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IDENTIFICATION OF AMINOGLYCOSIDE PHOSPHOTRANSFERASE RESISTANCE IN PATHOGENIC AND NON-PATHOGENIC BACTERIA : BIOINFORMATICS ANALYSIS ON NON-CODING MOTIF

SRIVASTAVA A.[1] AND GUPTA D.[2]*

[1]Center of Bioinformatics, Institute of Interdisciplinary Studies, University of Allahabad, Allahabad- 211 002, UP, India.
[2]Department of Biochemistry, University of Allahabad, Allahabad- 211 002, UP, India.
*Corresponding Author: Email- dwijenkumar@gmail.com

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Abstract- The treatment of infections is frustrating as it is increasingly compromised by the ability of bacteria to develop resistance towards antibiotics through mutations or through acquisition of resistance gene. Both pathogenic and non-pathogenic bacteria belonging to class gram- and gram+, like *Mycobacterium tuberculosis, Lactobacillus reuteri, Streptococcus pneumoniae* and a host of others show resistance towards aminoglycoside phosphotransferase (AGPT) activity. In the present study we have conducted an *in silico* study on the gene that confers resistance towards AGPT. The bioinformatics based study heads in the direction of developing a novel motif from sequences (taken from NCBI) of gene against AGPT taken from ARDB (Antibiotic Resistance Gene Database). We have observed a motif that could potentially be used for developing drugs.

Keywords- Antibiotic Resistance Gene Database (ARDB), Aminoglycoside phosphotransferase resistance, Antibiotic resistance

Introduction

Antibiotic resistance is a type of drug resistance in which a genetic change results in a microorganism becoming able to survive exposure to an antibiotic. This possibly is due to a spontaneous or induced genetic mutation, or by horizontal transfer of acquiring antibiotic resistance genes from other bacterial species through. conjugation, transduction, or transformation [1].

Aminoglycosides are among the most commonly used broad-spectrum antibiotics and act usually by binding to the bacterial 30S ribosomal subunit through some work by binding to the 50S subunit and thus inhibiting the step of peptidyl-tRNA translocation from the A-site to the P-site and consequently causing misreading of mRNA. This renders bacterium unable to synthesize essential growth proteins. Aminoglycoside antibiotics vary amongst themselves with regard to their binding specificities to different sites on the rRNA based on their mutual structural complementarit. The structural modifications of aminoglycosides affect drastically the ability of the modified antibiotic to bind to the target RNA [2]. In bacteria, resistance to aminoglycosides is often due to enzymatic inactivation by phosphotransferases (Aph). The Aph group from antibiotic resistance gene database (ARDB) contains several genes that confer resistance to antibiotics, like butirosin, kanamycin, neomycin, paromomycin, ribostamycin etc. The genes that are present in this group are Aph33Ia, Aph33Ib, Aph3IIIa, Aph3IVa Aph3Ia, Aph3Ib, Aph3Ic, Aph3VIIa, Aph3VIa, Aph3Va, Aph3Vb, Aph4Ib, Aph6Ia, Aph6Ib, Aph6Ic, Aph6Id(taken from ARDB). Products of genes are responsible for conferring aminoglycoside resistance and eventually destroying drug action [3].

The motifs are the stable arrangements of two dimensional structure of protein. Knowing secondary structures is always an important step for understanding substitutions, conserved nature of residues and for aligning sequences [4]. Hence, a set of conserved sequences motif has been employed for identification of aminoglycoside phosphotransferase resistance in the resistant sequences that are taken from National Center for Biotechnology Information (NCBI). The motif was adapted from the sequences of the resistant genes that has been taken from the group aminoglycoside phosphotransferase from ARDB. Appreciating motif' feature to carry conserved sequences, this motif has played a vital role in our study to identify the residues of the bacterial sequences that are extracted from NCBI.

Materials and Methods

The sequence of resistant gene from aminoglycoside phosphotransferase group has been retrieved from ARDB database (*ardb.cbcb.umd.edu*) which was used for designing motif. The bacterial sequences were downloaded from NCBI site-*www.ncbi.nlm.nih.gov>protein* in order to find residues that are mainly responsible for aminoglycoside phosphotransferase resistance in that particular bacterial sequences. Swiss-model (*swissmodel.expasy.org*) was used for building structure of these bacterial sequence. MEME (*meme.ncbr.net*) a tool was used for preparing motif. For determining the structure of motif, the server Phyre-2 (*www.sbg.bio.ic.ac.uk/phyre2/*) was used.

Sixteen sequences of resistant gene ascribed to group aminoglycoside phosphotransferase were taken into consideration. These 16 resistant genes are named as Aph33Ia, Aph33Ib, Aph3IIIa, Aph3IVa Aph3Ia, Aph3Ib, Aph3Ic, Aph3VIIa, Aph3VIa, Aph3Va, Aph3Vb, Aph4Ib, Aph6Ia, Aph6Ib, Aph6Ic and Aph6Id and all are resistant to particular antibiotic. [Table-1] carries details of resistance gene and corresponding antibiotics etc.

After retrieval of all these sequences from ARDB successfully the concern was shifted to select gram-positive and gam-negative path-

ogenic and non-pathogenic bacteria. Therefore, pathogenic gram positive bacteria that were taken are *Mycobacterium tuberculosis* and *Streptococcus pneumonia* and non-pathogenic gram-positive bacteria that was taken is *Lactobascillus reuteri*. The pathogenic gram-negative bacterial sequence that were taken are *Salmonella typhi* and *Klebsiella pneumonia* and non-pathogenic gram-negative bacterial sequence taken *was Escherichia coli.*

From the retrieved sequences a common motif was observed using MEME tool which to help appreciate a description for each pattern it discovers and reveal any similarity amongst the sequences. This was necessary to find a conserved sequence from all the sequences to suggest that motif found might also show aminoglycoside phosphotransferase resistance help one identify the residues from the extracted bacterial sequences. The parameters that were taken are as follows:-the minimum width was taken as 6 and the maximum width was set as 50 and overall 3 motifs were generated among those the best one was taken on the basis of e-value and no. of sites. Motif is shown in [Fig-1].

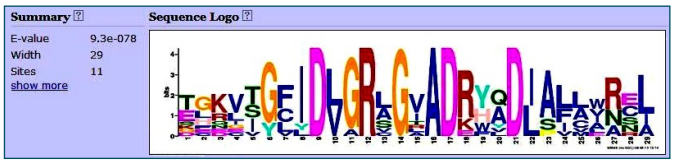

Fig. 1- Motif having 11 sites and e-value of 9.3e-078.

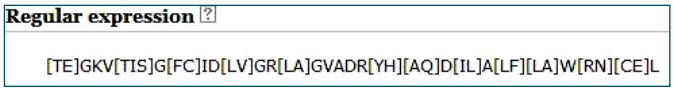

Fig. 2- The regular expression of motif.

The regular expression of motif is shown in [Fig-2].

For the structural comparison of the motif and bacterial sequences the structure of motif was generated using PHYRE2 server which is protein folding recognition server and is meant for predicting the structure of protein sequence. Structure of motif is shown in [Fig-3]. The structure from bacterial sequences was generated using SWISS-MODEL which is also a server and is meant for predicting three-dimensional structure of protein. The structure of motif and structure of bacterial sequences were aligned using PYMOL software and then the identification of amino acids showing aminoglycoside phosphotransferase resistance was done for 6 different bacterial sequences.

Table 1- Genes that confer resistance towards a particular antibiotic in a particular bacterial species listed below (data taken from ARDB)

Resistant type	Antibiotic resistance	Genus
Aph33Ia	Streptomycin	Streptomyces
Aph33Ib	Streptomycin	Escherichia
Aph3IIIa	Neomycin	Staphylococcus
Aph3IVa	Kanamycin	Bascillus
Aph3Ia	Neomycin	Klebsiella
Aph3Ib	Lividomycin	Escherichia
Aph3Ic	Neomycin	Escherichia
Aph3VIIa	Kanamycin	Camphylobacter
Aph3VIa	Ribostamycin	Pseudomonas
Aph3Va	Neomycin	Streptomyces
Aph3Vb	Neomycin	Streptomyces
Aph4Ib	Hygromycin b	Streptomyces
Aph6Ia	Streptomycin	Streptomyces
Aph6Ib	Streptomycin	Streptomyces
Aph6Ic	Streptomycin	Salmonella
Aph6Id	Streptomycin	Salmonella

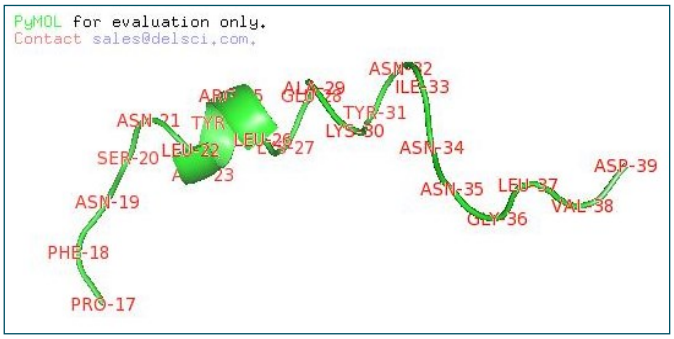

Fig. 3- Structure of motif visualized in pymol

Results and Discussion

After aligning the structures of bacterial strains and structure of motif one by one, the results that we got were very fruitful and also helped in identification of residues conferring resistance. The results are displayed below [Fig-4] to [Fig-15].

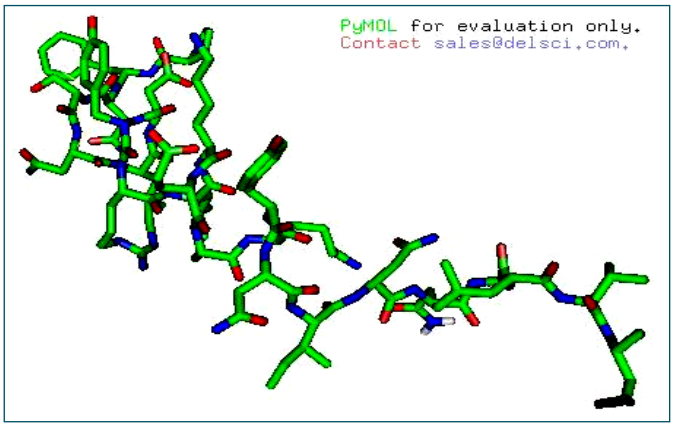

Fig. 4- Aligned structure of motif and *Mycobacterium tuberculosis*

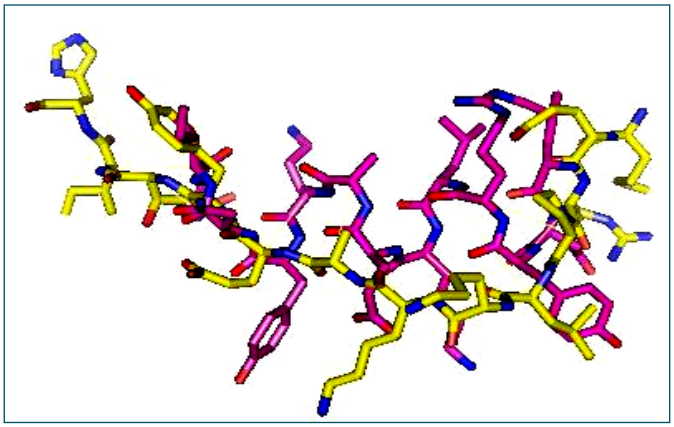

Fig. 5- Aligned structure motif and S*treptococcus pneumoniae*

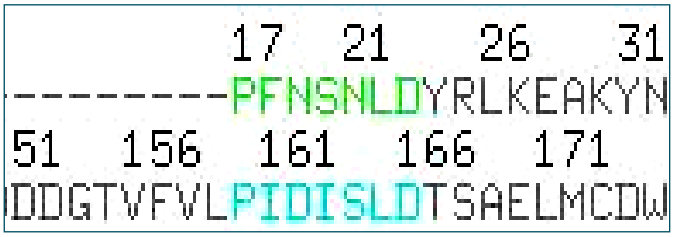

Fig. 6- Similar residues in motif and bacterial structure are found after alignment. (Green indicates residues of motif while blue indicates residues of bacterial structure *Mycobacterium tuberculosis*).

Fig. 7- Similar residues in motif and bacterial structure are found after alignment. (Pink indicates residues of motif while yellow indicates residues of bacterial structure of *Streptococcus pneumoniae).*

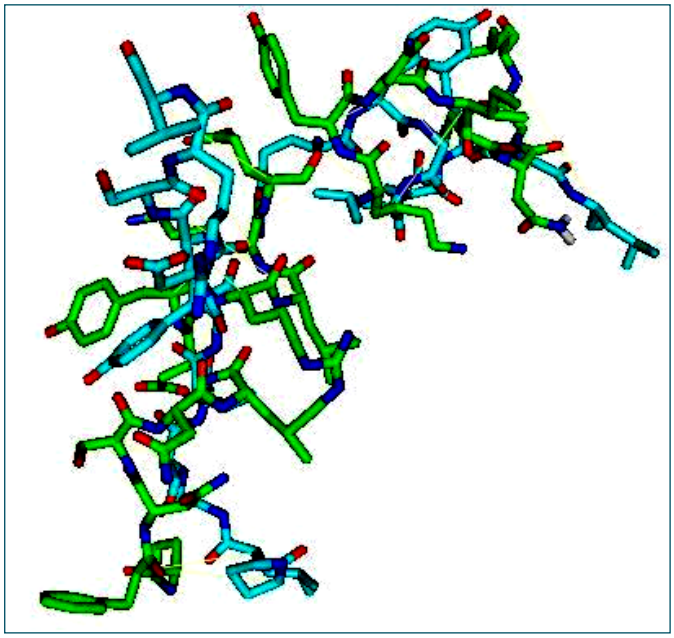

Fig. 8- Aligned structure of motif and *Lactobascillus reuteri.*

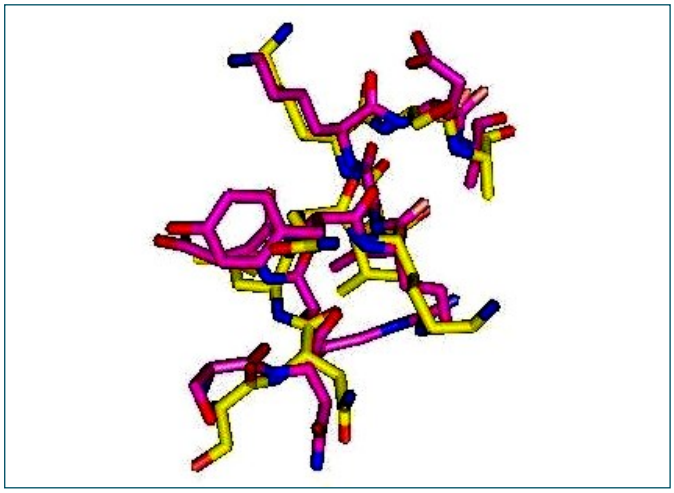

Fig. 9- Aligned structure of motif and *Salmonella typhi.*

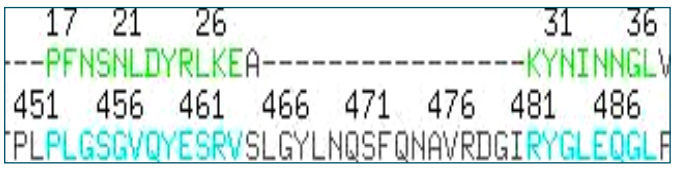

Fig. 10- Similar residues in motif and bacterial structure are found after alignment. Green indicates residues of motif while blue indicates residues of bacterial structure *Lactobascillus reuteri.*

Fig. 11- Similar residues in motif and bacterial structure are found after alignment. Pink indicates residues of motif while yellow indicates residues of bacterial structure of *Salmonella typhi*

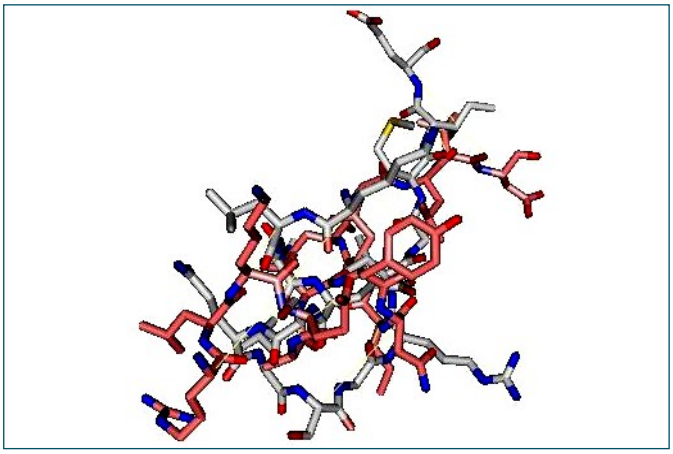

Fig. 12- Aligned structure of motif and *Klebsiella pneumonia*e.

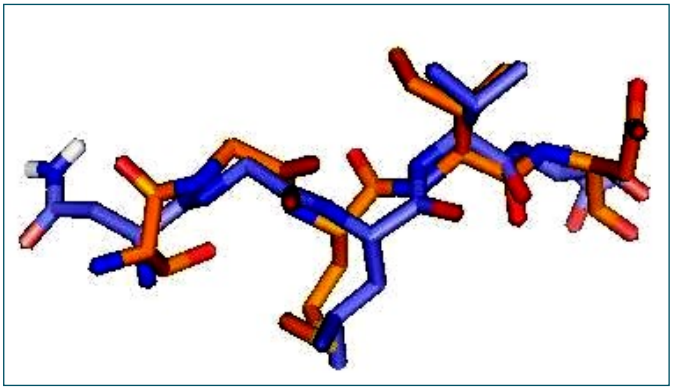

Fig. 13- Aligned structure motif and *Escherichia coli*

Fig. 14- Similar residues in motif and bacterial structure are found after alignment. pink indicates residues of motif while white indicates residues of bacterial structure *Klebsiella pneumonia*e.

Fig. 15- Similar residues in motif and bacterial structure are found after alignment. Purple indicates residues of motif while yellow indicates residues of bacterial structure of *Escherichia coli*

The aligned structure of motif and of respective bacteria shows identical residues as well as residues which are not identical probably due to divergent evolution. But the alignment shows that the non-identical residues are found superimposed on each other just because they belong to some similar groups of amino-acids like: polar, non-polar, neutral, acidic, basic, aliphatic and aromatic.

In [Fig-6] P-P, L-L, D-D (are conserved) while F-I, N-D, N-S (are not identical but are structurally similar. Also, [Fig-7] has 6 conserver residues like L-L, K-K, A-A,Y-Y, I-I, N-N (are conserved) and the rest residues were L-M, D-E, Y-R, R-P, E-K (not identical but structurally similar. In [Fig-10] P-P, L-L, D-D, G-G, Y-Y, Y-Y, S-S (6 residues are found conserved) while others are found structurally similar. In [Fig-11] A-A, L-L, L-L, S-S, N-N, K-K (6 residues are found conserved) while others are structurally similar. In [Fig-14] L-L, E-E (2 residues are found conserved) while others are structurally similar. In [Fig-15] G-G, D-D (2 residues are found conserved).

Hence the conserved residues in each bacterial strain suggests that all the bacteria that were taken into study show aminoglycoside phosphotransferase resistance and the residues suggestive of conferring antibiotic. We suggest that these conserved identical residues might also be part of active sites. Further investigation may be necessary to see how mutation affected the active sites.

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Contributors

Both authors have approved the final article and approved the manuscript for final publication.

Conflict of Interest: There is no conflict of interest or competing interests.

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