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Phylogenetic Evaluation of Antibiotic resistance conferring genes in Bacteria

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ABSTRACT
Antibiotic resistance genes in microbes refer to genetic elements that confer resistance to antibiotics. These genes can be naturally present in microbial populations or acquired through various mechanisms such as mutation, horizontal gene transfer, or gene amplification. These genes in bacteria pose a significant issue for human health due to their ability to undermine the effectiveness of antibiotic treatments. They are classified based on how they confer the resistance to the organism and on the antibiotic that they hydrolyse. In the present investigation the following types of antibiotic resistance genes were studied- Beta-lactamase genes, Methicillin resistance genes, Fluoroquinolone resistance genes, Aminoglycoside resistance genes, Tetracyclin resistance genes, Sulfonamide resistance genes. Many multidrug resistant super bugs also have these genes which help them gain protection against a broad spectrum of antibiotics. In the present investigation, alignment studies were done for 43 genes. Genes with of similar activity were chosen and evolutionary relationship between these genes was studied. .

Key Words: Antibiotic resistance genes, Multidrug resistance genes, Bioinformatics, Multiple sequence alignment, phylogenetic tree for antibiotic resistance genes.

Introduction

Antibiotic resistance genes (ARGs) can be found in various organisms, including bacteria, archaea, and even in some cases, eukaryotic organisms like fungi. Antibiotic resistance genes are commonly found in pathogenic bacteria such as Staphylococcus aureus, Escherichia coli,

Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterococcus faecalis/faecium. Resistance genes are also present in commensal bacteria that inhabit various niches in the human body, such as the gut microbiota (1). Antibiotic resistance genes have also been identified in fungi, particularly in medically relevant species such as Candida spp. Some Candida species have developed resistance to antifungal agents, and the genes responsible for this resistance are of interest for understanding and combating fungal infections (2).

The antibiotic resistant genes are categorized into various types based on the class of antibiotics they grant resistance. Some examples include to which include β-lactams (bla), fluoroquinolone (fca), tetracyclines (tet), sulfonamides (sul), macrolides (erm), aminoglycosides (aac), vancomycin (van), multidrug (mdr) etc (3). Antibiotic resistance genes are not solely confined to clinical settings but can also be found in environmental reservoirs such as soil, water, and plants. These environmental sources contribute to the spread and persistence of resistance genes in bacterial populations (4). Understanding the diversity and distribution of resistance genes across different organisms is crucial for developing strategies to mitigate the spread of antibiotic resistance. Few genes that confer antibiotic resistance and their mechanism of action is listed in table 1.

Table 1: Types of antibiotic resistance in bacteria, genes involved and their mode of action

Type	**Mode of action**	**Resistance conferred to**	**Genes**	**Found in**	**Reference**
Beta- lactamase genes	Hydrolyze beta-lactam ring	Penicillin, Cephalosporins	TEM SHV CTX-M	Gram negative bacteria	5
Methicillin resistance genes	Decreased affinity for beta lactams	Methicillin, beta-lactamases	mecA mecC	Staphylococcus aureua	6
Fluoroquinolone resistance genes	Alters target sites for the antibiotic on DNA gyrase and Topoisomerase IV	Fluoroquinolone	gyrA gyrB parC pare	Gram negative bacteria	7
Aminoglycoside Resistance genes	Degrade aminoglycoside antibiotics or modify them	Aminoglycoside modifying enzyme	aac(6')-Ib, aph(3')-Ia, and aph(6)-Id	Streptomyces sps and Microcystis sps	8
		reducing aminoglycoside binding affinity	rrs and rrl genes	Streptomyces sps and Microcystis sps	8

Tetracycline resistance genes	Encode efflux pumps that actively transport tetracyclines out of bacterial cells	Tetracycline	tetA and tetB	Gram negative bacteria	9
	encode ribosomal protection proteins that prevent tetracycline binding to ribosomes	Tetracycline	tetM tetO	Gram negative bacteria	9
Sulfonamide-resistant genes	Encode dihydropteroate synthase enzymes with reduced affinity for sulfonamide antibiotics	Sulfonamide antibiotics	sul1 sul2 sul3	Gram negative bacteria	10

Multidrug-resistant genes (MDRGs) refer to genes that confer resistance to multiple classes of antibiotics, making bacteria containing these genes resistant to a broad range of antimicrobial agents. These genes can arise through various mechanisms, including mutations, genetic recombination, and horizontal gene transfer etc. (11-14). Table 2 illustrates the categories of MDRGs, genes involved and the antibiotics that they offer resistance to. The emergence of multidrug-resistant and extensively drug-resistant bacteria limits treatment options, forcing clinicians to resort to more toxic, less effective, or expensive antibiotics (28).

Table 2: Multidrug resistant categories with genes involved and antibiotic that they offer resistance to.

Category of MDRGs	**Antibiotics they offer resistance to**	**Proteins encoded**	**Genes**	**Organism**	**Reference**
Methicillin-resistant Staphylococcus aureus (MRSA)	Multiple antibiotics	Encode altered penicillin-binding proteins (PBPs) or other resistance mechanisms	mecA	Staphylococcus aureus	15
		Variant of mecA acts same as above	mecC	Staphylococcus aureus	15
Carbapenem resistant	Carbapenem antibiotics	Hydrolyse carbapenem	blaKPC-2,	Enterobacteriaceae family	17

Enterobacteriaceae		antibiotics by producing carbapenemaes enzyme	blaKPC-3, and others		
Klebsiella pneumoniae carbapenemase (KPC) genes	Carbapenems and other beta-lactam antibiotics	KPC enzymes confer resistance to carbapenems and other beta-lactam antibiotics	blaKPC-2, blaKPC-3, and others	Klebsiella pneumoniae	18
New Delhi metallo-beta-lactamase (NDM) genes	Exhibit broad spectrum specificity to antibiotics	encode metallo-beta-lactamases that hydrolyze carbapenems and other beta-lactam antibiotics	NDM genes, including blaNDM-1 and others	Escherichia Coli, Klebsiella pneumonia etc	19
OXA-48-like genes	Beta lactam antibiotics and carbapenems	encode class D beta-lactamases, the OXA-48 enzymes	blaOXA-48, blaOXA-162, and others	Enterobacteriaceae	20
Verona integron-encoded metallo-beta-lactamase (VIM) genes	Beta-lactams and carbapenems	encode metallo-beta-lactamases that hydrolyze carbapenems	blaVIM-1, blaVIM-2, and others	Pseudomonas aeruginosa and some Enterobacteriaceae	21
Imipenemase (IMP) genes	Beta-lactams and carbapenems	Encode metallo-beta-lactamases that confer resistance to carbapenems	blaIMP-1, blaIMP-2, and others	Pseudomonas aeruginosa and some Enterobacteriaceae	22
OXA-23, OXA-24/40, OXA-58 Genes	Carbapenems antibiotics	Encode class D beta-lactamases that hydrolyze carbapenems	OXA-23, OXA-24/40, and OXA-58 genes	Acinetobacter species	23
TEM and SHV Genes	Penicillins and extended-spectrum cephalosporins	Extended-spectrum beta-lactamases (ESBLs)	blaCTX-M, blaSHV, and blaTEM	Enterobacteriaceae and some A. baumannii	24

AdeABC Efflux Pump	Beta-lactams, fluoroquinolones, and tetracyclines	Multidrug efflux system	adeA, adeB, and adeC	A. baumannii	25
AdeIJK Efflux Pump	Multiple antibiotics, including aminoglycosides, tetracyclines, fluoroquinolones, and chloramphenicol	Multidrug efflux system	adeI, adeJ, and adeK	A. baumannii	26
ArmA, RmtB, and AadA Genes	Aminoglycosides,	Encode 16S rRNA methyltransferases that modify the ribosomal target site of aminoglycosides	ArmA, RmtB, and AadA Genes	A. baumannii	27

Objective of the present investigation is about finding evolutionary relation between the genes listed in table 1 and narrate the possible evolution of antibiotic resistance genes.

Material and methods:

Selection of antibiotic resistance genes: The genes selected for the present investigation are from the categories listed in table 1. These include Beta- lactamase genes, Methicillin resistance genes, Fluoroquinolone resistance genes, Aminoglycoside resistance genes, Tetracyclin resistance genes and Sulfonamide resistance genes. Genes and their modes of action are identified and listed further according to their mode of action.

Retrieval of proteins conferring antibiotic resistance genes: The proteins encoded by the genes are retrieved from NCBI. The accession numbers and the FASTA sequences were saved for further use. These were saved as per the mode of action of the under each category of the antibiotic resistance genes.

Alignment studies: Multiple sequence alignment studies were carried out using multiple alignment option of BLAST in NCBI. This was done for each of the category of the genes. Phylogenetic tree was constructed for all the feasible ones under investigation.

Results and Discussion:

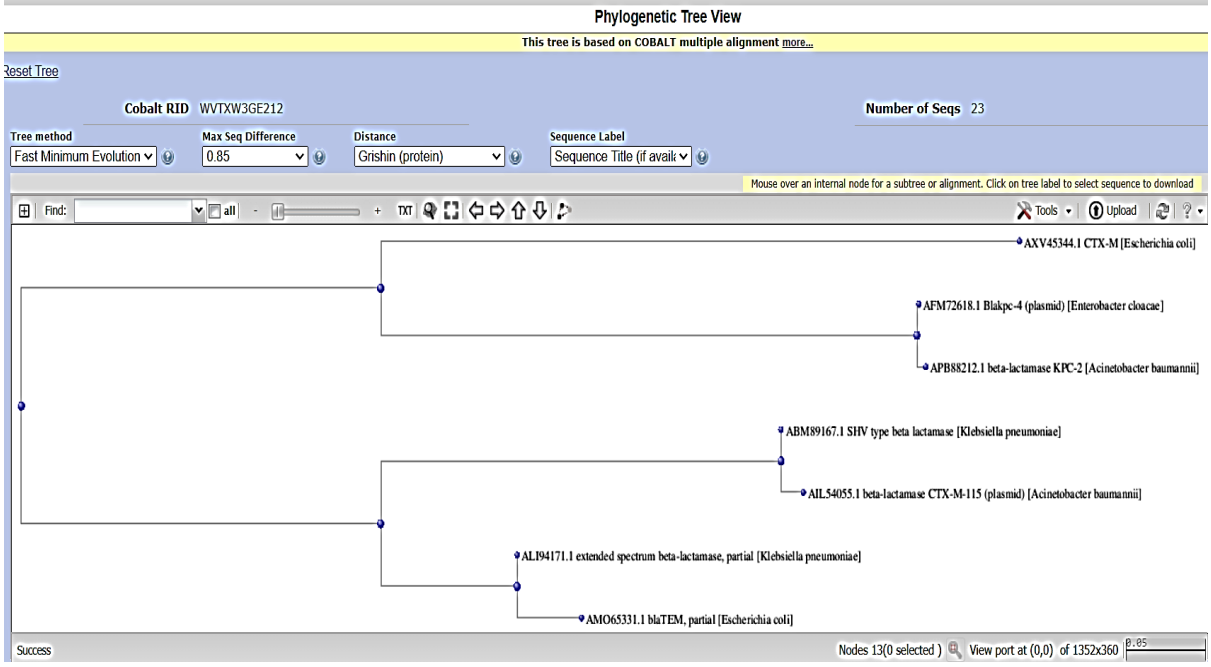
1. Beta-lactamase genes: These hydrolyze the beta lactam ring and confer resistance to pencillin and celphalosporin. The genes listed in tables were TEM, SHV, CTX-M, mecA, mecC from table 1 and NDM genes, blaOXA 48 and 162 genes, bla VIM-1, blaVIM-2, blaIMP-1, blaIMP-2, OXA-23, OXA-24/40, OXA 58, blaCTXM, blaSHV and blaTEM. FASTA

sequences of the proteins were taken for phylogenetic analysis. The accession numbers and description were mentioned in the Figure 1.

Figure 1. Beta-lactamase gene products considered for multiple sequence alignment.

Accession	Description
lcl|Query_10001	ALI94171.1 extended spectrum beta-lactamase, partial [Klebsiella pneumoniae]
lcl|Query_10002	ABM89167.1 SHV type beta lactamase [Klebsiella pneumoniae]
lcl|Query_10003	AXV45344.1 CTX-M [Escherichia coli]
lcl|Query_10004	AWK45681.1 adaptor protein MecA [Bacillus velezensis]
lcl|Query_10005	CDR19470.1 beta-lactam-inducible penicillin-binding protein [Staphylococcus aureus]
lcl|Query_10006	AFM72618.1 Blakpc-4 (plasmid) [Enterobacter cloacae]
lcl|Query_10007	APB88212.1 beta-lactamase KPC-2 [Acinetobacter baumannii]
lcl|Query_10008	ADY86699.1 metallo-beta-lactamase 1, partial [Acinetobacter baumannii]
lcl|Query_10009	OOM87954.1 subclass B1 metallo-beta-lactamase NDM-1 [Acinetobacter baumannii]
lcl|Query_10010	WP_043907054.1 MULTISPECIES: OXA-48 family carbapenem-hydrolyzing class D beta-lactamase OXA-232 [Bacteria]
lcl|Query_10011	WP_060613455.1 MULTISPECIES: OXA-48 family carbapenem-hydrolyzing class D beta-lactamase OXA-162 [Enterobacterales]
lcl|Query_10012	OTH01407.1 subclass B1 metallo-beta-lactamase VIM-1 [Pseudomonas aeruginosa]
lcl|Query_10013	AKQ98357.1 subclass B1 metallo-beta-lactamase VIM-46 [Pseudomonas aeruginosa]
lcl|Query_10014	RSE88359.1 subclass B1 metallo-beta-lactamase IMP-1 [Achromobacter denitrificans]
lcl|Query_10015	WP_063860578.1 subclass B1 metallo-beta-lactamase IMP-2 [Acinetobacter baumannii]
lcl|Query_10016	WP_005404206.1 MULTISPECIES: OXA-23 family carbapenem-hydrolyzing class D beta-lactamase OXA-813 [Acinetobacter]
lcl|Query_10017	UBS26085.1 OXA-24 family carbapenem-hydrolyzing class D beta-lactamase OXA-1040 [Acinetobacter baumannii]
lcl|Query_10018	WP_063862810.1 MULTISPECIES: OXA-58 family carbapenem-hydrolyzing class D beta-lactamase OXA-420 [Acinetobacter]
lcl|Query_10019	AIL54055.1 beta-lactamase CTX-M-115 (plasmid) [Acinetobacter baumannii]
lcl|Query_10020	AMO65331.1 blaTEM, partial [Escherichia coli]
lcl|Query_10021	QJC63655.1 AdeA [Acinetobacter baumannii]
lcl|Query_10022	WP_000987599.1 MULTISPECIES: multidrug efflux RND transporter permease subunit AdeB [Acinetobacter]
lcl|Query_10023	AXU17770.1 AdeC, partial [Acinetobacter baumannii]

Figure 2 indicates the phylogenetic tree for the 23 gene products chosen for study.



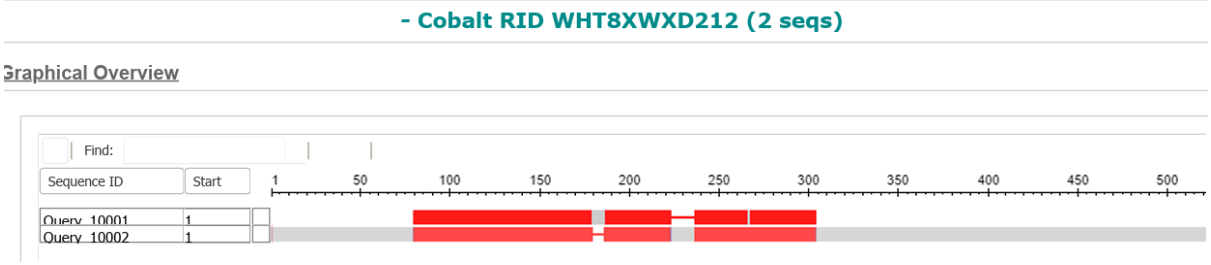
Among the selected 23 samples coding for beta lactamase and related genes, the phylogenetic tree showed 13 nodes and 2 clades. AXV45344.1CTX-M E.coli , AFM72618.1Blakpc-4 Enterobacteriaceae, APB88212.1KPC-2 Acinetobacter baumanni form a clade indicating evolutionary relationship, while the second clade shows- ABM89167.1 SHC Klebsiella pneumonia, AIL54055.1 CTX-M115 Acinetobacter baumanni, ALI94171.1 Klebsiella pneumonia and AMO65331.1 blaTEM E.coli. And all the listed above have a common point of ancestral evolution. The rest of the genes were not depicted indicating no such relationship.

Multiple sequence alignment was done for the above-mentioned sequences. Figure 3 shows the extent of conserved sequences (red) in them and hence confirming evolutionary relationship.

Figure 3. Multiple sequence alignment proving evolutionary relationship among few beta-lactamase gene products

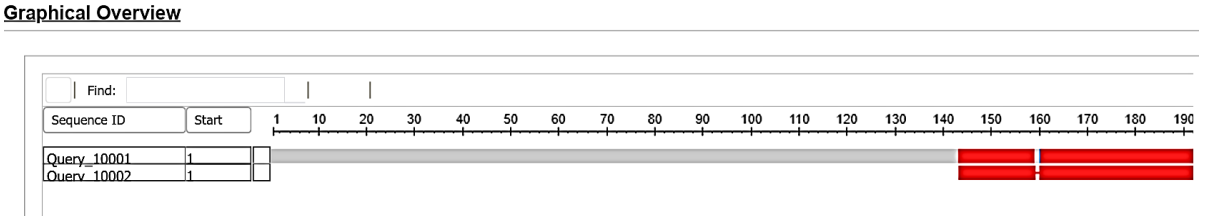
2. Methicillin antibiotic resistance genes: From the table mecA and mec C were the two genes that were identified to confer resistance to methicillin. These two were used for alignment studies. Figure 4 showing red regions indicate that there are some conserved regions, evolutionary relationship could not be drawn due to the extent of dissimilarity (>0.85).

Figure 4: Alignment studies for methicillin resistance gene products



3. Fluoroquinolone resistance genes: These alter the target sires for the antibiotics on the DNA gyrase and Toposiomerase IV. Two genes gyrA, gyrB were chosen for verifying sequence and their FASTA sequence for the proteins was used. Sequence similarity was seen for the two sequences as shown in figure 5, but phylogenetic tree could not be constructed.

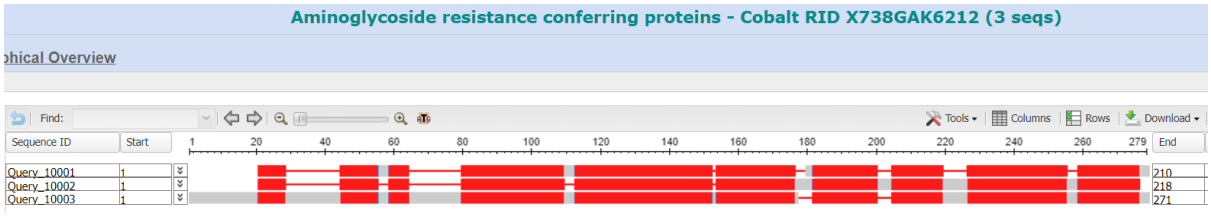
Figure 5. Protein sequence similarity for Fluoroquinolone resistance gene products



4. Aminoglycoside resistance genes: The products of these genes degrade aminoglycodie antibiotics or they modify them. Accordingly the modifying enzymes (coded by aac(6')-Ib, aph(3')-Ia, and aph(6)-Id genes) and the rrs and rrl genes encode 16S rRNA and 23S rRNA mutations there by reducing aminoglycoside binding affinity.

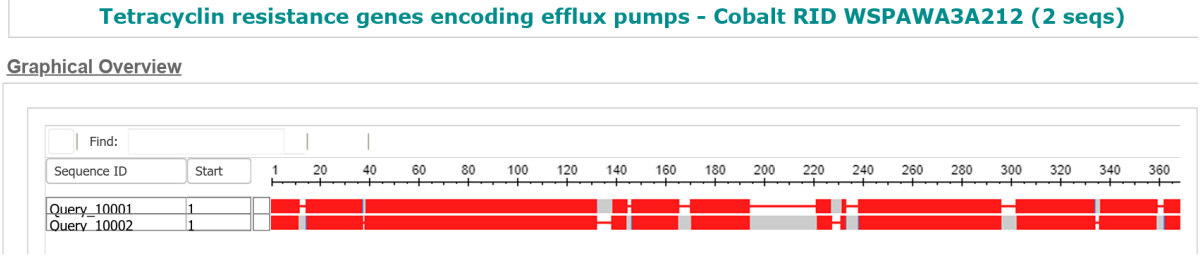
The modifying enzymes showed by sequence similarities as shown in the figure 6, but phylogenetic tree was not shown as dissimilarity exceeded maximum divergence.

Figure 6: Sequence similarity study for enzymes that confer aminoglycoside resistance by modification.



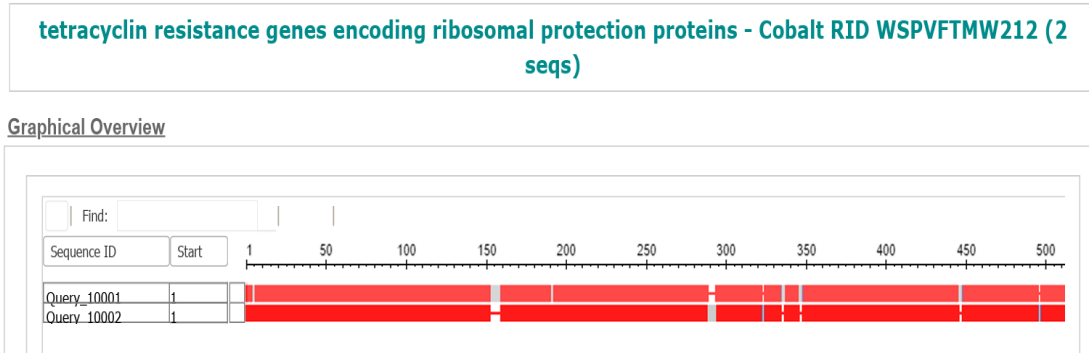
5. Tetracycline resistance genes: These genes encode for efflux pumps that actively transport tetracyclines out of the bacterial cells (tetA and tetB). The other set of genes (tetM and tetO) encode for ribosomal protection proteins that prevent tetracyclines from binding to ribosomes. Both the types were separately studied for sequence alignment. Figure 7 shows the sequence similarity between proteins of efflux

Figure 7: Sequence similarity between protein sequences of efflux pumps



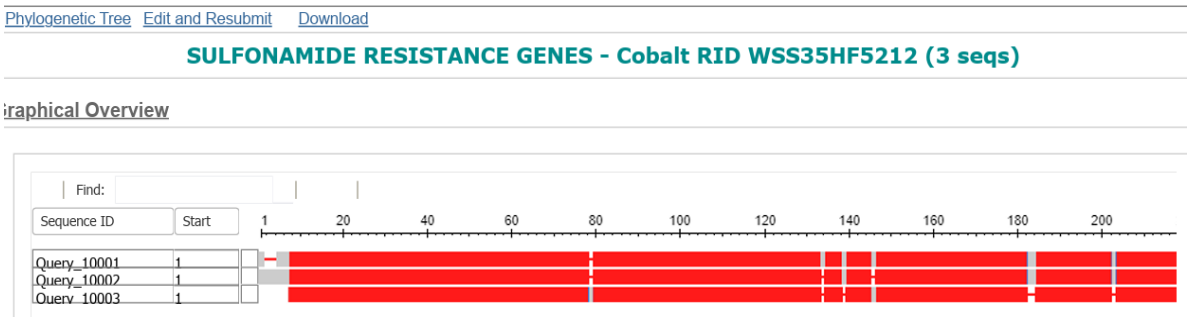
The other set of genes that offer tetracycline resistance are the ribosomal protection proteins and the sequence similarity between the two proteins are shown in Figure 9.

Figure 9. Sequence alignment between tetracycline resistance genes encoding ribosomal protection genes.



6. Sulfonamide resistance genes: The genes sul1, sul2 and sul3 encode for dihydropteroate synthase that reduce the affinity for sulfonamide antibiotics. Figure 11 shows the sequence similarity between the sequences and phylogenetic tree has indicated that enzymes encoded by sul 1 and sul2 are closer while all three are from the evolutionarily related.

Figure 11. Sequence alignment for the three genes products conferring resistance to Sulfonamide resistance



The evolutionary relationship between various subgroups of antibiotics confirms the role of gene mutations that could have led to the evolution of antibiotic resistance in genes (29, 30)). Resistance can emerge through gene mutations which can emerge through spontaneous mutations and also via horizontal gene transfer (31).

Conclusion:

Antibiotic resistance conferred to microbes due to the genetic elements that confer them the same is acquired through various mechanisms. These help them to survive in environments that could be natural or human induced. They have evolved to proliferate in the presence of antibiotics and leading to these antibiotic resistance strains and super bugs that confer multidrug resistance. This paper attempts to show the evolutionary relationship between the various classes of antibiotics resistance genes. There was evolutionary relationship between most of the gene products performing same action. Sequence similarity studies and phylogenetic trees incorporated proves the same.

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