

Complexity, Heterogeneity and Mutational Analysis of Antibiotic Inactivating Acetyl Transferases in MDR Conjugative Plasmids Conferring Multi-Resistance

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ABSTRACT

Drug acetylating transferases (AAC) are enzymes that inactivate aminoglycoside antibiotics by acetylating its O' and N' atom in the drug. AAC genes were detected in bacterial plasmids and integrons of many pathogenic bacteria rendering drug resistance. The unique CAT enzyme was discovered early that could acetylate chloramphenicol at 1' and 3' -OH group and largely used as reporter gene in expression vectors. Aminoglycoside 6'-N-acetylating enzymes were mainly classified as *acc6'-la* to *aac6'-lf* and genes were designated as *aacA1* to *aacA8* but *aacA16* or *aacA41* types isomers were also sequenced. The isomers *aacA3*, *aacA4* and *aacA8* are very identical contrary to other *aacA1* isomers. Aminoglycosides 3'-acetylating enzymes were designated as *aac3'-la* to *aac3'-Xa* and genes were designated as *aacC1* to *aacC10*. Sequence analysis suggested that *aac2'*, *aac4'* and bifunctional enzymes (*aac6'-aph2''*) were different class of acetylating enzymes. But *aac6'-1b-cr* protein that was involved in ciprofloxacin resistance resembled to *aac6'-1b* with point mutations. Interestingly, *cat* gene has no similarity to *aacA1* or *aacC1* genes and so far was ignored as being non-clinical origin. But now *catB3* gene was reported in many MDR plasmids of pathogens like *Shigella flexneri* (pR100), *Yersinia pestis* (pIP1202), *Escherichia coli* (pNR1), *Pseudomonas aeruginosa* (pOZ176), *Klebsiella pneumoniae* (pNDM-MAR) and *Salmonella enterica* (pHXY0908). Such plasmids were also frequently associated with *acc3'* and *aac6'* enzymes including diverged β -lactamase genes (*blaTEM*, *blaNDM*, *blaCTX-M* etc) and drugs efflux genes (*acrAB*, *mexAB/CD/XY*, *tetA/S*) as well as AG adenylyl transferases (*aad*) and AG phospho transferases (*aph*) genes. Surely, appearance of *cat*, *amp*, *tet* genes in conjugative plasmids of superbugs is frightening as those genes are randomly used in expression vectors for RDT work. Diversities among drug acetylating enzymes were found very high suggesting multiple mechanisms of their origin.

INTRODUCTION

The MDR is a unique phenomenon where bacteria acquire MDR genes in plasmids and chromosomes and can survive in stressful environment containing high concentration of antibiotics and other pollutants ^[1]. Such bacterial infections, on the other hand are hard to cure by antibiotics contributing huge life and wealth loss worldwide ^[2]. Aminoglycoside acetyl transferases modify the drug's structure by acetylating its N-atom or O-atom in the drug and as a result such acyl-drug could not bind the target site to inhibit bacterial replication, transcription or translation ^[3-8]. So patient could not get rid of bacterial infections by simply taking few tablets of ampicillin, streptomycin or gentamycin antibiotics because acetyl transferases were activated in plasmids that inactivate drugs.

The AMR mechanisms broadly classified into six major categories: (1) activation of β -lactamase (*bla*) (2) activation of drug modifying enzymes (*aac*, *aad*, *aph*) (3) activation of drug efflux genes (*acr*, *tet*, *cmr*, *mex*) including ABC drug transporter (4) alteration of target sites by mutations (rRNA, PBPs) (5) neutralizing antibiotic after binding with drug like *tetM* and (6) lower expression of porins restricting drug entry like imipenem. Thus it is very complex to stop gene creation in nature under stressful environment and none could even imagine such changes how created an acute problem in medicine today^[9].

The first acetylating enzyme was discovered as *cat* gene or chloramphenicol acetyl transferase. Chloramphenicol was first discovered in actinomycete isolated from soil by Ehrlich in 1947 followed by Tamura in 1971 from *Streptosporagium viridogriseum*. Chloramphenicol inhibits the bacterial 30S ribosome and is a very good antibiotic as it cures the common diseases caused by *S. aureus*, *E. coli*, *K. pneumoniae*, and more. However, soon *cat* gene was discovered in many bacterial pathogens those were found resistant to chloramphenicol^[10]. *Cat* enzyme is different from “*amp*” or “*tet*” gene’s mode of actions in that it acetylates the chloramphenicol drug (1’ and 3’ positions) in such a way that acetylated drug no longer able to bind the bacterial ribosome. Thus *cat* gene containing bacteria (in plasmid) easily grow containing MIC amount of chloramphenicol *in vitro* as well as *in vivo* in patient blood^[11,12].

Sequence analysis suggested that *cat* gene was a different enzyme in amino acid sequence than *aacA1* or *aacC1* and other isomers. Why *cat* gene was not considered as *aac* enzyme was not sure to say but *aacA1* enzymes are linked to clinical origin found in MDR plasmids with other MDR genes like *blaTEM*, *strA/B*, *sul1/2*, *tetA/S* type genes including *Tnp* and *Tra* genes^[13-15]. So *cat* gene (designated as *catB3* and was very famous for CAT *in vitro* transcription technology) was isolated from small R-plasmids in bacteria that were not clinically relevant^[10]. However, it was detected now in many large conjugative plasmids of common pathogenic bacteria^[16].

Similarly, *aac(6’)* enzymes was symbolized as *aacA1* to *aacA9* but other isomers like *aac(3’)* designated as *aacC1-aacC9*^[17-20], *aac2’’* and *aac4’* etc were kept aside and their divergence was labelled as *aac(2’’)-Ia/b/c* or *aac(2’’)-I/II* etc^[21-23]. Similarly, there are *catB2/4/8* or *catA_1/2* isomers in the literature. Mutations of *cat* gene as well as *aacC1* and *aacA1* genes were never investigated as compared to huge citations of β -lactamases mutations (*blaTEM*, *blaOXA*, *blaCTX-M* etc) that conferred PDR or XDR type resistance in bacteria^[10]. The origin of this review however, lies on the facts that GenBank data analysis of *aacA1/C1* genes contradicted highly for consensus primers that to be used to study the contamination of superbugs in Kolkata water bodies, particularly Ganga River water.

RESULTS

Similarities among *cat* gene isomers

GenBank data analysis clearly suggested that *cat* genes were widespread in bacterial plasmids and integrons (**Tables 1-4**). Class I integron mediated *catB3* genes of many bacteria like 2177bp *IntI1* integron of *Pseudomonas aeruginosa* (AN:EF660562); 2731bp Class I integron of *Escherichia coli* (AN:ABP35557), 5857bp class I integron of *Klebsiella oxytoca*, 2297bp Class I integron of *Acinetobacter baumannii* (AN:ADF59078), 1738bp Class I integron of *Aeromonas veronii* (AN:ALB07153), 4632bp *In846* integron of *Enterobacter cloacae* (AN:AGJ70489) and that of Proteobacteria (AN:WP_000186237) were identical in protein sequence^[14]. Interestingly, such integrons were also linked to other type’s aminoglycoside acetyl transferases like *aacA1*, *aacA4* and *aacA7*^[12].

We observed that *cat* genes were now associated in conjugative plasmids which were hard to rescue in absence in drugs and also could deliver MDR genes into household bacteria. So it seems AMR is a ubiquitous phenomenon in modern days. As for example, in conjugative MDR plasmid (pI1-34TF; 167198bp) of *Escherichia coli* (AN: LN850163) four acetylating enzymes had been accumulated in four positions of plasmid spreading all across same distance. Two *cat* genes (*cat_1* and *cat_2*; protein Ids. CRK62767 and CRK62815) were different where *cat_2* was *catB3* type but *cat_1* was related to xenobiotic acetylating enzyme. Moreover two other acetylating enzyme, N-6 hydroxylysine O-acetyl transferase (protein id. CRK62680; antibiotic_NAT-like) and SPBe2 prophage derived acetyl N-3’-transferase (*yokD*; protein Id. CRK62756) had roles in inactivating diverse drugs. Such plasmid did contain other *mdr* genes like *blaOXA-1*, *blaTEM-1*, *macB* (macrolide exporter), MFS, *aph* (phosphotransferase), *aad3’* (streptomycin adenyl transferase), *tetA* (tetracycline transporter) and *tmrB* (tunicamycin resistant protein). Further, such plasmid also accumulated HTH-type *cmtR* and *envR*, *tetR* transcription factors in close association with IS-elements and transposons. Multi-align and seq-2 sequence analysis were presented in **Figures 1 and 2** where *catB3* was found similar but *catB8* had many mutations and frequent carboxy-terminal deletions and substitutions^[14].

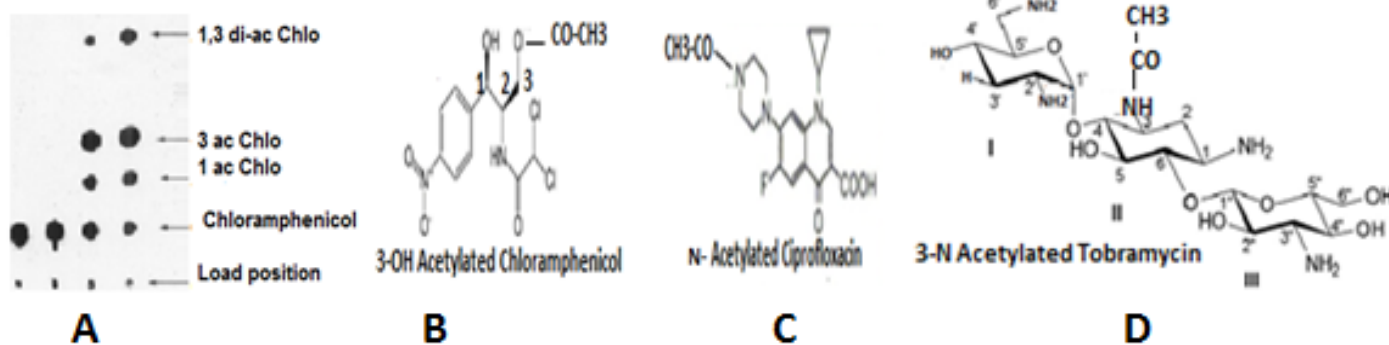


Figure 1. Acetylation of chloramphenicol by CATB2 plasmid transfection of HeLA cells with Lypofectamine reagent (panel, A). Lane, 1 free chloramphenicol, lane 2, cell extract with no plasmid, lane 3, 5 µl cell extract and lane 4, 10 µl cell extract with transfected plasmid. Structures of 3-acetylated chloramphenicol (panel-B), N-acetylated ciprofloxacin (panel, C) and tabromycin (panel, D).



Figure 2. Homologies among CAT Enzymes: (A) CatB3, catB4 & CatB8 similarities. (B) catA2 and CatA4 similarity and (C) catB3 vs. catA_1 similarity.

catB3 gene was present in many MDR plasmids including 249kb *Salmonella enterica* plasmid pHXY0908 (AN:KM877269) and in high molecular weight P1 plasmid of *Klebsiella pneumoniae* containing mphA (macrolide 2" phosphotransferase; protein Id. WP_000219391), aadA2 (ANT3"-1a), aph3'-1a (protein Id. WP_000018329), GCN5 acetyl transferase (protein Id. WP_003026803) and phosphonothricin acetyl transferase (protein Id. WP_004152096) including other mdr genes like drug transporter tetA/tetG, ABC transporter (protein Id. WP_004118832), MFS-type drug efflux proteins (protein Ids. WP_003846917 and WP_000214125) as well as CTX-M-24/KPC-2/VEB-3 types β-Lactamases. Similarly pOZ176 large plasmid (AN:KC543497) contains catB8a, neo (aminoglycoside 3'-O-phosphotransferase; protein Id. AGL46257) and aac6'-IId (aacA4-type) giving resistance to chloramphenicol, neomycin and amikacin respectively [16,24].

Table 1. Classification of major AAC(6') acetyl transferase; The accession numbers of plasmids, integrons and genomic fragments carrying aac(6')-type genes were demonstrated. The different types of pathogenic MDR bacteria and associated *mdr* genes were also described.

Classification of drug modifying aac(6') N-acetyl transferases							
Gene	Synonyme	AA length	Accession no.	Protein ID	Plasmid/ Bacteria	Associated MDR Genes	
aacA1	Aac6'	185aa	AB061794	BAB72153	pCMXR1/ <i>E. coli</i>	<i>Sul1</i> , <i>bla</i> CMY-9	
	Aac6'	185aa	AB116723	BAD11027	<i>Int1-1/ K. pneumoniae</i>	<i>bla</i> GES-4	
	Aac6'-Ia	185aa	AB901039	BAO48019	<i>Int1-1/ P. aeruginosa</i>	<i>bla</i> IMP-10	
	Aac6'	185aa	NC_014167	YP_0036573222	pJA144188/ <i>C. resistens</i>	<i>aphA</i> , <i>strA/B</i> , <i>sul1</i>	
	Aac6'-Ia	185aa	M18967	AAA98298	<i>Int1/ Citrobacter diversus</i>	nd	
	Aac6'-Ii	182aa	L12710	AAB63533	<i>Int1/ Enterococcus faecium</i>	nd	
aacA2	Aac6'-1b	199aa	JN420336	AEB82864	pNDM-MAR/ <i>K. pneumoniae</i>	OXA-1, CTXM-15	
aacA3	aac(6')	184aa	KM111260	AIP98294	<i>Int1-1/ P. aeruginosa</i>	nd	
aacA4	aac6'	184aa	EF138817	ABO21792	<i>Int1-1/P. aeruginosa</i>	<i>bla</i> VIM-3, <i>sul1</i>	
	Aac6'	184aa	EF488369	ABP35556	<i>Int1-1/ E. coli</i>	<i>catB3</i> ,	
	Aac6'	184aa	HM043570	ADH82126	<i>Int1-1/ K. pneumoniae</i>	<i>cmlA1</i>	
	aac6'	184aa	JN596279	AEZ05102	<i>Int1-1/ K. oxytoca</i>	<i>bla</i> GES11, <i>sul1</i>	
	Aac6'-Ib	201aa	M55547	AAA98404	<i>Int1-1/ P. aeruginosa</i>	nd	
	Aac6'-IIa	184aa	M29695	AAA25688	<i>Int1/ P. aeruginosa</i>	nd	
	Aac6'-1b	210aa	GQ293500	ADC80825	23kb plasmid	<i>cmlA</i> , <i>bla</i> TEM-24	
	Aac6'-IIb	180aa	L06163	AAA25680	<i>Int1/ P. aeruginosa</i>	nd	
	Aac6'-Ic	146aa	M94066	AAA26549	<i>Int1/ S. marcescens</i>	nd	
	Aac6'	172aa	KF977034	AHY00029	pDW16C2/ <i>K. pneumoniae</i>	<i>bla</i> VIM	
	Aac6'	172aa	KP870110	AKC98300	pRCPEC6335-2/ <i>E. coli</i>	<i>bla</i> C-5, <i>bla</i> TEM1	
	aacA5	aac6'	158aa	AM749810	CAO78542	<i>Int1-1/ P. aeruginosa</i>	<i>bla</i> VIM-2
		Aac6'	158aa	HQ005291	ADN22946	<i>Int1-1/ P. aeruginosa</i>	<i>bla</i> VIM-2, <i>dhfr</i>
	aacA6	Aac6'-Ib	172aa	AY686225	AAT94163	<i>Int1-1/ A. xylosoxidans</i>	<i>bla</i> VIM-2
aacA7	Aac6'	152aa	KJ679405	AID65189	<i>Int1-1/ P. aeruginosa</i>	OXA-2, <i>aadA6</i>	
	aac6'-1a	152aa	EF577406	ABQ65124	<i>In58/ P. aeruginosa</i>	<i>bla</i> VIN-2	
	Aac6'	152aa	FJ715943	ACN62402	<i>Int1-1/P. aeruginosa</i>	nd	
	Aac6'	152aa	KJ679406	AID65194	<i>Int11/P. aeruginosa</i>	<i>bla</i> VIM-2, <i>aacA4</i>	
	Aac6'	152aa	JX486753	AFV31445	<i>In 58/C. freundii</i>	<i>bla</i> VIM-2, <i>aacA4</i>	
	aac(6')-I1	152aa	KP754008	ALE32150	<i>In 903/P. aeruginosa</i>	<i>Sul1</i>	
	Aac6'	152aa	KJ679405	AID65190	<i>Int11/P. aeruginosa</i>	<i>bla</i> OXA-2. <i>aadA6</i>	
	Aac6'-I1	152aa	U13880	AAA90937	<i>Int1-1/ E. aerogenes</i>	nd	
aacA8	Aac(6')	172aa	KJ679405	AID65187	<i>Int11/P. aeruginosa</i>	OXA-2, <i>aacA6/7</i>	
	Aac6'-IIb	180aa	L06163	AAA25680	Genomic/ <i>P. fluorescences</i>	<i>ANT(3')-Ia</i>	
	Aac(3')	308aa	AXTL01000004	ESD46433	Genome/ <i>Escherichia coli</i>	nd	
aacA(?)	Aac6'-Ic	146aa	M94066	AAA26549	<i>Int1/ Serratia marcescens</i>	nd	
	Aac6'-If	144aa	X55353	CAA39038	<i>Int1/ Enterobacter cloacae</i>	nd	
	Aac6'-Ilg	145aa	L09246	AAA21889	<i>Int1/ Acinetobacter sp</i>	nd	
	Acc6'-Ij	146aa	L29045	AAC41392	<i>Int1/ Acinetobacter sp</i>	nd	
	Aac6'-Ik	145aa	L29510	AAA87229	<i>Int1/ Acinetobacter sp</i>	nd	
	Acc6'-Im	173aa	Z54241	CAA91010	<i>Int1/ Citrobacter freundii</i>	nd	
	Acc6'-Is	146aa	AF031327	AAD03491	<i>Int1/ Acinetobacter sp</i>	nd	
	Acc6'-Iq	183aa	AF047556	AAC25500	<i>Int1/ K. pneumoniae</i>	nd	
	Aac6'-Iw	146aa	AF031331	AAD03495	<i>Int1/ Acinetobacter sp</i>	nd	
	Aac6'-Ix	146aa	AF031332	AAD03496	<i>Int1/ Acinetobacter sp</i>	nd	
	Aac6'-Iy	145aa	AF144880	AAF03531	<i>Int1/ Salmonella enterica</i>	nd	
Aac6'-Iz	153aa	AF140221	AAD52985	<i>Int1/ S. maltophilia</i>	nd		
aacA16	Aac6'-Iip	173aa	Z54241	CAA91010	1153bp <i>Int1/Citrobacter</i>	nd	
aacA17	Aac6'-Iiq	183aa	AF047556	AAC25500	plasmid/ <i>K. pneumoniae</i>	nd	
aacA28	Aac6'-Iae	183aa	AB104852	BAD14386	Plasmid/ <i>P. aeruginosa</i>	<i>bla</i> IMP, <i>aadA1</i>	
aacA30	Aac6'-I30	184aa	AY289608	AAP43642	2220bp <i>Int1/S. enterica</i>	<i>bla</i> OXA-53	
aacA39	Aac6'-Iai	188aa	EU886977	ACI28880	pLQ1001/ <i>P. aeruginosa</i>	<i>aadA1</i> , <i>Sul1</i>	
aacA41	Aac6'-Iaf	183aa	AB462903	BAH66386	<i>Int1123/P. aeruginosa</i>	<i>bla</i> IMP, <i>sul1</i>	
aacA42	Aac6'-33	184aa	GQ337064	ACT99625	6816bp/ <i>P. aeruginosa</i>	<i>bla</i> GES, OXA-2	
aacA43	Aac6'-Ii	182aa	L12710	AAB63533	<i>Int1/Enterococcus faecium</i>	nd	

Table 2. Classification of major AAC(3') acetyl transferase; The accession numbers of plasmids, integrons and genomic fragments carrying aac(3')-type genes were demonstrated. Protein ids and types of MDR bacteria were also described.

Classification of different amino glycoside 3'-acetyl transferases (aacC1)						
Aac3' Genes	Enzyme types	No of AA	Accession number	Protein Id number	Plasmid/genomic	Name of bacteria
aacC1	Aac3'-Ia	177aa	X15852	CAA33850	pR1033(Tn1696)	Enterobacteriaceae
	Aac3'-Ia	154aa	AY577724	AAT51721	3035bp genomic	A. baumannii
	Aac3'-Ia	176aa	L06157	AAA88422	genomic	P. aeruginosa
	Aac3'-I	154aa	KJ688704	AID61151	2684bp genomic	K. pneumoniae
	Aac3'-I	154aa	AJ009820.2	CAA08847	6436bp pSEM	S. enterica
	Aac3'-Ia	154aa	KR028107	ALD03719	2427bp genomic	A. baumannii
	Aac3'-I	154aa	JX486753	AFV31447	3030bp In58 integron	C. freundii
aacC2	Aac3'-IIa	286aa	JQ364967	AFI72859	87kb pGuE-NDM	E. coli
	accC2	286aa	X54723	CAA38525	R plasmid	E. coli
	Aac3'-IId	286aa	EU022314	ABS70977	Plasmid fragment	E. coli
	Aac3'-IIe	286aa	EU022315	ABS70978	Plasmid fragment	E. coli
	Aac3'	286aa	JN202624	AFP55521	pFZ51; 15672bp	H. parasuis
	Aac3'	311aa	AXUL01000144	ESA99429	2663 bp genomic	K pneumoniae
	Aac3'	286aa	KJ187752	AJD77170	pTR2	K. pneumoniae
	Aac3'-III*	286aa	HF545433	CCN79846	pE66An	E. coli
	Aac3'-III*	286aa	NC_024983	YP_009061951	pSTm-A54650	S. enterica
	Aac3'	286aa	KP010147	AJN91221	pECO-HN; 18784bp	E. coli
	Aac3'	286aa	JX988621	AFZ84485	pNDM-OM	K. pneumoniae
	Aac3'	286aa	HQ840942	AES85952	pSRC27-H; 50129bp	S. enterica
aacC3	aacC3	286aa	X13543	CAA31895	Plasmid pWP113a	Enterobacteriaceae
	Aac(3')-III	208aa	JMVN01000059	KDG46702	5980bp genomic	E. coli
	Aac3'-IIIa	271aa	X55652	CAA39184	2336bp genomic	P. aeruginosa
	Aac3'-IIIb	245aa	L06160	AAA25682	2613bp genomic	P. aeruginosa
	Aac3'-IIIc	279aa	L06161	AAA25683	1234bp genomic	P. aeruginosa
	Aac3'-III	308aa	ADTS01000075	EGB89811	genomic	E. coli
	Aac3'-III	308aa	AXTL01000004	ESD46483	6803bp genomic	E. coli
	Aac3'-III	294aa	L0017738	CRH08791	143kb pRCS57	E. coli
aacC4	Aac3'-IV	261aa	X01385	CAA25642	1368bp genomic	E. coli
	Aac3'-IV	255aa	MPWC01000123	OKB98731	genomic	K. pneumoniae
	Aac3'-IV	172aa	AJ009820.2	CAA08845	pSEM	S. enterica
	AAC3'	206aa	CP015500	ANE70283	Genome	K. pneumoniae
	Aac3'	227aa	FLCN01000053	SAT62391	Genomic fragment	K. pneumoniae
aacC5	aac(3')-Vb	269aa	M97172	AAA26548	1572bp genomic	S. marcescens
aacC6	Aac(3')-VI	299aa	M88012	AAA16194	2077bp genomic	E. cloacae
	Aac3'-VIa	274aa	nd	WP_031611451	nd	E. coli
	Aac3'-VI	300aa	nd	WP_053271189	nd	E. coli
	Aac3'-VI	299aa	nd	WP-020837048	nd	S. enterica
aacC7	Aac3'-VIIa	288aa	M22999	AAA88552	1494bp genomic	S. rimosus
	Aac3'-VIIa	288aa	AJ749845	CAG44462	genomic	S. rimosus
	Aac3'-IIb	286aa	GG657754	EFL26570	genomic	S. himasstatiniosis
aacC8	Aac3'-VIIIa	286aa	M55426	AAA26685	1353bp genomic	S. fradiae
	Aac3'-VIIIb	287aa	nd	WP_063840271	nd	S. ribosidificus
aacC9	Aac3'-IXa	281aa	M55427	AAA25334	1149bp genomic	M. chalcea
	Aac3'-IXb	279aa	KB405056	EMF57573	genomic	S. bottropensis
aacC10	Aac3'-X-Ia	284aa	AB028210	BAA78619	genomic	S. griseus
	Aac3'-X-I	284aa	FMCP01000318	SCE16679	genomic	Streptomyces sp
	Aac3'-X-I	284aa	FLTQ01000010	SBU98351	genomic	Streptomyces sp

Similarities among the aac(6') isomers (aacA1-aacA8)

Aminoglycoside 6'-N-acetyl transferase actively acetylates 6-N atom of amikacin, kanamycin, and neomycin [25-30]. BLAST analysis suggested that aacA1 was unique enzyme and had no similarity to aacA2/3/4/5/6 but aacA3 has similarity to aacA4 and aacA8 lineages (Figure 3 and Table 1). Two types (184aa and 172aa) of aacA4 enzyme contained difference in NH2 terminal 13 amino acids indicating a precursor. Aminoglycoside 6'-N-acetyl transferases (aacA1) were located in plasmids and integrons of E. coli and other Enterobacteriaceae and were identical sequence [31-36]. As for example, plasmid NR79 (8298bp) of E. coli had aacA1 including aadA3, catB2, and sul1 genes. Another 8049bp small plasmid pCMXR1 contains blaCMY-9 and sul1 mdr

genes with *aacA1*. However, in *Pseudomonas aeruginosa*, *aacA1* gene was located in class I integron In831 with *blaIMP-10* that involved in imipenem resistance. All *aacA1* sequences were identical and no mutation was found. *Pseudomonas aeruginosa* class I integron contains *aacA3* gene (protein Id. AIP98294 and AN:KM111260). A conjugative MDR plasmid, pNDM-MAR (267242bp, AN:JN420336) of *Klebsiella pneumoniae* contains *aacA4* gene (protein Id. AFB82784) along with deadly *blaNDM-1* and *blaCTX-M-15* genes (Table 4)^[37]. Another *aacA4* gene (AN:ABP35556) was located in 2731bp class I integron (AN: EF488369) of *E. coli* with closely linked to *catB3* gene^[38]. Two *aacA4* genes (accession nos. AEZ05099) were located in 6061bp small plasmid pINCan01 (AN:JN596279) of *Klebsiella oxytoca* in association with *blaGES-11* and *sul1* *mdr* genes^[39]. Two genes for aminoglycoside acetyl transferases (*aacA5* and *aadA7*) were located in *Pseudomonas* 2903bp class I integron in association with *blaVIM-2* and *dhfr* genes, involved in carbapenem and trimethoprim resistance respectively^[40-44]. Two aminoglycoside 6'-acetyltransferase type Ib (*aac6'-1b*; 172aa) were cloned from *Achromobacter xylosoxidans* (AN:AY686225) as 3436bp class I integron with also *blaVIM-2* gene. A 281 amino acids *aacA8* gene was found with *aacA7* (152aa) isomers in 3608bp class I integron (AN:KJ679405) of *Pseudomonas aeruginosa* associated with *aadA6* and *blaOXA-2*. There was no similarity between *aacA1* vs. *aacA4* of *Klebsiella pneumoniae* (protein Id. ADH82126) and *Escherichia coli* (Protein Id. BAB72153) or *Pseudomonas aeruginosa* (protein Id. AIP98294) and therefore *Pseudomonas* enzyme designated as *aacA3* which was isolated in China (Figure 2 and Table 1). Neither 172aa 6'-N-acetyl transferase (*aac6'-1b*; protein Id. CAF18332) of *Morganella morgani* has similarity to *aacA1* and designated as *aacA4* and such enzyme in both plasmids were identical suggesting horizontal transfer but *Klebsiella* plasmid (AN:HM043570) was partially sequenced (38-44). The few new aminoglycoside resistance gene, designated *aac(6')-lae*, encoded a 183-amino-acid protein that shared 57.1% identity with AAC(6')-Iq. Such *Escherichia coli* expressing exogenous *aac(6')-lae* showed resistance to amikacin, dibekacin, isepamicin, kanamycin, netilmicin, sisomicin, and tobramycin but not to arbekacin, gentamycin or streptomycin (Figure 1 and Tables 1-4)^[45].

Table 3. Localization of cat genes and hybrid aac-aph genes. Popular cat genes in plasmids/integrations of pathogenic MDR bacteria were described with GenBank accession numbers and protein ids. Also activated hybrid aac-aph genes were described including less well known *aac2'* and *aac4'* type genes that destroy many aminoglycoside antibiotics.

Types of <i>aac2'</i> and <i>aac4'</i> acetyl transferases						
AAC2'	<i>aac(2')-I</i>	181aa	NC_000962.3	NP_214776	Genome	<i>M. tuberculosis</i>
	<i>aac(2')-Id</i>	210aa	U72743	AAB41701	Genomic fragment	<i>M. smegmatis</i>
	<i>Aac2''-IC</i>	181aa	CPO12506.2	ALB17378	Genome	<i>M. tuberculosis</i>
	<i>aac-2'</i>	198aa	CWKHO1000002	CVZ16866	genomic	<i>M. neworleansense</i>
AAC-APH hybrid acetyl transferases-phospho transferase						
AAC-APH	<i>aacA-aphD</i>	479aa	AB682805	BAM15583	6483bp genomic	<i>S. aureus</i>
	<i>aacA-aphD</i>	479aa	GZU565967	AAA88548	<i>pSK1</i>	<i>S. aureus</i>
	<i>aacA</i>	479aa	AP003367	BAB47534	<i>pVRSAp; 25107bp</i>	<i>S. aureus</i>
	<i>Aac6'-aph2'</i>	479aa	M13771	AAA26865	2120bp integron	<i>E. faecalis</i>
	<i>Aac6'-aph2''</i>	479aa	CP002120	ADL66016	2924344bp genomic; nt 2111743-2113182	<i>S. aureus</i>
Type of CAT genes or chloramphenicol acetyl transferases						
CAT	<i>catB3</i>	210aa	EF516991	ABP52023	2655bp integron	<i>E. coli</i>
	<i>catB3</i>	210aa	HX259086	AAD20921	<i>pHSH2</i>	<i>E. coli</i>
	<i>catB3</i>	210aa	DQ343904	ABC69169	1406bp integron	<i>M. morgani</i>
	<i>catB3</i>	210aa	HQ170516	ADX02581	3735bp integron	<i>A. media</i>
	<i>catB3</i>	210aa	KM278198	AIX48179	2208bp integron	<i>V. fluminis</i>
	<i>catB3</i>	210aa	JX885645	AGM38586	13241bp genomic	<i>S. enterica</i>
	<i>catB3</i>	210aa	KC237285	AGM38599	9983bp plasmid	<i>S. enterica</i>
	<i>catB3</i>	210aa	KX421096	AOR05996	253kb plasmid <i>pA32</i>	<i>S. enterica</i>
	<i>catB3</i>	210aa	EF488369	ABP35557	2731bp integron	<i>E. coli</i>
	<i>catIII</i>	213aa	JN202624	AFP55523	<i>pFZ51; 15672bp</i>	<i>H. parasuis</i>
	<i>catB4</i>	182aa	AP012056	BAN19548	<i>pKPX-2</i>	<i>K. pneumoniae</i>
	<i>catB8</i>	210aa	KC543497	AGL46467	<i>pOZ176</i>	<i>P. aeruginosa</i>
	<i>catA1</i>	219aa	AP012055	BAN19276	<i>pKPX-1</i>	<i>K. pneumoniae</i>
	<i>catA1</i>	219aa	KJ541071	AIV96857	<i>pG5A4Y217</i>	<i>E. coli</i>
	<i>cat_2</i>	219aa	LN850163	CRK62815	<i>pI1-34TF</i>	<i>E. coli</i>
	<i>catB2</i>	210aa	AF047479.2	AAC14737	<i>pNR79</i>	<i>E. coli</i>

Similarities among *aac(3')* acetyl transferases;

Aminoglycoside 3'-N/-O-acetyl transferases were isolated from various microorganisms where it was found both in plasmids and chromosomes^[46-55]. *aacC1* enzyme was isolated from 2324bp plasmid R1033 of many *Enterobacteriaceae* and were *aac(3')-1a* type (AN:CAA33850)^[46]. *aacC2* (EC:2.3.2.81) of *Escherichia coli* was cloned from a Moscow isolate (AN:X54723; Protein Id. CAA38525)^[47]. A 310 amino acids length *Escherichia coli aac3'* enzyme (protein Id. ESD46483) was isolated in genome fragments (Accession nos. AXTL01000004 and ADTS01000075). *Pseudomonas aeruginosa aac(3')-1b* enzyme was 176 aa and likely partial

(protein Id. AAA88422) [48]. *aacC3* isomers (*aac3'*-IIIa/b/c) were cloned from *Pseudomonas aeruginosa* (ANs. X55652, L06160 and L06161) [49] and *aac3'*-Vb was sequenced from *Serratia marcescens* genomic fragment and the enzyme had 72% similarity to *aac3'*-Va and gave high label resistance to gentamycin, netimicin and moderate resistance to tobramycin (protein Id. AAA26548) [53]. Similarly, *aac3'*-VIa gene (AN:M88012) was cloned from *Enterobacter cloacae* large plasmid with 39% and 48% similarities to *aac3'*-VII and *aac3'*-II respectively [51,52]. Similarly, *aac3'*-VIa enzyme was found 299 aa (protein Id. AAA16194). *aacC7* (protein Id. AAA88552), *aacC8* (protein Id. AAA26685) and *aacC9* (protein Id. AAA25334) genes were isolated from *Streptomyces rimosus* (AN:M22999), *Streptomyces fradiae* (AN:M55426) and *Micromonospora chalicea* (AN:M55427) (Figure 3 and Table 2) [53,54]. AAC(3)-XI, a new aminoglycoside 3-N-acetyltransferase from *Corynebacterium striatum* was isolated recently [55].

Similarities among 2'' acetyl transferases

An *Acinetobacter baumannii aac2''* acetyl transferase (286aa; protein Id. AAA21890) was identified [56]. Also a chromosomally encoded aminoglycoside 2'-N-acetyltransferase (AAC(2')-Ic) from *Mycobacterium tuberculosis* was isolated [57-61]. Similarities among *aac6'*-1b-cr enzymes that mediates fluoroquinolone resistance. Aminoglycoside acetyltransferase, AAC(6')-Ib reduces the activity of ciprofloxacin by N-acetylation at the amino nitrogen on its piperazinyl substituent [62]. AAC(6')-Ib-cr differs from AAC(6')-Ib, by two specific codon exchanges Trp102→Arg and Asp179→Tyr, which have been found to be necessary and sufficient for the ciprofloxacin resistance phenotype [63]. Among 313 Enterobacteriaceae from the United States, *aac(6')-Ib-cr* was detected in 15 (32%) of 47 *E. coli* isolates, 17 (16%) of 106 *K. pneumoniae* isolates, and 12 (7.5%) of 160 Enterobacter isolates [64]. ESBL *Escherichia coli* were isolated from poultry farm in Switzerland associated with fluoroquinolone resistance due to *aac6'*-Ib-cr acetyl transferase. *Aac6'*-1b-cr enzyme present in pKPS30 plasmid (AN:KF793937; nt. 22991-23509) of *Klebsiella pneumoniae* [65]. Enterobacter aerogenes isolates associated with variable expression of the *aac(6')-Ib-cr* gene was reported giving high label fluoroquinolones resistance [66]. A Korean study indicated the presence of *aac(6')-Ib-cr* variant enzyme in *E. coli* and *K. pneumoniae* and few strains simultaneously contained *aac(6')-Ib* and *aac6'*-1b-cr with resistant to tobramycin, kanamycin and amikacin (Figure 1) [66]. Other mechanisms for fluoroquinolone resistance were target protection by qnrA1/B1/S1 proteins (has high affinity for ciprofloxacin) and active drug efflux by QepA and OqxAB pumps (remove ciprofloxacin from bacterial cytoplasm) [63]. *Salmonella enterica* plasmid also detected *aac6'*-1b-cr gene (AN:KM877269; nt. 119381-119965, complement).

Table 4. Characteristics of large MDR conjugative plasmids carrying multiple *aac* and *cat* genes. Chloramphenicol acetyl transferases, AG acetyl transferases and other *mdr* genes were clustered in one plasmid and bacteria carrying such plasmid were usually XDR and PDR types. Further, mutations in *gyrAB*, 16S rRNA, *porB* and *ABC* genes have not been analyzed here.

Multi-drug resistant genes in Plasmids involving <i>aac</i> and <i>cat</i> genes				
Accession number	Plasmid Size	MDR Genes profiles with one or more <i>aac</i> and <i>cat</i> genes in large bacterial conjugative plasmids	GenBank Year	Pathogenic bacterial name
CP015725	210	aacA4 , blaOXA1, cat , arr2, sul1, aph2', mrx, mph2', blaNDM1, ANT3", dhfr, blaTEM1, RND, ANT3"-Ia, ble, blaCTXm-65, floR(MFS), sul1. Aac3'-IVa	2016	<i>S. enterica</i>
KF793937	61	tetA, blaOXA-30, catB3 , aac6'-1b-cr , arr3, sul1, SAPa, QnrB4, blaDHA, mex, mphA(2''), aphA1(3')	2016	<i>K. pneumoniae</i>
KM877269	249	Sul1, aphA1, aadA1, cmlA, aadA2, floR, hph, aac3'-IV , aac6'-1b-cr , , blaOXA1, catB , arr3, sul1, terA/C/F	2015	<i>S. enterica</i>
L0017736	124	tetA, aac3'-Ib , blaOXA-1, aac6'-Ia , blaCTX-M-15, blaTEM1, ABC	2016	<i>E. coli</i>
L0017738	143	tetA, blaTEM1, blaCTX-M-15, aac3'-III , cat , bla OXA-1. Dhfr, mrx(2''), mphA, ABC	2016	<i>E. coli</i>
LN794248	300	Sul1, strA/B, blaTEM1, tetA, aac6'-Ib , blaOXA1, catB3 , aacC3 , tmrB, blaCTXM-15, aadA1, catA1	2015	<i>S. enterica</i>
KF954759	73	<i>blaKPC-3</i> , strB, aac6' , chrB, ncrA/Y, <i>srbA</i>	2014	<i>K. pneumoniae</i>
KX421096	253	OqxB/A RND, sul1, dhfr, fosA3, sul2, aac3'-Iva , aac6'-1b-cr , blaOxa1, catB3 , arr3, sul1, TerC/D/A	2016	<i>S. enterica</i>
AB61665	47	<i>blaIMP-2</i> , aacA4 , aadA2, tetA, blaCTXm, sul1	2012	<i>K. pneumoniae</i>
KC354802	41	aacA4 , aadA1, blaOXA-9, blaTEM-1	2013	<i>K. pneumoniae</i>
NC_021087	26	<i>blaGIM-1</i> , aacA4 , aadA1, blaOXA-2, sul1	2015	<i>E. cloacae</i>
NG_035843	15	<i>blaOXA-30</i> , catB3 , arr-3, sul1, qnr, blaDHA-1	2014	<i>E. coli</i>
LN555650	299	terF, sul1, strA, catB , blaACC-1, aacA4 , blaVIM-1	2015	<i>S. enterica</i>
JN420336	267	blaNDM1, blaOXA1, aac6' , qnrB1, catB , blaCTX-M,	2012	<i>K. pneumoniae</i>
KC543497	501	Ter2, sul1,, MFS,, blaIMP-9, ble, catB8 , aac6'-IId	2016	<i>P. aerogenosa</i>
NC_022078	317	ABC, merB, cat, aph*, aac3' , cmr, tetA, blaKPC	2015	<i>K. pneumoniae</i>
JQ64967	87	aad, sul1, blaNDM1, aac3'-II , blaOXA1, aac6'-1b-cr	2016	<i>E. coli</i>
AP012056	141	Aac3' , aac6' , catB4 , tetA, sul2, blaOXA/CTX, strB,	2016	<i>K. pneumoniae</i>
CP011634	227	<i>blaOXA</i> , aad*, <i>blaTEM</i> , merC, sul1, aac	2015	<i>K. oxytoca</i>
NC_010795	15	Sul1, aacC2 , catB3 , strA, blaROB-1 , aph3'-I	2014	<i>A. p-pneumoniae</i>
CP009116	95	Aph, blaTEM, aac3' , MFS, dhfr, aad, arr2, blaNDM1	2014	<i>K. pneumoniae</i>

NC_019889	87	Aac3'-II , blaNDM1, sul1, MsrE, mphE	2014	<i>K. pneumoniae</i>
AP012055	250	blaNDM1, ccdA/B, aadA2, catA1 , qacA1	2013	<i>K. pneumoniae</i>
KF250428	151	blaIMP-4, aacA4 , MerC, cmr, flo ^R	2013	<i>K. pneumoniae</i>
HG530658	223	blaACC-1, strA, aadA2, aac3'	2015	<i>E. coli</i>
NC_019375	180	blaVIM, aacA7 , dhfr, ANT3', blaSHV-5, sul1, aph3'	2014	<i>P. stuartii</i>
KF705205	134	hph, strA, aac3'-IV , tetA, blaTEM-1	2015	<i>S. enterica</i>
NC_022522	168	blaCTX-M-25, aacA4* , strB, strA, aadB, blaOXA-21	2014	<i>S. enterica</i>
LC055503	160	blaSHV-12, aac6' , blaOXA-10, aadA1, sul, blaDHA	2015	<i>K. pneumoniae</i>
HG941719	135	blaTEM/CTX/OXA, aadA5, mphA, aac6' , sull, tetA	2014	<i>Escherichia coli</i>
KJ541071	44	sul1, blaOXA-2, aadA/B, blaTEM, catA1 , blaGES-5	2014	<i>E. coli</i>
GU256641	110	sul2, strA, blaTEM, blaSCO, aacC2 , blaACC-4	2011	<i>E. coli</i>
KJ541681	90	tetA, sul1, aadA1, aac3'-Ia , aac6'-Ib , aadA2, blaSHV5	2015	<i>K. oxytoca</i>

Similarities among bifunctional (aac-aph) drugs acetylating enzymes

Discovery of *Enterococcus faecium* aac6'-aph2'' bifunctional aminoglycoside modifying enzyme in 70 kb plasmid containing transposon Tn5281 and IS256 element was phenomenon [67]. All *E. faecium* strains from United Kingdom had showed high level gentamycin resistance with many MDR genes and multiple conjugative plasmids were detected as demonstrated by Pulse-Field Gel Electrophoresis (PFGE) and hybridization studies [68-71]. A French study isolated *Staphylococcus aureus* strains with aminoglycoside resistance and had both aac6'-aph2'' hybrid and aph3'-III genes (phospho transferases) located chromosomally [40]. A Turkish study with 358 gentamycin resistant *Staphylococcus aureus* showed 334 aac-aph type bifunctional acetylating enzyme [72]. A US-study indicated high level aminoglycoside resistance in *Enterococcus faecalis* due to aac6'-aph2'' enzyme causing both gentamycin and streptomycin resistance but streptomycin resistance conferred due to other aph3'-III/aph5''-III type genes [73]. *Shigella sonnei* genome has aac6'-aph2'' bifunctional enzyme (protein Id. CS006978) with similarity to aacA4 enzyme (EC:2.3.1.82) of *Proteus vulgaris* (protein Id. WP_058127929), *Escherichia coli* (protein Id. CRL66321), *Acinetobacter johnsoni* (protein Id. ALV74709) or *Pseudomonas aeruginosa* (protein Ids. CBI63199, CBL95252 and ALI59095). *Enterococcus faecalis* chromosome islands carried bi-functional acetyl transferases (aacA-aphD; protein Id. ANNO2929), streptothricin acetyl transferase (Sat4; Protein Id. ANNO2919) including many adenylyl transferases (Protein Ids. ANNO2918, ANNO2921, ANNO2922 and ANNO2927), streptomycin phosphotransferase (aphA3) and ISA(E) gene that conferred resistant to pleuromycin, streptogramin and incosamide antibiotics [74]. Bifunctional aminoglycoside-modifying enzyme like aminoglycoside (6')acetyltransferase-Ie/aminoglycoside 2''-phosphotransferase-Ia (AAC(6')-Ie-APH(2'')-Ia) from Gram-positive cocci, (was isolated that conferred resistance to the 4,6-disubstituted aminoglycosides kanamycin, tobramycin, dibekacin, gentamycin, and sisomicin, but not to arbekacin, amikacin, isepamicin, or netilmicin [75]. A recently discovered bifunctional antibiotic-resistance enzyme named AAC(3)-Ib/AAC(6')-Ib', from *Pseudomonas aeruginosa*, catalyzes the acetylation of aminoglycoside antibiotics. The AAC(3)-Ib domain appears to be highly specific to fortimicin A and gentamicin as substrates, while the AAC(6')-Ib' domain exhibits a broad substrate spectrum [76-79].

Similarities of drug acetylating enzymes with strA/B and other phospho transferases

strA and strB genes could inactivate the streptomycin by phosphorylation. Phospho-streptomycin could not bind bacterial ribosome and such bacteria could grow at as high as 100 µg/ml streptomycin giving AMR [80]. Sequence analysis suggested there was no similarities between catB3 vs. strA/B and aac6'/3' vs. strA/B. The aminoglycoside phosphotransferase (aph gene) phosphorylate the antibiotics so that phosphorylated kanamycin, amikacin and neomycin could not kill the bacteria. There was also no similarity between AG acetyl transferase (aacA1/aacC1) with 264aa neomycin phosphotransferase (aph3'-Ia, Protein Id. CAA23892) and 341aa hygromycin phospho transferase (hygA, protein Id. AHC55481). However, streptomycin phosphotransferases (strA, 278aa and strB, 276aa; protein Ids. AAA26443 and CED95338) have only 28% similarities within 72% and 37% cover respectively [81,82]. Similarity of dug acetylating enzymes with drug adenylation enzymes. The aminoglycoside adenylyl transferase [EC:2.7.7.47] was present in many bacterial plasmids of diverse bacterial species of *Escherichia* (ANs:HG41719, KJ484637, KM377239), *Klebsiella* (ANs:KF914286 and KF719970), *Salmonella* (AN:JQ899055) and *Acinetobacter* (AN: KM401411), but also present in some bacterial chromosome as in *Salmonella enterica* [83].

Antibiotic adenylyl transferase (~263aa) adenylylate drugs at 6-N position and conferred bacteria resistant to aminoglycoside antibiotics like streptomycin and amikacin [84]. Such enzymes seem Rel-Spo-like super family and do not have much similarity to the AG 3'/6' N-acetyl transferases and catB3 enzyme. Interestingly, adenylyl transferases have similarity across the species and also notably exists as different isomers (aadA1 to aadA17) with 50-80% sequence similarities among itself [85,86].

Similarities of drug acetylating enzymes with β-lactamases

Beta-lactamases are very diverged enzymes with at least twenty distinct isomers including TEM, OXA, NDM1 and CTX-M [1]. Metallo β-lactamases (VIM, IMP, NDM1, SPM, GIM, DIM) are very deadly as resistant to imipenem and beta-lactamase inhibitors cavulinate and sulbactam [40]. BLAST analysis found no similarities between AG acetyl transferases and β-lactamases [87].

Conjugative plasmids have multiple drug acetylating enzymes

GenBank (www.ncbi.nlm.nih.gov) search indicated (Table 4) that each single conjugative plasmid carried multiple drug

acetylating enzymes (cat, aac) in association with many beta-lactamase genes as well as strA/B, sul1/2 genes (10). As for example, *Salmonella enterica* plasmids (plncH12 and pHXY40908) acquired four (aac6'-1b, catB3, aac3, catA1) and three (aac3'-IV, aac6'-1b-cr, catB3) drug acetylating enzymes (accession nos. LN794248, KM877269). **Table 4** profoundly indicated how seriously multiple *mdr* genes had gathered in plasmids of many common pathogens that no drug would work to cure such bacterial infections.

Chromosomal localization of drug acetyl transferases

A 143aa long acetyl transferase was isolated from *A. baumannii* (AN:JFWP02000007; nt. 103508-103939) with similarity to aac6'-like enzyme (protein Id. EXT17036). *Acinetobacter* genomospecies aac6'-I_r enzyme (protein Id. WP_063840327) has 79% similarity to aac6'-I_s (protein Id. AHD03491) but aac6'-I_t, aac6'-I_w, aac6'-I_x and aac6'-I_u have 83%, 85%, 90% and 94% similarities to aac6'-I_s respectively (protein Ids. WP_063840329, WP_005296085, ALB75422 and WP_063840330) ^[88,89]. *Aeromonas hydrophila* genome (AN:LNUR01000009; nt. 843037-843495) has an unique 152aa 6'-acetyl transferase with limited similarity (47%) to aac6'-I_h of *A. baumannii* (protein Id. ALB75422). *Pseudomonas saponiphila* genome (AN:FNTJ01000001; nt. 257741-258187) also has 148aa long 6'-acetyl transferase (protein Id. SEB43247) with 51% similarity to *A. baumannii* aac6'-I_h enzyme (protein Id. ALB75422).

Chromosomally located AAC(2')-I_c of *Mycobacterium tuberculosis* catalyzes the coenzyme A (CoA)-dependent acetylation of the 2' hydroxyl or amino group of a broad spectrum of aminoglycosides. The aac(2')-I_c gene was cloned and expressed in *Escherichia coli*, and was purified. Recombinant AAC(2')-I_c was a soluble protein of 20,000 Da and acetylated all aminoglycosides substrates tested *in vitro*, including therapeutically important antibiotics like tobramycin, amikacin, kanamycin and ribostamycin. MRSA bacteria were initially discovered as methicillin resistant microbe named *Staphylococcus aureus* gram positive circular bacteria that notoriously known as for skin infections. The methicillin resistance gene (*mecA*) encodes a methicillin-resistant penicillin-binding protein and activated with mobile genetic element, the staphylococcal cassette chromosome *mec* (SCC*mec*), of many MRSA isolates which also associated with *bla*, *aac*, *aad*, ANT and sul1/2 types MDR genes. Such multi-drug resistant bacteria are susceptible only to glycopeptides antibiotics such as colistin and tigicycline ^[90,91]. Mutations among CAT enzymes

catB3 gene has no similarity to chloramphenicol drug efflux gene *cmlA* (protein Id. AKG90151), or acetyl transferases (aac3'-IV; Protein Id. AKG90173) or kanamycin phosphotransferases (protein Id. AKG90144) or hygromycin phosphotransferase (protein Id. AKG90172). No mutations were reported among the class I integron mediated catB3 genes of many Enterobacteriaceae (see, ANs: EF660562; ABP35557; ADF59078; AGJ70489). However, 217 aa long *Proteus mirabilis* CAT enzymes (protein Ids. WP_049194799 vs. WP_049197252) had two point mutations (V24A, N130D) and 201 aa long CAT enzymes in *Escherichia coli* had two point mutations (Y16N, D195N) and 9aa NH2-terminal substitutions (protein Ids. WP_050436713 vs. WP_050558894)

Mutation among aacA1 type enzymes

We see seven mutations in *Aeromonas hydrophila* aac6'-I enzymes (ANT67440 vs. KWR67119) with 95% similarities. However, 26 mutations in *Aeromonas piscicola* aac6' enzyme (protein Id. KWR67119) at the NH2-terminal 60 amino acid region (WP_065401184 vs. KWR67119). A 194aa long aac6'-I_a enzyme of *Wohlfahrtiimonas chitiniclastica* has 9aa signalling peptide at the NH2 terminus and very similar to aacA1 enzyme of *E. coli* plasmid pCMXR1 (AN:AB061794) except one point mutation (V84I). But 185 aa *E. coli* aacA1 enzyme (protein Id. BAB72153) has only 55% similarity with 185 aa long *Klebsiella pneumoniae* aac6'-I_a enzyme (protein Id. WP_032495046). Such unusual aacA4 enzyme has two different mutations in *A. baumannii* (I2S, K141N; protein Id. WP_024437351) and *P. aeruginosa* (R20Q, R95K; protein Id. WP_071846376).

Several mutations in many Enterobacteriaceae aacA4 enzymes (aac6'-1b type) were reported with respect to *Escherichia coli* enzyme (protein Id. ABP35556). Different point mutations were found in *Pseudomonas aeruginosa* (A75G; protein Id. WP_071846301), (S83G; protein Id. WP_071846385) and (T132A, K133R; protein Id. WP_071593232). Similarly in *E. coli* aacA4 enzyme, a single mutation (Q101L; protein Id. WP_069985732) was reported and also in *Enterobacter hormachei* (R181C; protein Id. WP_07220113) and in *Pseudomonas putida* (Q49R; protein Id. WP_071984682). A 197aa long aacA4 enzyme (protein Id. AKJ19116) in *P. aeruginosa* plasmid pMRVIM0713 (AN:KP975076) had extended 13 aa and two mutations (M1V and S102L) were reported. Such NH2-terminal fusion were found frequently as in *P. aeruginosa* integron-mediated 203aa long aac6'-1b enzyme (protein Id. AAC46343; AN:U59183) and also in 210 aa long enzyme (protein Id. CBI63203; AN:FN554980). A chromosome mediated *Acinetobacter baumannii* 216aa long aacA4 enzyme (protein Id. EKA73751) with similar M1V and S102L mutations was also reported (**Figure 3**).

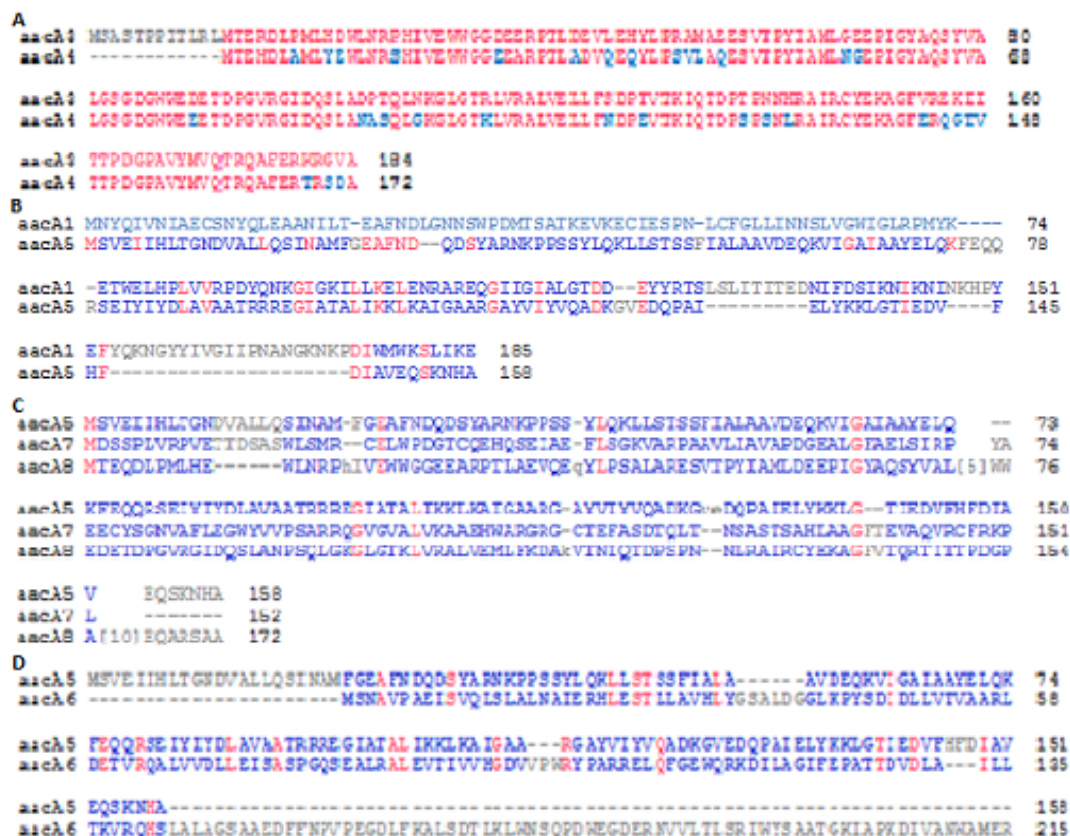


Figure 3. Similarities among aacA1 enzymes: (A) aacA1 vs. AacA4 with terminal truncation with point mutations. (B) aacA1 vs. aacA5 with internal deletions and no similarity. (C) No similarities among aacA5, aacA6 and aacA8 and (D) no similarity between aacA5 vs. aacA6.

A 183aa long aacA16 N(6')-acetyl transferase (protein Id. WP_001109644) has only 60% similarity with aacA43 enzyme of many Enterobacteriaceae (protein Ids. WP_063840279; WP_024437351). Such enzyme has 60% similarity with the *Citrobacter freundii* aac6'-I1 enzyme (protein Id. CAA91010; AN:Z542441) with most divergent at the NH2-terminus. As such enzymes have only 55-65% similarity with the aacA1 enzymes (protein Id. BAB72153), their association in aac6'-I class was not therefore justified (>60 mutations).

Mutations among aacC1 type enzymes

AacC1 enzyme of Enterobacteriaceae is 177 amino acids (protein Id. CAA33850) and has similarity to Acinetobacter baumannii gentamycin N-3'-acetyl transferases having different mutations reported in different isolates: In one isolate (protein Ids. WP_031950771, EXD70625 and WP_000441892) with three mutations (R98K, P102A, T175P). In *Pseudomonas aeruginosa*, aacC1 enzyme has many mutations reported. As for example, one isolate has S23R, K74R, D83E mutations (protein Id. WP_052158612); in another K38Q, D83E, E165D mutations (protein Id. WP_063840256) and in another (protein Id. ALE32149) five mutations (K38Q, D83E, R98K, P102A, and E165D) with few common mutations. However, less conserved enzymes have 71-73% similarities (protein Ids. ABN10340 and CRQ60998). *Salmonella enterica* aacC1 enzyme (protein Id. WP_032491356) has three mutations with two very common (S104I, R98K and P102A) and in another, two mutations were detected (P152T, S81I; protein Id: WP_032491356). An *A. baumannii* and *P. aeruginosa* AAC(3)-I enzyme have P152T common mutation (protein Ids: WP_052133400 and WP_063840258).

E. coli 158aa long AAC3'-acetyl transferase has two mutations (A53G, R234Q) that induces apramycin resistance (protein Id. WP_064756331) and also in *K. pneumoniae* (W5L, A53G; protein Id. WP_064735602). But in another *E. coli* mutant three mutations (A53G, H241R, G246E ; protein Id. WP_072833186) and in another four mutations (A53G, K177N, L178C, D182E; protein Id. WP_072739411) were reported. AAC(3)-I enzyme of *Serratia marcescens* (178aa; protein Id. OC095380) and *Enterobacter cloacae* (178aa; protein Id. WP_032663836) have only 73% similarity with 22aa signalling protein at the NH2 terminus suggesting those enzymes are diverged aac3'-Ia type. Even both enzymes are 178aa long with 88% identity, have 22 mutations demonstrating drug acetyl transferases are indeed involved rapidly similar to β -lactamases (**Figure 4**).

ESD46483	M[4]	TER[4]	SVLQFRGDIAMH-TRKAITEALQKLGVTGTDLLMVHASKAIGPVEGGAETVVAALRSA	VGPTGTVM	78
AAA21890	-	----	-----MH-TRKAITEALRKLGVQGTGDDLLMVHASKAIGPVEGGAETVVAALRSA	VGPTGTVM	56
CAD27711	M[2]	----	-----EwRKAELIGQLLNLGVTGPGVLLVHSSFRSVPLEDPGLGLEIALRAA	LGPGGTLV	59
AAA88422	M	--L[4]	DVTQQGSRPKTK-LGGSSMSIATVTKIGPDEISAMRAVLDLFGKEFEDIPITYSDRQPTN[5]	LLHSETFI	76
CAA93850	M	--L[4]	DVTQQGSRPKTK-LGGSSMGIIRTICRLGPDQVKSMAALDLFGREFGQVATYSQHQPDS[5]	LLRSKTFI	77
CAA38525	-	----	-----MH-TRKAITEALQKLGVTGTDLLMVHASKAIGPVEGGAETVVAALRSA	VGPTGTVM	56
CAA39184	M	TDL[3]	-----H-THAHLVDAFQALGIRAGQALMLHASVKAVGAVMGCPNVILQALMDA	LTPDGTLM	62
AAA25682	-	----	-----MVHAAVSRVGRLLDGPDTIIAALRDT	VGPGSTVL	34
AAA25683	M[4]	SKP[3]	-----AAVTR---ASLAADLAALGLAAGDAVMHAAVSKVGRLLDGPDTIIAALSDA	GRPAGTIL	68
AAA26548	-	----	-----MN-TIESITADLHGLGVRPGDLMVHASKAIGPVEGGAASVVSALRAA	VGSAGTLM	56
AAA16194	M	TDP[4]	DLHEPATAPATPwSKSELVLRQLRDLGVRSQDMMPHVSLRAVGPLADGPQLVLDALIEA	VGPTGNIL	75
AAA88552	M	DEL	ALLKRSDDGPVT---RTRLARDLTALGLGDGDTVMFHTRMSAVGYVAGGPEIVIGALRDV	VERGRTLM	68
AAA26685	M	DEK	ELIERAGGPVT---RGRLLVRDLREALGVAGDVTVMVHTRMSAIGYVVGSPQIVDAVRDA	VGADGTLM	68
AAA25334	M	EEM	SLLNHSGGPVT---RSRIKHDLDLGLKGDGDDVIFHTRMSAIGYVAGGTQTIIGALLDV	VGARGTLM	68
BAA78619	M	DET	ELLRRSDGPVT---RDRIRHDLAALGLVPGDVTVMFHTRLSAIGYVSGSPQIVDALLDV	VGPTGTLT	68
ESD46483	GYASWDR	-SPYEETLNGARLDDEARRIWL	PFDPATAGTY-RGFGLINQFLVQAPGARRSAHPDASMVAVGPIAETLTEPH	156	
AAA21890	GYASWDR	-SPYEETLNGARLDKARRIWL	PFDPATAGTY-RGFGLINQFLVQAPGARRSAHPDASMVAVGPIAETLTEPH	134	
CAD27711	MP-SWSC	-----LDDEP	-----FDBATSPVT-PDLGVVSDTFWRLPNVKSRAHP-FAFAAAGPQAEQIISDP	118	
AAA88422	ALAAAFDR	-----GTAIGGLAAYVLPKFEQARSEIYIYDLAVASSHRRRLGVATALISHLKRVAVELGAYVIYVQAD-	146		
CAA93850	ALAAFDQ	-----EAVVGALAAAYVLPKFEQARSEIYIYDLAVSSEHRRQGTATLINALKHEANALGAYVIYVQAD-	147		
CAA38525	GYASWDR	-SPYEETLNGARLDKTRRIWL	PFDPATAGTY-RGFGLINQFLVQAPGARRSAHPDASMVAVGPIAETLTEPH	134	
CAA39184	MYAGWQD	-IPDFIDSLPDALKAVYLECHP	PFDPATARAV-RENSVAEFLRTWPCVHRSANPEASMVAVGPAALLTANH	140	
AAA25682	AYADWEA	rYEDLDVDDAG-RVPPQEWREHVP	PFDPRRSRAI-RDNGVLPFLRITPCTLRSGNPGASLVALGAKAWEFTADH	112	
AAA25683	AYADWEA	rYEDLDVDEDC-RVPQEWREHVP	PFDPRRSRAI-RDNGVLPFLRITPCTLRSGNPGASLVALGAKAWEFTADH	146	
AAA26548	GYASWDR	-SPYEETLNGARMDEELRRW	PFDLATSQTY-PGFGLINRFLLEAPDARRSAHPDASMVAVGPIAATLTEPH	134	
AAA16194	AFVSWRD	-SPYEQLGHDAAPAAIAQSW	PAFDPDHPAY-PGFGAINEFIRTYPGCRRTAHPDASMAAIGPDAAWLVAPH	153	
AAA88552	VTCGWND	aPPYDFLDWPDWQDARAEHP	PAFDPDLSEAD-HNNGRLPEALRRRPGAVRSRHPDASFAALGAAATLADH	147	
AAA26685	AYCGWND	aPPYDLAEWPPAWREAAAEW	PAFDPDLSEAD-RGNRVEALRHPGAVRSRHPDASFAALGAAAEIAMDH	147	
AAA25334	VPCGWNN	aPPYDFLDWPDWQDARAEHP	PAFDPDLSEAD-YNNGRLPEALRRRPGAVRSRHPDASFAALGAAAEIAMDH	147	
BAA78619	VTCGWND	aPPYDFLDWPPAWQEAARAH	PAFDPRTSEAE-HANGRLPEALRRRPGAVRSRHPDVSLAALGASAPAIMDAH	147	
ESD46483	ELGHALGEGS	PVERFVRLGCKALLLGAPLNSVTL	ALHYAEAVADIPNKRRTVYEMPMLGRDGEVAVKNTASDYDSNGILDCE	236	
AAA21890	ELGHALGKGS	PVERFVRLGCKALLLGAPLNSVTL	ALHYAEAVADIPNKRRTVYEMPMLGRNGEVAVKNTASEYDSNGILDCE	214	
CAD27711	LPLPHSPAS	PVARVHIELDQVLLLVGVDHANT	LHLAELMAKVPYGG--VPRHCTIL-QDGKLVVRVDYLENHCCERFAL	195	
AAA88422	-----	YGDDPAVALYTKLGVREDDVMHFD	IDPRTAT-----	176	
CAA93850	-----	YGDDPAVALYTKLGRREEVMHFD	IDPSTAT-----	177	
CAA38525	KLGHALGEGS	PVERFVRLGCKALLLGAPLNSVTL	ALHYAEAVADIPNKRRTVYEMPMLGRNGEVAVKNTASDYDSNGILDCE	214	
CAA39184	ALDYGYGES	PIAKLVAIEGYVIMLGAPLDTIT	TLHHAAYLAKMRHKNVVRVPCPII-RDGRKVVWTVEDYDTGDPDDY	219	
AAA25682	FLDYGYGES	PIAKLVEAGKVMILGAPLDTLTL	LHHAELADIPGKRIKRIEVPFA-TPTGTQWRMIEEFDTGDPPIVAG	191	
AAA25683	FLDYGYGES	PIARLVEAGKVMILGAPLDTLTL	LHHAELADIPGKRIRRIEVPFA-TPTGTQWRMIEEFDTGDPPIVAG	225	
AAA26548	RLGQALGEGS	PIERFVGHGKVVLLGAPLDSVT	VLHYAEAIAPIPNKRRTVYEMPMLGRDGRVWRWELAEFDNSNGILDCE	214	
AAA16194	EMGAAYGFRS	PIARFLAHAGKILSIGAGPDAVT	ALHYAEAVARIEGKRRTVYSMPLL-REGKRVWTVSDWDSNGILDEY	232	
AAA88552	FWDDHGPDS	PIARLVAMGCRVLLLGAPLEALT	LHHAELADAPGKRFRVYEQPII-VDGEVWRRRHDIDSDGAFDY	226	
AAA26685	FWDDHGPDS	PIARLAGAGCRVLLLGAPLDTL	TLHHAELAEAPGKRFRVYEQPVT-VGGRRVWRRFRDVTISRGV-PY	225	
AAA25334	FWDHHPGPD	PIARLVAHSCRVLLLGAPLDTMT	LHHAELADVRSKRFRVYEQPII-VNQQRVWRQFRDIDSDGAFDY	226	
BAA78619	FWDDHGPDS	PIARLVALGCRVLLLGAPLDTMT	LHHAELAQAPGKRFRVYEQPIE-VAGEVWVRTFRDIDSDGAFDY	226	
ESD46483	AI---	EGkPDAVETIANAYVKLGRHREGV	VVGFACCYLFDQAQDIVTFGVTYLEKHF	GTTPI[15]	308
AAA21890	AI---	EGkPDAVETIANAYVKLGRHREGV	VVGFACCYLFDQAQDIVTFGVTYLEKHF	GATPI[15]	286
CAD27711	AD---	rWLKExSLQKEGFPVGHAFARL	IRSRDIVATALGQLGRDPLIFLHP	PEGGMRRMRCSRSP[6]	261
AAA88422	-----	-----	-----	-----	-----
CAA93850	-----	-----	-----	-----	-----
CAA38525	AI---	EGkPDAVETIANAYVKLGRHREGV	VVGFACCYLFDQAQDIVTFGVTYLEKHF	GTTPI[15]	286
CAA39184	S-----	FEQIARDYVAQCGGTRGKVGDA	DAYLFAAQDLTRFAVQWLES	RFSGDSAS[2]	271
AAA25682	IA-----	EDYFAGIVTEFLASQGRQGLI	GAAPSVLVDAAAITA	FGVWLEKRF	FTSP
AAA25683	IA-----	EDYFAEIVTAFIAGGRGRQGLI	GAAPSVLVDAAAITA	FGVWLES	RFSGPSS
AAA26548	AV---	DGkPDAVETIAKAYVELGRHREGV	VGRAPSYLFEAQDIVTFGVTYLE	QHFG-AP-	269
AAA16194	AA--	pdG-PDAVERIARDYLARTRVA	QGVVGAQSRLLIDAADIVS	FGIEWLEARHAAPAA[10]	299
AAA88552	SA	lvpEG-TEAFEIIGRDMRAAGI	GRRGTVGAADSHLFEARDVVD	FGVAMMEKLG	RRER[3]
AAA26685	GR	vvpEG-VVPFTVIAQDMLAAGI	GRTGRVAAA	PVHLFEAADVVR	FGVEWIESRMGGAAG[2]
AAA25334	ST--	vrRG-VEPFEAIRDMLSAGI	GRQGRVGAADSYLFDAGPFVFN	FAINWIEAK	LKR---
BAA78619	SS	avpEG-QDPFAVIVGSMLAAGI	GRGFRVGAARSRLLFDAAPAVE	FGVRWIEEHLNRDR-	284

Figure 4. Sequence alignment of different AAC3' drug acetyl transferases. ESD46483 (*E. coli* chromosomal aacC1 protein, 308aa, AN:AXTL01000004); AAA21890 (*A. baumannii* aacC2 protein, 286aa; AN:M62833); CAD27711 (*E. coli* aacC4 protein, 261aa, pHK11-apra vector; AN:AJ438947); CAA33850 (*Enterobacteriaceae* plasmid mediated aacC1 protein, 177aa; AN:X15852); CAA38525 (*E. coli* aacC2 protein, 286aa; AN:X54723); CAA39184 (*P. aeruginosa* aacC3, 271aa; AN:X55652); AAA26682 (*P. aeruginosa* aacC3b protein, 245aa; AN:L06160); AAA25683 (*P. aeruginosa* aacC3c protein, 279aa; AN:L06161); AAA26548 (*C. marcescens* aacC5b protein, 269aa; AN:M97172); AAA16194 (*E. cloacae* aacC6 protein, 299aa; AN:M88012); AAA88552 (*S. rimosus* aacC7 protein, 288aa, AN:M22999); AAA26685 (*S. fradiae* aacC8 protein, 286aa; AN:M55426); AAA25334 (*M. Chalcea* aacC9 protein, 281aa; AN:M55427); BAA78619 (*S. greuseus kan* gene. 284aa, AN:AB028210).

The 286 amino acids length *Escherichia coli* aacC2 (protein Id. CAA38525) was found similar to aac(3')-IIC enzyme of *E. coli* (protein Id. WP_063840266) but no similarity to aacC1 enzyme. *Escherichia coli* aacC2 enzyme (protein Id. AFI72859) had four (L14F, H275Q, E276K) and ten mutations (T11L, R12Q, K78E, P84L, A162T, N194D, E204D, A268P, A274V, Q278E) with the similar aac3'-IId (protein Id. ABS70977) and aac3'-Ile (protein Id. ABS70978) enzymes respectively. Among the Enterobacteriaceae

aac3'-II enzymes several mutations were reported (T87A, A112T, T132S, A245V; see Protein Ids. WP_00988063; WP_051421733; KTQ31168). In *Salmonella enterica* enzyme 4bp deletion and T132S mutation were reported (protein Id. WP_060588432). No mutation was found between *K. pneumoniae* and *E. coli aacC2* enzymes (AGP03376 vs. ODH13880). Other *aac(3')-II* enzymes reported in *Klebsiella pneumoniae* and *Acinetobacter baumannii* were shown very similar mutations indicating a horizontal transfer of such genes from *E. coli* plasmid by conjugation. The mutations in *K. pneumoniae* (protein Ids. AGP03376 and WP_031944095) were; L11I, Q12R, R70L, T79A, R183W, S193R, D204E, T270A, V277A, E279Q, and C280R. The mutations in *A. baumannii* (protein Ids. WP_057690920 vs. WP_002063884) were E142K, G184V, D186X but with *Escherichia coli* plasmid-mediated *aacC2* enzyme (protein Id. CAA38525) 14 mutations were reported (L11I, Q12R, R70L, T79A, K135E, R183W, V184G, Y186D, S193R, D204E, T270A, V277A, E279Q, and C280R). In *Enterobacter* sp strain 50858885 similar mutations were reported as follows: L11I, Q12R, R70L, K78E, K135E, T79A, P84L, K135E, R183W, S193R, T270A, V277A, E279Q and C280R. Although *Shigella flexneri* (protein Id. ADY02606) had very similar mutations but much more mutations (only 76% similarity) were found in *Salmonella enterica* enzyme (protein Id. WP_061873001) and 85% similarity to *Sinorhizobium meliloti* (protein Id. WP_003525983) *aac3'-II* enzyme.

However, numerous mutations were reported in *aac(3')-III* enzymes (EC:2.3.1.81) of *E. coli* conjugative MDR-plasmid pRCS57 (143225bp; AN:L0017738) as follows: L11I, Q12R, R70L, T79A, K135E, R183W, S193R, D204E, T270A, V277A, E279Q, and C280R (protein Id. CRH08791). Such plasmid has also had *mrx* macrolide resistant protein, *mphR* repressor, *bla*TEM-1 and *tetA* tetracycline efflux protein as well as *Tnp* and *Tra* genes including many IS-elements. Interestingly, a 308aa *aac3'-III*-like *E. coli* enzyme (protein Id. EGB89811) had extended 9aa at the NH2 terminus and very similar to other Enterobacteriaceae 294aa *aac3'-III* enzyme (protein Id. WP_013023858).

An 172aa long *aacC4* enzymes of *E. coli* (protein Id. ACS75040) and *P. aeruginosa* (protein Id. AGG23542) were found two mutations R107Q, D170S and M54L, D170S respectively. *Stenotrophomonas maltophilia* had D170M point mutation (protein Id. ABN48565; AN:EF210035). *Acinetobacter baumannii* genome had reported 210aa 38aa N-terminal extended *aacA4* enzyme with D170S, O171V mutations. *Citrobacter freundii* plasmid pMRVIM1012 had 34aa N-terminal extended *aacC4* enzyme with L90S, D170S mutations. *Achromobacter xylosoxidans* 19.8kb plasmid mediated 210aa *aacA4* enzyme (protein Id. BAV17747) had also similar mutations (L90S, D170S). Similar mutation further reported in *Serratia marcescens* class-3 integron 188aa long *aacC4* enzyme likely due to 16aa extension (protein Id. AAL10408) and also in *Vibrio cholerae* 192aa *aacA4* enzyme (protein Id. AAM52493), suggesting similar integron/plasmid involved in conjugation to transfer *aacA4* genes. Certainly such extended enzymes had not proved by the protein product analysis and reflects wrong reporting as judged by plasmid mediated shorter active enzymes reported (**Table 1**).

Other *Escherichia coli* extended *aac3'-IV* enzymes (258aa) have several point mutations (Y188H, R236Q; WP_064770919 vs. WP_064756331) and (A53G, A216G; WP_064769430 vs. WP_064769895) and (A53G, A241R, G247E; WP_064769430 vs. WP_072833186). A genomic clone of *S. enterica* may code 266 aa enzyme with NH2 terminal extension of 8aa and M1V and common A53G mutations (AN:LHLZ0100022; protein Id. KNT82816). Similarly, in *K. pneumoniae* genome (AN: MPWC0100123 and JMXV0100021) two *aacC4* enzymes (protein Ids. OKB98731 and KDJ63161) were predicted with 255 and 254 aa long and had common A53G point mutation.

Aac3'-VI enzyme of *E. coli* differs with single point mutations at T132S and A244V of *E. cloacae aacC6* enzyme (protein Ids. WP_053271189 vs. AAA16194) and insertions in *Salmonella enterica* genome may code for different acetyl transferases (protein Ids. KNK91744 and EHC71407).

Drug acetylating enzymes in expression vectors

Cat gene was introduced in many DNA vectors: As for example BAC vector pHL931 (protein Id. ALV82398); Gateway vector pB4cCGW for plant (protein Id. BAV44483); pDONRpEX18Gm expression vector (protein Id. AJW82929); orf selection vector pSOS (protein Id. ABK62679) and so many to state. *AacC1* gene (177aa) was cloned in varieties of expression vectors like pMQ175 (AN:FJ380062), pCVD001 (AN:KM017942), pSX (AN:JN703735), pUCP24 (AN:HM368668), and also in association with Beta-lactamase gene like pEX18gmGW (AN:KM880127), pLOXGen4 (AN:AJ401048); and in association with *cat* gene like pMpGWB236 (ANLC057515), pJM101 (AN:KX782328); and in association with AG phospho transferase gene like pBG51 (AN:KT192133) and pVZ324 (AN:AF100177). *AacA4* gene (267aa) was also cloned in suicidal vector pSUI3 (AN:KX863720) and in BAC vector pHL931 (AN:KT362048) and in cloning vector pHK11-apra (AN: AJ438947) 263aa long *aacA4*-type gene was dissimilar at amino and carboxy terminals. Wide spread use of *mdr* genes in plasmids should be controlled as recombinant drug resistant bacterial isolates might be released into environment.

DISCUSSION

We see wide spread presence of AAC enzymes in plasmids and chromosomes of household bacteria. Recently Hasani et al. evaluated aminoglycoside resistance in 87 *Acinetobacter baumannii* strains isolated from four hospitals of Iran and was found *aac(3')-Ia* predominant sequence group (SG) including ANT(2')-Ia and APH(3')-Via. APH(3')-Ia related to resistance against amikacin and kanamycin, whereas ANT(2')-Ia was related to the resistance for gentamycin and tobramycin in SG2 and tobramycin

resistance was correlated with *aac(6)-Ib* [92]. Many cellular N-acetyl transferases (NAT2) are known but are different than CAT and AAC enzymes.

Corynebacterium striatum BM4687 was resistant to gentamicin and tobramycin but susceptible to kanamycin A and amikacin. A novel 3-N-acetyltransferase type XI was purified and sequenced with 60% amino acid identity with acetyltransferases [93]. The purified protein acetylated dibekacin to the amine at the C-3 position. Many chromosomal enzymes designated as *aacA9*, *aacA16*, *aacA30*, *aacA41*, *aacA43* etc. were reported but further needed for placement [94]. Further, a unique bi-functional acetylating enzyme, *aac(6')-Ie-aph2''-I* was detected in *Staphylococci cassette* chromosome [95] as well as Enterococci clinical isolates in China [93] and *Campilobacter* isolates in USA.

CONCLUSION

Thus it was concluded that drug acetyl transferases were highly diversified. Cat gene although had minimum divergence but 3'- and 6'- acetyl transferases (*aacA1/C1*) were arose very highly similar to very diversified β -lactamase (*bla*) genes (Chakraborty, 2016). Functional analysis of aminoglycoside acetyl transferases mutants, however less explored. Because such genes were associated with MBLs and *acrAB/CD* or *mexAB/XY* diverged tripartite proton drug efflux genes, Never the less, *cmlA2* chloramphenicol transporter was also detected in 23kb plasmids like pRYC103T24 (AN:GQ293500; protein Id. ADC80829) of *E. coli* indicating chloramphenicol highly contaminated in nature and *mdr* gene evolution was maximum. 3-D structures and critical active site changes with better drug acetylating motifs and enlarged drugs selectivity must be addressed to design new drug against superbugs. AMR had reached an alarming label worldwide and all *mdr* genes must be assessed carefully at the molecular level. More importantly, three rings of aminoglycosides with many -OH and -NH₂ groups of acetylation were designated as (I=1'-6', upper), (II=1-6, middle) and (III=1''-6'', lower) but the number of acetylating preferences would be determined carefully. Although drug phospho transferases and adenylyl transferases could act very similarly to various O- and N-atom of the drugs but such enzymes had no similarity to *aacC1* or *aacA1* type enzymes indicating drug modifying enzymes indeed strongly diversified [50]. Presently, no de-acetylase enzyme was reported in MDR plasmids. Thus super conjugative plasmids with deacetylase genes could be used as a control measure to combat drug modifying superbugs that were contaminated highly in air and water. We are studying acetyl transferases in multi-drug resistant bacteria from Kolkata Ganga River and data indicates multiple isomers are present. In essence, *mdr* genes like drug acetyl transferases have created a very serious problem in human health and safety. It appears new drug development should be accelerated but alternate strategies like development of phyto-antibiotics, gene medicines (antisense, ribozyme, dicer-casper, miRNA) and DNA nanotechnology applications also should adapted in R&D research of India and other Asian countries with population burden.

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REFERENCES

1. Chakraborty AK. Multi-drug resistant genes in bacteria and 21st Century problems associated with antibiotic therapy. *Biotechnol Ind J* 2016;12:114.
2. Shaw KJ, et al. Molecular genetics of aminoglycoside resistant genes and familial relationships of the aminoglycoside-modifying enzymes. *Microbiol Rev.* 1993;57:138-163.
3. Courvalin P, et al. Resistance towards aminoglycoside-aminocyclitol antibiotics in bacteria. *J Antimicrob Chemother.* 1981;8:57-69.
4. Chinault AC, et al. Characterization of transferable plasmids from *Shigella flexneri* 2a that confer resistance to trimethoprim, streptomycin, and sulfonamides. *Plasmid.* 1986;5:119-131.
5. Shimizu K, et al. Comparison of aminoglycoside resistance patterns in Japan, Formosa, and Korea, Chile, and the United States. *Antimicrob Agents Chemother.* 1985;28:282-288.
6. Teran FJ, et al. Cloning, sequencing, and use as a molecular probe of a gene encoding an aminoglycoside 6'-N-acetyltransferase of broad substrate profile. *Antimicrob. Agents Chemother.* 1991;35 (4):714-719.
7. Wybenga-Groot LE, et al. Crystal structure of an aminoglycoside 6'-N-acetyltransferase: defining the GCN5-related N-acetyltransferase superfamily fold. *Structure.* 1999;7:497-507.
8. Hasani A, et al. Frequency of aminoglycoside-modifying enzymes and *armA* among different sequence groups of *Acinetobacter baumannii* in Iran. *Microb. Drug Resist.* 2016;22:347-353.
9. Li X-Z, et al. The challenge of efflux-mediated antibiotic resistance in Gram-negative bacteria. *Clin Microbiol Rev* 2015;28:337-418.
10. Chakraborty AK. Complexity, heterogeneity, 3-D structures and transcriptional activation of multi-drug resistant clinically relevant bacterial beta-lactamases. *Trends Biotechnol-open access.* 2016;2:1-001.

11. Bolivar F and Backman K. Plasmids of *Escherichia coli* as cloning vectors. *Methods Enzymol* 1979; 68:245-267.
12. Bunny KL, et al. New mobile gene cassettes containing an aminoglycoside resistance gene, *aacA7*, and a chloramphenicol resistance gene, *catB3*, in an integron in pBWH301. *Antimicrob Agents Chemother.* 1995;39:686-693.
13. Moura A, et al. Novel gene cassettes and integrons in antibiotic-resistant bacteria isolated from urban wastewaters. *Res Microbiol.* 2012;163:92-100.
14. Wang M, et al. Plasmid-mediated quinolone resistance in clinical isolates of *Escherichia coli* from Shanghai, China. *Antimicrob Agents Chemother.* 2003;47:2242-2248.
15. Boyd D, et al. Complete sequence of four multidrug-resistant MOBQ1 plasmids harboring *bla*GES-5 isolated from *Escherichia coli* and *Serratia marcescens* persisting in a hospital in Canada. *Microb Drug Resist.* 2015;21:253-260.
16. Xiong J, et al. Complete sequence of pOZ176, a 500-Kilobase IncP-2 plasmid encoding IMP-9-mediated carbapenem resistance, from outbreak isolate *Pseudomonas aeruginosa* 96. *Antimicrob Agents Chemother.* 2013;57:3775-3782.
17. Courvalin P and Carlier C. Resistance towards aminoglycoside-aminocyclitol antibiotics in bacteria. *J Antimicrob Chemother.* 1981;8:57-69.
18. Lovering AM, et al. AAC(1): a new aminoglycoside-acetylating enzyme modifying the C1 amino group of apramycin. *J Antimicrob Chemother.* 1987;20: 803-813.
19. Teran F, et al. Cloning, sequencing, and uses as a molecular probe of a gene encoding an aminoglycoside 6-N-acetyltransferase of broad substrate profile. *Antimicrob. Agents Chemother.* 1991;35: 714-719.
20. Champion HM, et al. Cloning and characterization of an AAC(6') gene from *Serratia marcescens*. *J Antimicrob Chemother* 1988;22:587-596.
21. Tenover F, et al. Cloning and sequencing of a gene encoding an aminoglycoside 6-N-acetyltransferase from an R factor of *Citrobacter diversus*. *J Bacteriol.* 1988;170:471-473.
22. Chevereau M, et al. Aminoglycoside resistance in bacteria mediated by gentamicin acetyltransferase II, an enzyme modifying the 2'-amino group of aminoglycoside antibiotics. *J Biol Chem.* 1974;13:598-603.
23. Aínsa JA, et al. Aminoglycoside 2'-N-acetyltransferase genes are universally present in mycobacteria: characterization of the *aac(2')-Ic* gene from *Mycobacterium tuberculosis* and the *aac(2')-Id* gene from *Mycobacterium smegmatis*. *Mol Microbiol.* 1997;24: 431-441.
24. Villa L, et al. Complete sequencing of an IncH plasmid carrying the *bla*NDM-1, *bla*CTX-M-15 and *qnrB1* genes. *J Antimicrob Chemother.* 2012;67:1645-1650.
25. Meyer JF and Wiedemann B. Characterization of aminoglycoside 6'-N-acetyltransferases [AAC(6')] from gram-negative bacteria and *Streptomyces kanamyceticus*. *J Antimicrob Chemother.* 1985;15:271-282.
26. Nobuta K, et al. Sequencing and expression of the 6-N-acetyltransferase gene of transposon Tn1331 from *Klebsiella pneumoniae*. *J Bacteriol.* 1988;170:3769-3773.
27. Lambert T, et al. Characterization of *Acinetobacter haemolyticus aac(6)-Ig* gene encoding an aminoglycoside 6-N-acetyltransferase which modifies amikacin. *Antimicrob. Agents Chemother.* 1993;37: 2093-2100.
28. Thungapathra M, et al. Occurrence of antibiotic resistance gene cassettes *aac(6')-Ib*, *dfrA5*, *dfrA12*, and *ereA2* in class I integrons in non-O1, non-O139 *Vibrio cholerae* strains in India. *Antimicrob Agents Chemother.* 2002;46:2948-2955.
29. Tolmasky ME, et al. Genetic organization of antibiotic resistance genes (*aac(6)-Ib*, *aadA*, and *oxa9*) in the multiresistance transposon Tn1331. *Plasmid.* 1993;29: 31-40.
30. Tran Van Nhieu G, et al. 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.* 1992;36:83-88.
31. Schroder J, et al. Complete genome sequence, lifestyle, and multi-drug resistance of the human pathogen *Corynebacterium resistens* DSM 45100 isolated from blood samples of a leukemia patient. *BMC Genomics.* 2012;13:141-146.
32. Hannecart-Pokorni E, et al. Characterization of the 6-N-aminoglycoside acetyltransferase gene, *aac(6)-II*, associated with a *sull* type integron. *Antimicrob Agents Chemother.* 1997;41:314-318.
33. Casin IF, et al. Aminoglycoside 6-N-acetyltransferase variants of the Ib type with altered substrate profile in clinical isolates of *Enterobacter cloacae* and *Citrobacter freundii*. *Antimicrob. Agents Chemother.* 1998;42:209-215.
34. Shaw KJ, et al. Isolation, characterization, and DNA sequence analysis of an AAC(6')-II gene from *Pseudomonas aeruginosa*. *Antimicrob. Agents Chemother.* 1989;33:2052-2062.
35. Tada T, et al. Novel 6'-N-Aminoglycoside Acetyltransferase AAC(6')-Iaj from a Clinical Isolate of *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother.* 2013;57:96-100.

36. Tenover FC, et al. Cloning and sequencing of a gene encoding an aminoglycoside 6'-N-acetyltransferase from an R factor of *Citrobacter diversus*. *J Bacteriol.* 1988;170:471-473.
37. Villa L, et al. Complete sequencing of an IncH plasmid carrying the blaNDM-1, blaCTX-M-15 and qnrB1 genes. *J Antimicrob Chemother* 2012;67:1645-1650.
38. Wang GQ, et al. Characterization of integrons-mediated antimicrobial resistance among *Escherichia coli* strains isolated from bovine mastitis. *Vet Microbiol* 2008;127:73-78.
39. Barrios H, et al. A plasmid-encoded class 1 integron contains GES-type extended-spectrum beta-Lactamases in Enterobacteriaceae clinical isolates in Mexico. *Antimicrob. Agents Chemother.* 2012;56:4032-4034.
40. el Solh N, et al. Molecular cloning and analysis of *Staphylococcus aureus* chromosomal aminoglycoside resistance genes. *Plasmid.* 1986;15:104-118.
41. Bunny K, et al. New mobile gene cassettes containing an aminoglycoside resistance gene, aacA7, and a chloramphenicol resistance gene, catB3, in an integron in pBWH301. *Antimicrob. Agents Chemother.* 1995;39:686-693.
42. Lambert T, et al. Characterization of the chromosomal aac(6')-Ij gene of *Acinetobacter* sp. and the aac(6')-Ih plasmid gene of *Acinetobacter baumannii*. *Antimicrob. Agents Chemother.* 1994;38:1883-1889.
43. Bunny KL, et al. New mobile gene cassettes containing an aminoglycoside resistance gene, aacA7, and a chloramphenicol resistance gene, catB3, in an integron in pBWH301. *Antimicrob. Agents Chemother.* 1995;39:686-693.
44. Centron D and Roy P. Characterization of the 6-N-acetyltransferase gene aac(6)-Iq from the integron of a natural multiresistance plasmid. *Antimicrob. Agents Chemother.* 1998;42:1506-1508.
45. Sekiguchi J, et al. Multidrug-resistant *Pseudomonas aeruginosa* strain that caused an outbreak in a neurosurgery ward and its aac(6')-Iae gene cassette encoding a novel aminoglycoside acetyltransferase. *Antimicrob. Agents Chemother.* 2005;49:3734-3742.
46. Salauze D, et al. Characterisation of aminoglycoside acetyltransferase-encoding genes of neomycin-producing *Micromonospora chalybeata* and *Streptomyces fradiae*. 1991;101: 143-148.
47. Lopez-Cabrera M, et al. Isolation and nucleotide sequencing of an aminocyclitol acetyltransferase gene from *Streptomyces rimosus* forma *paromomycinus*. *J Bacteriol.* 1989;171:321-328.
48. Vliegenthart JS, et al. Nucleotide sequence of the aacC3 gene, a gentamicin resistance determinant encoding aminoglycoside-(3)-N-acetyltransferase III expressed in *Pseudomonas aeruginosa* but not in *Escherichia coli*. *Antimicrob Agents Chemother.* 1991;35:892-897.
49. Nemeč A, et al. Diversity of aminoglycoside re-sistance genes and their association with class 1 integrons among strains of pan-European *Acinetobacter baumannii* clones. *J Med Microbiol.* 2004;53:1233-1240.
50. Wolf E, et al. Crystal structure of a GCN5-related N-acetyltransferase: *Serratia marcescens* aminoglycoside 3-N-acetyltransferase. *Cell.* 1998;94:439-449.
51. Wright GD and Ladak P. Over expression and characterization of the chromosomal aminoglycoside 6'-N-acetyltransferase from *Enterococcus faecium*. *Antimicrob. Agents Chemother.* 1997;41:956-960.
52. Weems JJ, et al. Molecular epidemiology of nosocomial, multiply aminoglycoside resistant *Enterococcus faecalis*. *J Antimicrob Chemother.* 1989;24:121-130.
53. Rather PN, et al. Cloning and DNA sequence analysis of an aac(3)-Vb gene from *Serratia marcescens*. *Antimicrob Agents Chemother.* 1992;36:2222-2227.
54. Rather PN, et al. Analysis of the aac(3)-Vla gene encoding a novel 3-N-acetyltransferase. *Antimicrob Agents Chemother.* 1993; 37:2074-2079.
55. Galimand M, et al. AAC(3)-XI, a new aminoglycoside 3-N-acetyltransferase from *Corynebacterium striatum*. *Antimicrob Agents Chemother.* 2015;59:5647-5653.
56. Tolmasky ME, et al. Transposon-mediated amikacin resistance in *Klebsiella pneumoniae*. *Antimicrob. Agents Chemother.* 1988;32:1416-1420.
57. Tolmasky ME and Crosa JH. Tn1331, a novel multiresistance transposon encoding resistance to amikacin and ampicillin in *Klebsiella pneumoniae*. *Antimicrob. Agents Chemother.* 1987;31:1955-1960.
58. Payie K, et al. Contribution of gentamicin 2'-N-acetyltransferase to the O acetylation of peptidoglycan in *Providencia stuartii*. *J Bacteriol.* 1995;177:4303-4310.
59. Hegde SS, et al. Overexpression and mechanistic analysis of chromosomally encoded aminoglycoside 2'-N-acetyltransferase (AAC(2')-Ic) from *Mycobacterium tuberculosis*. *J Biol Chem.* 2001;276:45876-46881.

60. Vetting MW, et al. Aminoglycoside 2'-N-acetyltransferase from *Mycobacterium tuberculosis* in complex with coenzyme A and aminoglycoside substrates. *Nat Struct Biol.* 2002;9:653-658.
61. Miller GH, et al. The most frequently occurring aminoglycoside resistance mechanisms combined results of surveys in eight regions of the world. The Aminoglycoside Resistance Study Groups. *J Chemother.* 1995;7:17-30.
62. Robicsek A, et al. Fluoroquinolone-modifying enzyme: a new adaptation of a common aminoglycoside acetyltransferase. *Nat Med.* 2006;12: 83–88.
63. Compain F, et al. Complete Nucleotide Sequence of Two Multidrug-Resistant IncR Plasmids from *Klebsiella pneumoniae*. *Antimicrob Agents Chemother.*2014;58:4207–4210.
64. Park CH, et al. Prevalence in the United States of *aac(6)-Ib-cr* Encoding a Ciprofloxacin-Modifying Enzyme. *Antimicrob Agents Chemother.* 2006;50:3953-3955.
65. Rodríguez-Martínez JM, et al. Plasmid-mediated quinolone resistance: Two decades on. *Drug Resist Updat.* 2016;29:13-29.
66. Ruiz E, et al. Changes in ciprofloxacin resistance levels in *Enterobacter aerogenes* isolates associated with variable expression of the *aac(6)-Ib-cr* gene. *Antimicrob Agents Chemother.* 2012;56:1097-1100.
67. Simjee S, et al. Plasmid heterogeneity and identification of a Tn5281-like element in clinical isolates of high-level gentamicin-resistant *Enterococcus faecium* isolated in the UK. *J Antimicro Chemother.* 1999;43:625-635.
68. Ferreti J, et al. 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.* 1986;167:631–638.
69. Rouch DA, et al. The *aacA-aphD* gentamycin and kanamycin resistance determinant of Tn4001 from *Staphylococcus aureus*: expression and nucleotide sequence analysis. *J Gen Microbiol.* 1987;133:3039-3052.
70. Khan SA, et al. Detection of *aacA-aphD*, *qacEd1*, *marA*, *flor*, and *tetA* genes from multidrug-resistant bacteria: Comparative analysis of real-time multiplex PCR assays using EvaGreen(®) and SYBR(®) Green I dyes. *Mol Cell Probes.* 2011;25:78-86.
71. Boehr DD, et al. The molecular basis of the expansive substrate specificity of the antibiotic resistance enzyme aminoglycoside acetyltransferase-6'-aminoglycoside phosphotransferase-2". The role of ASP-99 as an active site base important for acetyl transfer. *J Biol Chem.* 2003;278:12873-12880.
72. Yildiz Ö, et al. Antimicrobial susceptibility and resistance mechanisms of methicillin resistant *Staphylococcus aureus* isolated from 12 Hospitals in Turkey. *Ann Clin Microbiol Antimicrob.* 2014;13:44.
73. Weems JJ Jr, et al. Molecular epidemiology of nosocomial, multiply aminoglycoside resistant *Enterococcus faecalis*. *J Antimicrob Chemother.* 1989;24:121-130.
74. Mir AR, et al. Identification of genes coding aminoglycoside modifying enzymes in *E. coli* of UTI patients in India. *Scientific World J.* 2016:1875865.
75. Kim C, et al. Mechanistic characterization of the bifunctional aminoglycoside-modifying enzyme AAC(3)-Ib/AAC(6)-Ib' from *Pseudomonas aeruginosa*. *Biochemistry.* 2007;46:5270-5282.
76. Toth M, et al. Novel aminoglycoside 2"-phosphotransferase identified in a gram-negative pathogen. *Antimicrob Agents Chemother.* 2013;57:452-457.
77. Davies J and Wright GD. Bacterial resistance to aminoglycoside antibiotics. *Trends Microbiol* 1997;5:234-240.
78. Thompson CJ and Gray GS. Nucleotide sequence of a streptomycete aminoglycoside phospho-transferase gene and its relationship to phosphotransferases encoded by resistance plasmids. *Proc Natl Acad Sci. U.S.A.* 1983;80:5190-5194.
79. Matsushashi Y, et al. Aminoglycoside 3'-phosphotransferases I and II in *Pseudomonas aeruginosa*. *J Antibiot (Tokyo)* 1975;28:442-447.
80. Hollingshead S and Vapnek D. Nucleotide sequence analysis of a gene encoding a streptomycin/spectinomycin adenyltransferase. *Plasmid.* 1985;13:17–30.
81. Lambert T, et al. Characterization of the chromosomal *aac(6)-Iz* gene of *Stenotrophomonas maltophilia*. *Antimicrob Agents Chemother.* 1999;43:2366–2371.
82. Shaw KJ, et al. Characterization of the chromosomal *aac(6)-Ic* gene from *Serratia marcescens*. *Antimicrob. Agents Chemother.* 1992;36:1447-1455.
83. Costa Y, et al. Characterization of the chromosomal *aac(6)-Ii* gene specific for *Enterococcus faecium*. *Antimicrob Agents Chemother.* 1993;37:1896-1903.
84. Rudant E, et al. Characterization of the *aac(6)-Ik* gene of *Acinetobacter* sp. 6. *FEMS Microbiol. Lett.* 1994;124:49-54.
85. Lambert T, et al. 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.* 1994;38:1883–1889.

86. Chakraborty AK. In silico analysis of hotspot mutations in the bacterial NDM-1 and KPC-1 carbapenemases that cause severe MDR phenotypes. *Biochem Biotechnol Res.* 2016;4:17-26.
87. Ilin AI, et al. Complete genome sequence of multidrug-resistant new medicinal drug FS-1. *Genome Announc.* 2015;3:e01272-15.
88. Chakraborty AK. High mode contamination of multi-drug resistant bacteria in Kolkata: mechanism of gene activation and remedy by heterogenous phyto-antibiotics. *Ind J Biotechnol.* 2015;14:149-59.
89. Compain F, et al. Complete nucleotide sequence of two multidrug-resistant IncR plasmids from *Klebsiella pneumoniae*. *Antimicrob Agents Chemother.* 2014;58:4207-4210.
90. Ito T, et al. Staphylococcal Cassette Chromosome mec (SCCmec) analysis of MRSA. *Methods Mol Biol.* 2014;1085:131-148.
91. Hasani A, et al. Frequency of aminoglycoside-modifying enzymes and ArmA among different sequence groups of *Acinetobacter baumannii* in Iran. *Microb Drug Resist.* 2016;22:347-353.
92. Galimand M, et al. AAC(3)-XI, a new aminoglycoside 3-N-acetyltransferase from *Corynebacterium striatum*. *Antimicrob Agents Chemother.* 2015;59:5647-5653.
93. Perumal N, et al. Distribution of genes encoding aminoglycoside-modifying enzymes among clinical isolates of methicillin-resistant staphylococci. *Indian J Med Microbiol.* 2016;34:350-352.
94. Niu H, et al. The prevalence of aminoglycoside-modifying enzyme and virulence genes among enterococci with high-level aminoglycoside resistance in Inner Mongolia, China. *Braz J Microbiol.* 2016;47:691-696.
95. Zhao S, et al. Novel gentamicin resistance genes in *Campylobacter* isolated from humans and retail meats in the USA. *J Antimicrob Chemother.* 2015;70:1314-1321.