRANSPOSON Tn*1331*

We were originally interested in studying the basis of resistance to Ak in *Klebsiella pneumoniae* clinical isolates. The semisynthetic aminoglycoside Ak is a derivative of kanamycin A obtained through acylation with the L(-)-gamma-amino-alpha-hydroxybutyryl side chain at the C-1 amino group of the deoxystreptamine moiety (1). This antibiotic is refractory to most Ag-modifying enzymes and has been a powerful weapon against *Klebsiella* and other Enterobacteriaceae infections (34, 35). However, AAC(6')-I type enzymes can utilize Ak as substrate and confer resistance to this antibiotic (20). Furthermore, resistance to this antibiotic has been on the raise reaching alarming levels. Lately, there have been several reports of outbreaks of multiresistant *Klebsiella* that in some cases include resistance to Ak (36, 37, 38, 39, 40). In a study of one hundred and sixty *Klebsiella sp.* isolated from patients with nosocomial infections in the Children Hospital of Buenos Aires, Argentina during a 1 year period (1981-1982) it was found that all were multiresistant with 7% being resistant to the Ag amikacin (40). Further studies showed that the percentage of Ak resistant *Klebsiella sp.* kept increasing yearly. Therefore, years ago we initiated a project to characterize the nature of this resistance as well as its mechanism of dissemination. A clinical *K. pneumoniae* strain isolated from a diseased neonate was used as a model for these studies. An 11 kilobase pairs (kb) plasmid, pJHCMW1, was found to harbor a gene encoding an Ag-modifying enzyme responsible for resistance to Ak as well as other Ags such as Km, Tm, Nm, and others (40). Characterization of this enzyme indicated that this enzyme was the acetyltransferase AAC(6')-Ib (40, 41). The AAC(6') enzymes acetylate the Ag molecule at the 6' position (figure 1), and bacteria possessing AAC(6')-I enzymes are resistant to several Ags such as Ak, Km, Nm, sisomicin, Tm, and others, but not to gentamicin.

Analysis of the DNA region where *aac(6')-Ib* is located in pJHCMW1 led to the identification of integron-like sequences as well as a transposable element, Tn*1331*, related to Tn*3* (42). Further studies on this transposon showed that it mediates resistance to the aminoglycosides mentioned before and in addition it mediates resistance to streptomycin, and some beta-lactams. Resistance to streptomycin is mediated by *ant(3'')-Ia* which encodes an adenylyltransferase (43). The sequence of this gene is related to those of other adenylyltransferase genes described in other systems (44, 45, 46, 47). Resistance to beta-lactams is due to the presence in Tn*1331* of two beta-lactamase genes: bla_{TEM} identical to that one present in Tn*3* (48) and $bla_{\text{OXA-9}}$, which codes for an oxacillinase-carbenicillinase (43, 49, 50). The OXA-9 enzyme has a pI of 6.9 and the optimal pH for its activity is 7.7 - 8.2 (51). Stability studies showed that enzyme activity decayed to approximately 56% of the original value after 6 hours pre-incubation of total soluble extracts of *E.coli* harboring $bla_{\text{OXA-9}}$ at 37°C. Instead, upon pre-incubation at 42°C, enzyme activity decayed to approximately 50% of the original value after 30 minutes (51). The activity of the OXA-9 enzyme was inhibited in the presence of *p*-chloromercuribenzoate, cloxacillin, and clavulanic acid, but not by 200 mM sodium chloride. The inhibition by *p*-chloromercuribenzoate may indicate the presence of a cysteine residue playing a role in the catalytic action of the enzyme (51).

The genetic map of Tn*1331* and its closely related tranposon Tn*3* are shown in figure 2.

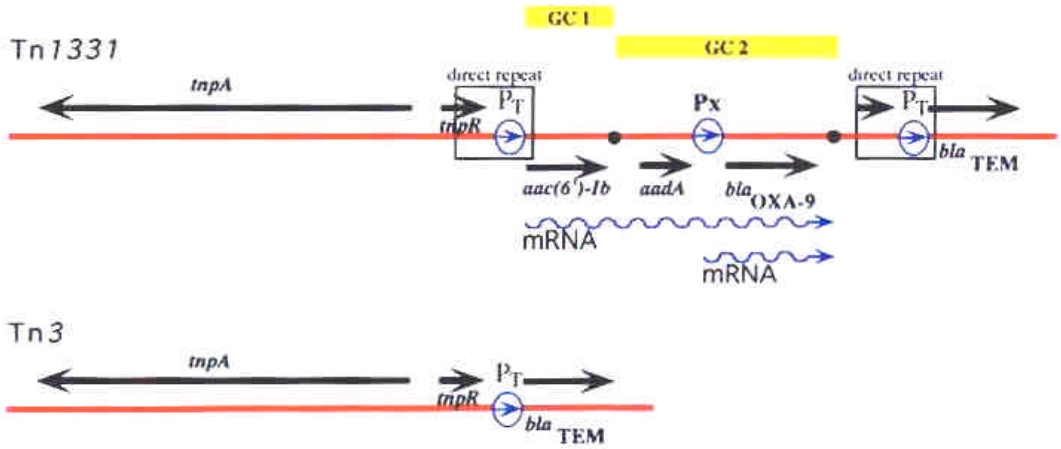

Figure 2. Genetic map of Tn*1331*. The genetic map of Tn*1331* and its comparison to a genetic map of Tn*3* are shown in the diagram. Filled circles downstream of *aac(6')-Ib* and bla_{OXA-9} represent the 59-base element. The arrow inside the rightmost direct repeat represents a DNA fragment corresponding to amino acids #81 to #185 of *tnp*R. IR: inverted repeats. PT and Px represent the promoters. GC: gene cassette.

Nucleotide sequence and mutant complementation analyses demonstrated that Tn*1331* can be considered as Tn*3* (52, 53) with the addition of a DNA region carrying the antibiotic resistance genes and a 520-bp direct repeat (42, 43) (figure 2). The genes *aac(6')-Ib*, *ant(3'')-Ia* and bla_{OXA-9} are flanked by the direct repeats which include a portion of *tnpR*, the promoter (P_T) as well as the sequence encoding the first 6 amino acids of the protein encoded by bla_{TEM} (see figure 2). As can be seen in figure 2, this duplication resulted in a fusion of the first 6 amino acids of the TEM beta-lactamase to the rest of the polypeptide that forms the AAC(6')-Ib protein (43, 49, 54).

The DNA region encompassing the three resistance genes *aac(6')-Ib*, *ant(3'')-Ia* and bla_{OXA-9} resembles the variable region of the integrons (33, 55). The nucleotide sequence of this DNA region defines two portions with the characteristics described for the gene cassettes found within the variable region of the integrons (56). In the case of *aac(6')-Ib* the 5' sequence of the gene cassette does not precede the structural gene but rather is part of it (50). The two genes located downstream of *aac(6')-Ib*, *ant(3'')-Ia* and bla_{OXA-9} , are included in a structure that can be considered a single gene cassette (figure 2) because most of the 59-base element that commonly follows the resistance gene is missing following *ant(3'')-Ia* (50). Although this is a rare phenomenon, other cases like this have now been described. The *aac(6')-Ia* gene in Tn*2424* (57) is followed, in the same gene cassette, by an ORF with no homology to any sequence in the database (58). This ORF has a Shine-Dalgarno sequence and therefore it may be translated. Also, Roy recently found an integron with the arrangement *aadB-ant(3'')-Ia-*bla_{OXA-9} in which the *ant(3'')-Ia* and bla_{OXA-9} are arranged as in Tn*1331* with no 59-base element at the end of *ant(3'')-Ia*. (P. Roy, personal communication).

Another unusual feature of the gene cassette including *ant(3'')-Ia* and bla_{OXA-9} is the presence of the sequence AAACAAA preceding bla_{OXA-9}. These are the last nucleotides found in the 5' conserved region of the integrons just before the variable region starts with the sequence GTT. This gene cassette could have been generated by an illegitimate recombination event between the end of *ant(3'')-Ia* in one integron and the 5' conserved segment in another integron carrying bla_{OXA-9} generating a new integron that includes a gene cassette with the two genes (50).

This putative new integron may have been the target for a Tn*3* transposition event. Two different mechanisms for genesis of Tn*1331* starting with transposition of Tn3 into the integron have been postulated based on the mechanism for generation of transposons postulated by Grinsted (figure 3) (43, 59). In one case a defective transposition event consisting of a deletion of the appropriate DNA fragments in the cointegrate could have led to the formation of Tn*1331* (figure 3a). The other possibility considers a complete transposition event followed by duplication and deletion of DNA regions that led to the generation of Tn*1331* (figure 3b) (43).

The genetic organization of the DNA region encompassing the genes *aac(6')-Ib*, *ant(3'')-Ia*, and bla_{OXA-9} is shown in figure 2. Analysis of insertion and deletion mutants used to analyze expression of *ant(3'')-Ia*. indicated that the *ant(3'')-Ia* transcript most probably originates at the promoter P_T that also directs transcription of *aac(6')-Ib*. Expression of the bla_{OXA-9} gene was studied using another set of mutants and subclones. Minimal inhibitory concentrations and primer extension experiments suggested that transcription of the bla_{OXA-9} gene can originate at the promoter P_T as well as at Px, a promoter located immediately upstream bla_{OXA-9} (50). In general, genes inserted in integrons are expressed from a common promoter region located in the adjacent 5'-conserved segment (60, 61).

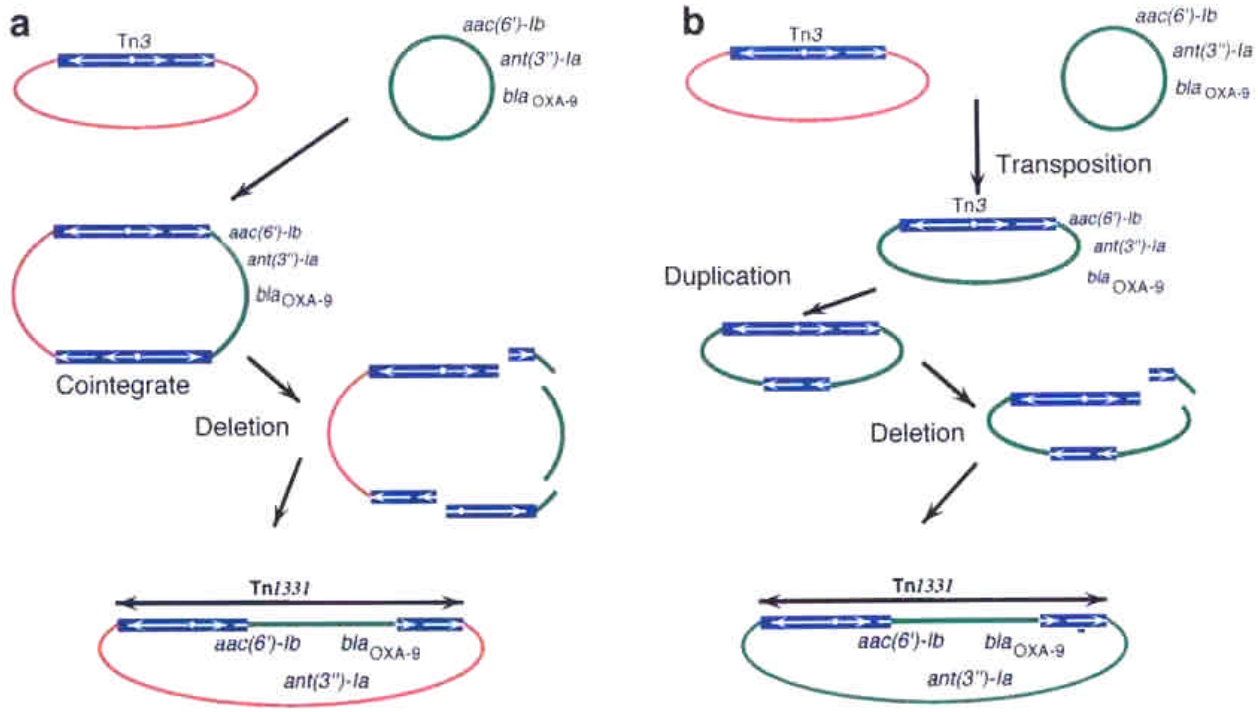

Figure 3. Two possible mechanisms for the generation of Tn*1331* from Tn*3* (interpreted from 43). a. A plasmid carrying Tn*3* acts as a donor. The first step of the transposition process, formation of the cointegrate, takes place but before the regular resolution occurs there are two deletion events. The DNA fragments deleted are those indicated in the diagram, as a result of the loss of a copy of the res site the structure is locked generating a new transposon, Tn*1331*. The box with three arrows represents Tn*3*, and the three arrows represent the direction of transcription of *tnpA*, *tnpR*, and bla_{TEM} genes. The res site is represented by a dot. b. This is a slight modification of one of the mechanisms postulated by Grinsted (59). Tn*3* transposes from the donor to a location upstream of the resistance genes in the recipient recipient. Following this event the Tn*3* portion encompassing nucleotides 3449 and 4957 undergoes a duplication followed by a deletion starting at nucleotide 3969 of Tn*3*. The product of this deletion event is the new transposon Tn*1331*.

Therefore, the expression of bla_{OXA-9} from two different promoters is an unusual albeit not unique fact. Parent and Roy recently found that the*cat* gene present in the Tn*2424* integron also possesses a promoter immediately upstream the resistance gene (58).

The *aac(6')-Ib* gene was also found associated with other integron and transposon sequences in different bacteria (62, 63, 64, 65, 66).

4. BIOLOGICAL PROPERTIES OF PJHCMW1 AND DISSEMINATION OF Tn*1331*

Tn*1331* was first detected in the multiresistance *Klebsiella pneumoniae* plasmid pJHCMW1. The replication functions of this plasmid and a functional *oriT* were located in a 2.4 kb *Eco*RI (67). In addition a site homologous to *cer* (*mwr*) was found in a 0.6 kb *Eco*RI-*Sac*I fragment (68). The plasmid pJHCMW1 replicates using the RNA-regulated general mechanism described for ColE1, p15A, and several other plasmids (67, 69, 70, 71, 72, 73, 74). Although a very high degree of homology was found between the pJHCMW1 and the p15A origins of replication, compatibility experiments demonstrated that they are compatible indicating that even when both replicons are highly related they are not sufficiently similar

to be incompatible. The pJHCMW1 2.4 kb *Eco*RI fragment also includes a sequence with homology to the origin of transfer of other plasmids (67). Conjugation experiments using the helper plasmid pRK2073 (75), a recombinant plasmid that carries the RK2 *tra* genes and the ColE1 *mob* genes, indicated that in the presence of the appropriate elements, i.e. the *tra* and *mob* encoded products, this origin of transfer is functional making pJHCMW1 a mobilizable plasmid (67).

It is noteworthy to mention that pJHCMW1 possesses a locus, *mwr*, with homology to *cer* and *psi* (76, 77) but that is unable to confer stability by multimer resolution in *E. coli* (68). Analysis of *mwr* showed that XerC binds poorly to the *mwr* XerC-binding site and dimers carrying two *mwr* regions are not resolved efficiently. This fact plus a substandard ArgR binding site could be the reasons why *mwr* fails to stabilize pJHCMW1. Multimer resolution in pJHCMW1 is achieved by the Tn*1331* resolvase at the *res* site. A site-directed

mutagenesis experiment generating the substitution D17Stop in *tnpR* resulted in destabilization of the mutant derivative which is almost completely lost from *E. coli* JC8679 after 60 generations (68).

Since transposition and conjugation are important mechanisms of gene exchange we analyzed a number of Ak-resistance clinical isolates for their plasmid content and the presence of Tn*1331*. The results showed that most strains harbored different Ak-resistance encoding plasmids but in all of them Tn*1331* (or its closest relative Tn*1331.2*) was present. This results showed that Tn*1331* was spreading very rapidly (78). It was of interest that some strains harbored a relative of Tn*1331*, called Tn*1331.2*, that has the DNA portion including the three genes *aac(6')-Ib*, *ant(3'')-Ia*, bla_{OXA-9} duplicated (79). In particular, a conjugative low copy number plasmid, pMET1, was isolated from a clinical *Klebsiella pneumoniae* strain that caused a devastating outbreak of hospital infection that led to the deaths of several neonates carried Tn*1331.2* (79).

5. CHARACTERIZATION OF AAC(6')-Ib

AAC(6')-I enzymes can inactivate several clinically important aminoglycosides including Ak by acetylation of the 6'-amino group of the molecule (20). Several genes encoding AAC(6')-I enzymes have been described to date (20, 49, 54, 57, 63, 66, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90 , 91, 92, 93, 94, 95, 96, 97). Based on deduced amino acid sequences, three subfamilies have been defined(20). AAC(6')-Ib which has been detected in the *K. pneumoniae* transposon Tn*1331* (43, 49), *Pseudomonas aeruginosa* (98), *K. oxytoca* (65), and *Serratia marcescens* (54) is included in subfamily 1(20).

Early studies on AAC(6')-I enzymes clarified different aspects of the biochemical reaction mechanism of acetylation (25, 26). Alignment of the amino acid sequences of several AAC(6')-I and AAC(6)-II enzymes permitted to identify conserved regions among the three subfamilies and in particular, 6 motifs were defined within the four members of subfamily 1 (20). Mutational studies carried out by Rather et al showed that when AAC(6')-Ib was subjected to a modification Leu120Ser the new protein had the resistance profile of AAC(6')-II enzymes, i.e. it could not mediate resistance to Ak but it was capable to mediate resistance to gentamicin (99). Later a spontaneous mutation in *aac(6')-Ib* that led to conversion of the AAC(6')-I profile to that of AAC(6')-II was found to be identical to that one generated in vitro (63). Interestingly, in vivo mutagenesis experiments carried out in our lab showed that after random mutagenesis followed by selection for resistance to gentamicin, only colonies carrying the Leu120Ser could be isolated. These results confirmed the importance of the role played by this amino acid in substrate specificity with respect to Ak and gentamicin (Tolmasky, unpublished results).

In vivo mutagenesis of *aac(6')-Ib* also permitted the isolation of other mutants of interest (24). A transition in nucleotide 511 changing the codon TTT to CTT which specifies the amino acid Leu generated the mutant AAC(6')-Ib_{DP1} which has the Phe171Leu modification. Minimal inhibitory concentration (MIC) analyses of *E. coli* carrying either the mutant AAC(6')-Ib_{DP1} or the wild type showed that, as it is the case for the wild tpye, the mutant enzyme confers a high level of resistance to Km and Tm at either 37°C or 42°C. However, in the case of Ak or Nm there was a clear difference in the behavior of *E. coli* strains carrying either of the enzymes. While the wild type enzyme conferred high levels of resistance to these Ags at both temperatures, the MICs for the same antibiotics dropped sharply at 42°C for *E. coli* harboring the mutant derivative. In vitro experiments to determine the acetylating activity of the wild type and mutant enzymes were performed using total soluble protein extracts of *E. coli* harboring either *aac(6')-Ib* or *aac(6')-Ib* $_{DP1}$. Both enzymes had higher specific activity at 42°C than at 30°C when Km or Tm were used as substrates. However, AAC(6')-Ib_{DP1} had a drastically lower specific activity for Ak or Nm at 42°C than at 30°C (24). Preincubation of the enzymes at different temperatures demonstrated that AAC(6')-Ib_{DP1} irreversibly looses its ability to modify Ak after a short incubation at 37 or 42°C. The behavior of the mutant derivative suggested that AAC(6')-Ib_{DP1} specifically looses Ak and Nm acetylating efficiency at 42°C. A common feature of the chemical structure of both antibiotics is the substitution of the C-1 amino group of the deoxystreptamine moiety (1, 3). AAC(6')-Ib_{DP1} may suffer a conformational change at 42°C that makes it unable to efficiently modify Ags molecules with a substitution at the C-1 amino group of the deoxystreptamine moiety. Further mutagenesis analysis of the Phe171 showed that this amino acid position has low tolerance to substitutions confirming that it may play an important role in the structure and/or function of AAC(6')-Ib (100). Furthermore, sequence comparisons among AAC(6') enzymes (20) and members of the N-acetyltransferase superfamily (101) indicated that Phe171 is included within the conserved motif B (or motif 5 according to an older nomenclature) and it is one of the most conserved amino acids in this region (20, 101). Mutant AAC(6')-Ib_{DP1} has this nonpolar amino acid replaced by Leu, another nonpolar amino acid. The phenotype of the Phe171Leu substitution taken together with the high conservation of Phe_{171} observed among AAC(6')-I proteins suggests that this amino acid may be critical for the spatial folding of these acetyltransferases (24). It was described that the hydrophobic effect is an important factor in the three dimensional conformation of proteins (102). Therefore, the nonpolar Phe_{171} residue may contribute to the structure of AAC(6')-Ib as well as other AAC(6')-I enzymes. The Phe171Leu change decreases the residue volume (103) and eliminates an aromatic ring. These anomalies may contribute to the lower stability of the mutant enzyme, which may be unable to keep the proper spatial conformation at 42°C. It has been also described that substitutions between Phe and Leu perturb hydrogen bonds that contribute to the conformation of the protein (104). These factors can contribute to the reduction in specific activity of AAC(6')-Ib_{DP1} and its stability at higher temperatures (24).

Another mutant, AAC(6')-Ib_{DP6}, has the Tyr80Cys (24) substitution. Although *E. coli* carrying this

mutant gene showed a certain level of resistance to some aminoglycosides, the enzyme AAC(6')-Ib_{DP6} had only marginal levels of acetylating activity in vitro. The change Tyr80Cys is a replacement of a polar residue for a nonpolar amino acid that could not be tolerated by the protein, which lost most of its enzymatic activity. Tyr_{80} is located within the region encompassing amino acids 77 - 81 (Val-Thr-Pro-Tyr-Ile) which is conserved among three members of the subfamily 1, AAC(6')-Ib, AAC(6')-IIa, and AAC(6')-IIb but not in AAC(6')-Ie (20) where a Val residue is present in the position equivalent to Tyr_{80}. Since Val is hydrophobic, this data suggests that the nonpolar nature of the substituting amino acid is not enough to explain the loss of activity.

Recently, Casin et al. characterized variants of AAC(6')-Ib from clinical *Enterobacter cloacae* and *Citrobacter freundii* isolates (105). These variants had substrate profiles intermediate between those of the AAC(6')-Ib and AAC(6')-IIa enzymes. Characterization of these enzymes demonstrated that two of them are 177 or 184 amino acids long while the other is composed of 196 amino acids. All three proteins exhibited a Ser residue at position 119 instead of the Leu found in the wild type AAC(6')-Ib. Size variation in AAC(6')-Ib like proteins was also found in a study of 70 clinical Ak-resistance strains by Tran Van Nhieu et al. (106). These authors found that most amikacin-resistant clinical isolates carried the *aac(6')-Ib* gene. However, western blots demonstrated that the proteins were not identical, they had molecular weights ranging between 24 and 26 kD. In addition, another AAC(6')-Ib variant with an N terminus modification was published (107). Based on these facts Casin et al. proposed that AAC(6')-Ib variants may have flexible structural requirements for the N terminus(105). This variations in the N terminus, together with the possible alternatives for translation or lack of translation (108, 109) (Brassard and Roy, unpublished results) may indicate that this gene is "hunting" for a good expression element. Fusion to the initial portion of the bla_{TEM} seems to be a good strategy for expression of *aac(6')-Ib* utilizing the bla_{TEM} promoter (43, 49, 54).

Studies on other AAC(6')-I enzymes showed that the *Acinetobacter haemolyticus* enzyme AAC(6')-Ig looses acetylating activity against Tm when the Met56 is substituted by an Arg residue (90). In addition these authors found two other natural derivatives unable to confer resistance to Tm. These mutants have either an insertion of 19 thymines in the gene which results in frameshift, or an insertion of a copy of IS17 (90). Wright and Ladak recently purified and characterized the AAC(6')-Ii enzyme from *Enterococcus faecium*. They concluded that this enzyme is not optimally evolved for Ag inactivation indicating that it probably plays another physiological role(s) (110).

6. CONCLUDING REMARKS

The transposon Tn*1331* is a paradigm of the molecular evolution of drug resistance mechanisms. A transposon carrying only one resistance gene like Tn*3* which unlike other tranposons (like the Tn*21* subfamily) had shown to be very resilient to changes evolved into a new more dangerous element carrying four genes. This event could represent an important pathway of evolution of transposition elements of the Tn*3* family besides other known mechanisms. The addition of the *aac(6')-Ib* gene to the structure of Tn*1331* provided a very effective vehicle for dissemination of the gene with it resistance to very important antibiotics like Ak. In addition the presence of Tn*1331* in mobilizable and conjugative plasmids that in some cases can have a wide host range indicates that the gene will continue to spread to a large variety of bacteria. It is therefore necessary to continue the characterization of different aspects of the mechanism of resistance to antibiotics and dissemination of these traits to design rational methods to overcome the problem.

7. ACKNOWLEDGEMENTS

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8. REFERENCES

1. H. Kawaguchi: Discovery, chemistry, and activity of amikacin. *J. Infect. Dis.* 134(Suppl.), 242-248 (1976)
2. R. L. Herting, A. Z. Lane, R. R. Lorber & J. J. Wright: Netilmicin: chemical development and overview of clinical research. *Scandinavian J. Infect. Dis.* Suppl 23, 20-29 (1980)
3. G. H. Miller, G. Arcieri, M. J. Weinstein & J. A. Waitz: Biological activity of netilmicin, a broad-spectrum semisynthetic aminoglycoside antibiotic. *Antimicrob. Agents Chemother.* 10, 827-836 (1976)
4. J. E. Davies: Resistance to aminoglycosides: mechanisms and frequency. *J. Infect. Dis.* 5 (Suppl. 2), S267 (1983)
5. B. Davis: Mechanisms of bactericidal action of aminoglycosides. *Microbiol. Rev.* 51, 341-350 (1987)
6. D. Moazed & H. Noller: Interaction of antibiotics with functional sites in 16S ribosomal RNA. *Nature* 327, 389-394 (1987)
7. J. Woodcock, D. Moazed, M. Cannon, J. Davies & H. F. Noller: Interaction of antibiotics with A- and P-site-specific bases in 16S ribosomal RNA. *Embo J* 10, 3099-103 (1991)
8. J. Davies & G. Wright: Bacterial resistance to aminoglycoside antibiotics. *Trends Microbiol.* 5, 234-240 (1997)
9. D. Fourmy, M. Recht, S. Blanchard & J. Puglisi: Structure of the A site of *Escherichia coli* 16S ribosomal RNA complexed with an aminoglycoside antibiotic. *Science* 274, 1367-1371 (1996)
10. B. Davis, L. Chen & P. Tai: Misread protein creates membrane channels: an essential step in the bactericidal action of aminoglycosides. *Proc. Natl. Acad. Sci. USA* 83, 6164-6168 (1986)
11. B. Davis: The lethal action of aminoglycosides. *J. Antimicrob. Chemother.* 22, 1-3 (1988)
12. J. Yao & R. Moellering: Antibacterial agents. In: Manual of Clinical Microbology Eds: P. Murray, E. Baron, M. Pfaller, F. Tenover, R. Yolken, ASM Press, Washington DC, 1281-1307 (1995)

13. M. Cohen: Epidemiology of drug-resistance: implications for a post-antimicrobial era. *Science* 257, 1050-1055 (1992)
14. J. Davies: Inactivation of antibiotics and the dissemination of resistance genes. *Science* 264, 375-382 (1994)
15. H. Neu: The crisis in antibiotic resistance. *Science* 257, 1064-1072 (1992)
16. D. Schlaes, S. Levy & G. Archer: Antimicrobial resistance : new directions. *ASM news* 57, 455-458 (1991)
17. R. Benveniste & J. Davies: Mechanims of antibiotic resistance in bacteria. *Ann. Rev. Biochem.* 42, 471-506 (1973)
18. T. Foster: Plasmid-determined resistance to antimicrobial drugs and toxic metal ions in bacteria. *Microbiol. Rev.* 47, 361-407 (1983)
19. R. Quintiliani & P. Courvalin: Mechanisms of resistance to antimicrobial agents. In: Manual of Clinical Microbology Eds: P. Murray, E. Baron, M. Pfaller, F. Tenover, R. Yolken, ASM Press, Washington DC, 1308-1326 (1995)
20. K. Shaw, P. Rather, R. Hare & G. H. Miller: Molecular genetics of aminoglycoside resistance genes and familial relationships of the aminoglycoside-modifying enzymes. *Microbiol. Rev.* 57, 138-163 (1993)
21. J. R. Cox & E. H. Serpersu: Biologically important conformations of aminoglycoside antibiotics bound to an aminoglycoside 3'-phosphotransferase as determined by transferred nuclear Overhauser effect spectroscopy. *Biochemistry* 36, 2353-2359 (1997)
22. G. McKay, P. Thompson & G. Wright: Broad spectrum aminoglycoside phosphotransferase type III from *Enterococcus*: overexpression, purification, and substrate specificity. *Biochemistry* 33, 6936-6944 (1994)
23. G. McKay, J. Roestamadjl, S. Mobasheri & G. Wright: Recognition of aminoglycoside antibiotics by Enterococcal-Staphylococcal aminoglycoside 3'-phosphotransferase type IIIa: role of substrate amino groups. *Antimicrob. Agents Chemother.* 40, 2648-2650 (1996)
24. D. Panaite & M. Tolmasky: Characterization of mutants of the 6'-N-Acetyltransferase encoded by the multiresistance transposon Tn*1331*: effect of Phe_{171}-to-Leu_{171} and Tyr_{80}-to-Cys_{80} substitutions. *Plasmid* (1998)
25. K. Radika & D. Northrop: The kinetic mechanism of kanamycin acetyltransferase derived from the use of alternative antibiotics and coenzymes. *J. Biol. Chem.* 259, 12543-12546 (1984)
26. K. Radika & D. Northrop: Substrate specificities and structure-activity relationships for acylation of antibiotics catalyzed by kanamycin acetyltransferase. *Biochemistry* 23, 5118-5122 (1984)
27. J. Siregar, S. Lerner & S. Mobashery: Purification and characterization of aminoglycoside 3'-phosphotransferase type IIa and kinetic comparison with a new mutant enzyme. *Antimicrob. Agents Chemother.* 38, 641-647 (1994)
28. W. Hon, G. McKay, P. Thompson, R. Sweet, D. Yang, G. Wright & A. Berghuis: Structure of an enzyme required for aminoglycoside antibiotic resistance reveals homology to eukaryotic protein kinases. *Cell* 89, 887-895 (1997)
29. L. Pedersen, M. Benning & H. Holden: Structural investigation of the antibiotic and ATP-binding sites in kanamycin nucleotidyltransferase. *Biochemistry* 34, 13305-13311 (1995)
30. L. E. Wybenga-Groot, K. Draker, G. D. Wright & A. M. Berghuis: Crystal structure of an aminoglycoside 6'-N-acetyltransferase: defining the GCN5-related N-acetyltransferase superfamily fold. *Structure Fold. Des.* 7, 497-507 (1999)
31. E. Wolf, A. Vassilev, Y. Makino, A. Sali, Y. Nakatani & S. Burley: Crystal structure of a GCN5-related N-acetyltransferase: Serratia marcescens aminoglycoside 3-N-acetyltransferase. *Cell* 94, 439-49 (1998)
32. N. Kleckner: Transposable elements in prokaryotes. *Ann. Rev. Genet.* 15, 341-404 (1981)
33. R. Hall & C. Collis: Mobile gene cassettes and integrons: capture and spread of genes by site-specific recombination. *Mol. Microbiol.* 15, 593-600 (1995)
34. K. Ryan & S. Falkow: Enterobacteriaceae. In: Medical Microbiology, an introduction to infectious diseases. Ed: K. Ryan, Appleton & Lange, Stanford, 321-344 (1994)
35. R. Meyer, R. Lewis & S. Finegold: Amikacin therapy for gram-negative septicemia. *The Amer. J. Med.* 62, 930-935 (1977)
36. O. Flidel-Rimon, E. Leibovitz, A. Juster-Reicher, M. Amitay, A. Miskin, Y. Barak & B. Mogilner: An outbreak of antibiotic multiresistant *Klebsiella* at the Neonatal Intensive Care Unit, Kaplan Hospital, Rehovot, Israel, November 1991 to April 1992. *Amer. J. Perinatology* 13, 99-102 (1996)
37. T. A. R. S. Groups: The most frequently occurring aminoglycoside resistance mechanisms--combined results of surveys in eight regions of the world. *J. Chemother.* 7 Suppl 2, 17-30 (1995)
38. G. Miller, F. Sabatelli, R. Hare, Y. Glupezynski, P. Mackey, K. Shlaes & A. R. S. Groups: The most frequent aminoglycoside resistance mechanisms-changes with time and geographic area: a reflection of aminoglycoside usage pattern? *Clin. Infect. Dis.* 24(Supplement 1), 846-862 (1997)
39. A. Yinnon, A. Butnaru, D. Raveh, Z. Jerassy & B. Rudensky: *Klebsiella* bacteremia: community versus nosocomial infection. *QJM* 89, 933-941 (1996)
40. M. Woloj, M. E. Tolmasky, M. Roberts & J. H. Crosa: Plasmid-encoded amikacin resistance in multiresistant strains of *Klebsiella pneumoniae* isolated from neonates with meningitis. *Antimicrob. Agents Chemother.* 29, 315-319 (1986)
41. M. E. Tolmasky, M. Roberts, M. Woloj & J. H. Crosa: Molecular cloning of amikacin resistance determinants from a *Klebsiella pneumoniae* plasmid. *Antimicrob. Agents Chemother.* 30, 315-320 (1986)
42. M. E. Tolmasky & J. H. Crosa: Tn*1331*, a novel multiresistance transposon encoding resistance to amikacin and ampicillin in *Klebsiella pneumoniae*. *Antimicrob. Agents Chemother.* 31, 1955-1960 (1987)
43. M. E. Tolmasky: Sequencing and expression of *aadA*, *bla* and *tnpR* from the multiresistance transposon Tn*1331*. *Plasmid* 24, 218-226 (1990)
44. A. Chinault, V. Blakesley, E. Roessler, D. Willis, C. Smith, R. Cook & R. Fenwick: Characterization of transferable plasmids from *Shigella flexneri* 2a that confer resistance to trimethoprim, streptomycin, and sulfonamides. *Plasmid* 15, 119-131 (1986)
45. M. Fling, J. Kopf & C. Richards: Nucleotide sequence of the transposon Tn*7* gene encoding an aminoglycoside-

modifying enzyme, 3"(9)-O-nucleotidyltransferase. *Nucleic Acids Res.* 13, 7095-7106 (1985)
46. S. Hollingshead & D. Vapnek: Nucleotide sequence analysis of a gene encoding a streptomycin/spectinomycin adenyltransferase. *Plasmid* 13, 17-30 (1985)
47. K. Leung, S. Ruschkowski & B. Finlay: Isolation and characterization of the *aadA* aminoglycoside-resistance gene from *Salmonella choleraesuis*. *Mol. Microbiol.* 6, 2453-2460 (1992)
48. F. Heffron, J. McCarthy, H. Ohtsubo & E. Ohtsubo: DNA sequence analysis of the transposon Tn*3*: three genes and three sites involved in transposition of Tn*3*. *Cell* 18, 1153-1164 (1979)
49. K. Nobuta, M. E. Tolmasky, L. Crosa & J. Crosa: Sequencing and expression of the 6'-N-acetyltransferase gene of transposon Tn*1331* from *Klebsiella pneumoniae. J. Bacteriol.* 170, 3769-3773 (1988)
50. M. E. Tolmasky & J. H. Crosa: Genetic organization of antibiotic resistance genes *aac(6')-Ib*, *aadA*, and *oxa9*) in the multiresistance transposon Tn*1331*. *Plasmid* 29, 31-40 (1993)
51. D. Bojorquez, M. Belei, S. DeLira, S. Sholly, J. Mead, & M. E. Tolmasky: Characterization of OXA-9, a beta-lactamase encoded by the multiresistance transposon Tn*1331*. *Cell. Mol. Biol.* 44, 483-491 (1998)
52. F. Heffron: Tn*3* and its relatives. In: Mobile Genetic Elements. Ed: J. Shapiro, Academic Press, New York, 223-260 (1983)
53. D. Sherratt: Tn3 and related transposable elements: site-specific recombination and transposition. In: Mobile DNA. Eds: D. Berg & M. Howe, American Society for Microbiology, Washington DC, 163-184 (1989)
54. G. Tran Van Nhieu & E. Collatz: Primary structure of an aminoglycoside 6'-N-acetyltransferase, AAC(6')-4, fused in vivo with the signal peptide of the Tn3-encoded b-lactamase. *J. Bacteriol.* 169, 5708-5714 (1987)
55. R. Hall: Mobile gene cassettes and integrons: moving antibiotic resistance genes in gram-negative bacteria. *Ciba Foundation Symposium* 207, 192-202 (1997).
56. R. Hall, D. Brookes & H. Stokes: Site-specific insertion of genes into integrons: role of the 59-base element and determination of the recombination cross over point. *Mol. Microbiol.* 5, 1941-1959 (1991)
57. J. Meyer, B. Nies & B. Wiedemann: Amikacin resistance mediated by multiresistance transposon Tn*2424*. *J. Bacteriol.* 155, 755-760 (1983)
58. R. Parent & P. Roy: The chloramphenicol acetyltransferase gene of Tn*2424*: a new breed of *cat. J. Bacteriol.* 174, 28891-2897 (1992)
59. J. Grinsted: Evolution of transposable elements. *J. Antimicrob. Chemother.* 18 Suppl C, 77-83 (1986).
60. C. Levesque, S. Brassard, J. Lapointe & P. Roy: Diversity and relative strength of tandem promoters for the antibiotic-resistance genes of several integrons. *Gene* 142, 49-54 (1994)
61. C. Collis & R. Hall: Expression of antibiotic resistance genes in the integrated cassettes of integrons. *Antimicrob. Agents Chemother.* 39, 155-162 (1995)
62. J. Hopkins, A. Flores, M. Pla, S. Lester & T. O'Brien: Nosocomial spread of an amikacin resistance gene on both a mobilized, nonconjugative plasmid and a conjugative plasmid. *Antimicrob. Agents Chemother.* 35, 1605-1611 (1991)
63. T. Lambert, M. Ploy & P. Courvalin: A spontaneous point mutation in the *aac(6')-Ib'* gene results in altered substrate specificity of aminoglycoside 6'-N-acetyltransferase of a *Pseudomonas fluorescens* strain. *FEMS Microbiol. Lett.* 115, 297-304 (1994)
64. C. Levesque, L. Piche, C. Larose & P. Roy: PCR mapping of integrons reveals several novel combinations of resistant genes. *Antimicrob. Agents Chemother.* 39, 185-191 (1995)
65. K. Preston, M. Kacica, R. Limberger, W. Archinal & R. Venezia: The resistance and integrase genes of pACM1, a conjugative multiple-resistance plasmid, from *Klebsiella oxytoca. Plasmid* 37, 105-118 (1997)
66. H. Wu, G. Miller, M. Guzman Blanco, R. Hare & K. Shaw: Cloning and characterization of an aminoglycoside 6'-N-acetlytransferase gene from *Citrobacter freundii* which confers an altered resistance profile. *Antimicrob. Agents Chemother.* 41, 2439-2447 (1997)
67. K. Dery, R. Chavideh, V. Waters, R. Chamorro, L. Tolmasky & M. E. Tolmasky: Characterization of the replication and mobilization regions of the multiresistance *Klebsiella pneumoniae* plasmid pJHCMW1. *Plasmid* 38, 95-105 (1997)
68. M. E. Tolmasky, S. Colloms, G. Blakely & D. J. Sherratt: Stability by multimer resolution of pJHCMW1 is due to the Tn*1331* resolvase and not to the *Escherichia coli* Xer system. *Microbiology* in press (2000)
69. U. Kues & U. Stahl: Replication of plasmids in gram negative bacteria. *Microbiol. Rev.* 53, 491-516 (1989)
70. B. Polisky: ColE1 replication control circuitry: sense from antisense. *Cell* 55, 929-932 (1988)
71. D. Helinski, A. Toukdarian & R. Novick: Replication control and other stable maintenance mechanisms of plasmids. In: *Escherichia coli* and *Salmonella*. Ed: F. Neidhardt, ASM Press, Washington, DC, 2295-2324 (1996)
72. J. Tomizawa: Control of ColE1 plasmid replication. Intermediates in the binding of RNA I and RNA II. *J Mol Biol* 212, 683-94 (1990)
73. J. Tomizawa: Control of ColE1 plasmid replication: intermediates in the binding of RNA I and RNA II. *J. Mol. Biol.* 212, 683-694 (1990)
74. J. Tomizawa: Control of ColE1 plasmid replication. Interaction of Rom protein with an unstable complex formed by RNA I and RNA II. *J. Mol. Biol.* 212, 695-708 (1990)
75. S. Leong, G. Ditta & D. Helinski: Heme biosynthesis in *Rhizobium*. *J. Biol. Chem.* 257, 8724-8730 (1982)
76. D. Summers & D. Sherrat: Multimerization of high copy number plasmids causes instability: ColE1 encodes a determinant essential for plasmid monomerization and stability. *Cell* 36, 1097-1103 (1984)
77. F. Cornet, I. Mortier, J. Patte & J. M. Louarn: Plasmid pSC101 harbors a recombination site, *psi*, which is able to resolve plasmid multimers and to substitute for the analogous chromosomal *Escherichia coli* site dif. *J. Bacteriol.* 176, 3188-95 (1994)
78. R. Chamorro, L. Actis, J. Crosa & M. E. Tolmasky: Dissemination of plasmid-mediated amikacin resistance among pathogenic *Klebsiella pneumoniae. Medicina* 50, 543-547 (1990)

79. M. E. Tolmasky, R. Chamorro, J. H. Crosa & P. Marini: Transposon-mediated amikacin resistance in *Klebsiella pneumoniae*. *Antimicrob. Agents Chemother.* 32, 1416-1420 (1988)
80. D. Centron & P. Roy: Characterization of the 6'-N-Acetyltransferase gene *aac(6')-Iq* from the integron of a natural multiresistance plasmid. *Antimicrob. Agents Chemother.* 42, 1506-1508 (1998)
81. H. Champion, P. Bennet, D. Lewis & D. Reeves: Cloning and characterization of an AAC(6') gene from *Serratia marscecens*. *J. Antimicrob. Chemother.* 22, 587-596 (1988)
82. J. Ferreti, K. Gilmore & P. Courvalin: Nucleotide sequence analysis of the gene specifying the bifunctional 6'-aminoglycoside acetyltransferase 2''-aminoglycoside phosphotransferase enzyme in *Streptococcus faecalis* and identification and cloning of gene regions specifying the two activities. *J. Bacteriol.* 167, 631-638 (1986)
83. T. Lambert, G. Gerbaud & P. Courvalin: Characterization of Acinetobacter haemolyticus *aac(6')-Ig* gene encoding an aminoglycoside 6'-N-acetyltransferase which modifies amikacin. *Antimicrob. Agents Chemother.* 37, 2093-2100 (1993)
84. T. Lambert, G. Gerbaud & P. Courvalin: Characterization of the chromosomal *aac(6')-Ij* gene of *Acinetobacter sp*. 13 and the *aac(6')-Ih* plasmid gene of *Acinetobacter baumannii*. *Antimicrob. Agents Chemother.* 38, 1883-1889 (1994)
85. T. Lambert, M. C. Ploy, F. Denis & P. Courvalin: Characterization of the chromosomal aac(6')-Iz gene of stenotrophomonas maltophilia. *Antimicrob Agents Chemother* 43, 2366-71 (1999)
86. S. Magnet, P. Courvalin & T. Lambert: Activation of the cryptic *aac(6')-Iy* aminoglycoside resistance gene of *Salmonella* by a chromosomal deletion generating a transcriptional fusion. *J. Bacteriol.* 181, 6650-6655 (1999)
87. J. Meyer & B. Wiedemann: Characterization of aminoglycoside 6'-N-acetyltransferases (AAC(6')) from gram-negative bacteria and *Streptomyces kanamyceticus*. *J. Antimicrob. Chemother.* 15, 271-282 (1985)
88. E. Rudant, P. Bourlioux, P. Courvalin & T. Lambert: Characterization of the *aac(6')-Ik* gene of *Acinetobacter* sp. 6. *FEMS Microbiol. Lett.* 124, 49-54 (1994)
89. E. Rudant & P. Courvalin, T. Lambert: Characterization of IS18, an element capable of activating the silent *aac(6')-Ij* gene of *Acinetobacter* sp. 13 strain BM2716 by transposition. *Antimicrob. Agents Chemother.* 42, 2759-61 (1998)
90. E. Rudant, P. Courvalin & T. Lambert: Loss of intrinsic aminoglycoside resistance in *Acinetobacter haemoly*ticus as a result of three distinct types of alterations in the *aac(6')-Ig* gene, including insertion of IS17. *Antimicrob. Agents Chemother.* 41, 2646-51 (1997)
91. E. Rudant, P. Bouvet, P. Courvalin & T. Lambert: Contribution of *aac(6')-I* gene and their deduced amino acid sequences to the phylogenetic analysis of proteolytic *Acinetobacter* strains. *accesion number AF031328-32* (1998)
92. E. Rudant, P. Bouvet & P. Courvalin, T. Lambert: Phylogenetic analysis of proteolytic *Acinetobacter* strains based on the sequence of genes encoding aminoglycoside 6'-N-acetyltransferases. *Syst. Appl. Microbiol.* 22, 59-67 (1999)
93. K. Shaw, P. Rather, F. Sabatelli, P. Mann, H. Munayyer, R. Mierzwa, G. Petrikkos, R. Hare, G. Miller & P. Bennet, P. Downey: Characterization of the chromosomal *aac(6')-Ic* gene from *Serratia marcescens*. *Antimicrob. Agents Chemother.* 36, 1447-1455 (1992)
94. F. Schmidt, E. Nucken & R. Henschke: Nucleotide sequence analysis of 2''-aminoglycoside nucleotidyl-transferase ANT(2'') from Tn*4000*: its relationship with AAD(3'') and impact on Tn*21* evolution. *Mol. Microbiol.* 2, 709-711 (1988)
95. F. Tenover, D. Filpula, K. Phillips & J. Plorde: Cloning and sequencing of a gene encoding an aminoglycoside 6'-N-acetyltransferase from an R factor of *Citrobacter diversus*. *J. Bacteriol.* 170, 471-473 (1988)
96. F. Teran, J. Suarez & M. Mendoza: Cloning, sequencing, and uses as a molecular probe of a gene encoding an aminoglycoside 6'-N-acetyltransferase of broad substrate profile. *Antimicrob. Agents Chemother.* 35, 714-719 (1991)
97. G. Tran Van Nhieu & E. Collatz: Heterogeneity of 6'-N-acetyltransferases of type 4 conferring resistance to amikacin and related aminoglycosides in members of the family *Enterobacteriaceae*. *Anitmicrob. Agents Chemother.* 32, 1289-1291 (1988)
98. M. Galimand, T. Lambert, G. Gerbaud & P. Courvalin: Characterization of the *aac(6')-Ib* gene encoding an aminoglycoside 6'-N-acetyltransferase in *Pseudomonas aeruginosa* BM2656. *Antimicrob. Agents Chemother.* 37, 1456-1462 (1993)
99. P. Rather, H. Munayyer, P. Mann, R. Hare, G. Miller & K. Shaw: Genetic analysis of bacterial acetyltransferases: identification of amino acids determining the specificities of the aminoglycoside 6'-N-acetyltransferase Ib and IIa proteins. *J. Bacteriol.* 174, 3196-3203 (1992)
100. R. Chavideh, S. Sholly, D. Panaite & M. E. Tolmasky: Effects of F171 mutations in the 6'-N-acetyltransferase type Ib [AAC(6')-Ib] enzyme on susceptibility to aminoglycosides. *Antimicrob. Agents Chemother.* 43, 2811-2 (1999)
101. A. F. Neuwald & D. Landsman: GCN5-related histone N-acetyltransferases belong to a diverse superfamily that includes the yeast SPT10 protein. *Trends Biochem. Sci.* 22, 154-5 (1997)
102. G. Rose, A. Geselowitz, G. Lesser, R. Lee & M. Zehfue: Hydrophobicity of amino acid residues in globular proteins. *Science* 229, 834-838 (1985)
103. F. Richards: Areas, volumes, packing and protein structure. *Ann. Rev. Biophys. Bioengin.* 6, 151-176 (1977)
104. A. Aleshin, A. Golubev, L. Firsov & R. Honzatko: Crystal structure of glucoamylase from *Aspergillus awamori* var. X100 to 2.2-A resolution. *J. Biol. Chem.* 267, 19291-19298 (1992)
105. I. Casin, F. Bordon, P. Bertin, A. Coutron, I. Podglajen, R. Brasseur & E. Collatz: Aminoglycoside 6'-N-acetyltransferase variants of the Ib type with altered substrate profile in clnical isolates of *Enterobacter cloacae* and *Citrobacter freundii*. *Antimicrob. Agents Chemother.* 42, 209-215 (1998)
106. G. Tran Van Nhieu, F. Bordon & E. Collatz: Incidence of an aminoglycoside 6'-N-acetyltransferase,

ACC(6')-Ib, in amikacin-resistant clinical isolates of gram-negative bacilli, as determined by DNA-DNA hybridisation and immunoblotting. *J. Med. Microbiol.* 36, 83-88 (1992)
107. C. Mabilat, S. Lourencao-Vital, S. Goussard & P. Courvalin: A new example of physical linkage between Tn*1* and Tn*21*: the antibiotic multiple-resistance region of plasmid pCFF04 encoding extended-spectrum beta-lactamase TEM-3. *Mol. Gen. Genet.* 235, 113-121 (1992)
108. B. Sallen, A. Rajoharison, S. Desvarenne & C. Mabilat: Molecular epidemiology of integron-associated antibiotic resistance genes in clinical isolates of enterobacteriaceae. *Micro.b Drug Resist.* 1, 195-202 (1995)
109. N. Larak, G. Rossolini, M. Galleni & J. Frere: *Pseudomonas aeruginosa* plasmid genes, *cat*, *aac(6')*, *imp* and *tnpI*. *Accession number X98393* (1996)
110. G. D. Wright & P. Ladak: Overexpression and characterization of the chromosomal aminoglycoside 6'-N-acetyltransferase from *Enterococcus faecium*. *Antimicro.b Agents Chemother.* 41, 956-60 (1997)

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