

Antibiotic Resistance Genotyping in Bacteria

Introduction

Antibiotics are medicines used to prevent and treat bacterial infections. Antibiotic resistance happens when bacteria develop the ability to defeat the drugs designed to kill them. This results in the complete dominance of bacteria over the drug, leading to an exponential growth of bacteria and untreated infection. According to the Centers for Disease Control and Prevention 2019 Antibiotic Resistance Threats Report, more than 2.8 million antimicrobial-resistant infections occur in the U.S., and out of which, 35000 die each year. According to WHO, antibiotic resistance is one of today's biggest threats to global health, food security, and development. A growing number of infections such as pneumonia, tuberculosis, gonorrhea, and salmonellosis are becoming harder to treat as the antibiotics used to treat them become less effective. It can affect anyone, of any age, in any country. It occurs naturally, but misuse of antibiotics in humans and animals is expediting the process, resulting in longer hospital stays, higher medical costs, and increased mortality.

Aim and Relevance of the Project

The project aims to find the resistance genotypes of three antibiotics: Beta-lactam; Aminoglycoside; and Tetracycline in *Mycobacterium tuberculosis* samples taken from five different countries representing five different regions of the world. These are India, Nigeria, the USA, Germany, and Australia. The result of this project will provide clues on the pattern of resistance in each region and what antibiotics should be prescribed to avoid the risk of antibiotic resistance.

Methodology

Data Collection

The source for collecting the *Mycobacterium tuberculosis* samples is the Sequence Read Archive (SRA) of the National Center for Biotechnology Information (NCBI). It is the largest publicly available repository and contains high throughput sequencing data. Sequence reads were downloaded through the links generated by SRA explorer.

Country	Accession Number	Study Runs
India	SRP367420	SRR18593225, SRR18593226, SRR18593227, SRR18593228, SRR18593229
Nigeria	ERP124986	ERR4829978, ERR4829979, ERR4829980, ERR4829981, ERR4829982
USA	SRP276100	SRR18913746, SRR18913747, SRR18913748, SRR18913749, SRR18913750
Australia	SRP319622	SRR14520035, SRR14520036, SRR14520037, SRR14520038, SRR14520039
Germany	ERP109929	ERR2704675, ERR2704676, ERR2704677, ERR2704678, ERR2704679

Assembling and Processing

All the samples were assembled and processed with a bioinformatics tool called SPAdes.py. To save time and increase efficiency, a 'for loop' is created that assembles & processes every sample with respect to their country and saves them in contigs.fasta format.

Results and Discussion

Country	Study Runs	Antibiotic(s)	Resistance Gene
INDIA	SRR18593225	Aminoglycoside (gentamicin, tobramycin, dibekacin, netilmicin)	aac(2')-Ic
	SRR18593226	Aminoglycoside (gentamicin, tobramycin, dibekacin, netilmicin)	aac(2')-Ic

	SRR18593227	Aminoglycoside (gentamicin, tobramycin, dibekacin, netilmicin)	aac(2')-Ic
	SRR18593228	Aminoglycoside (gentamicin, tobramycin, dibekacin, netilmicin)	aac(2')-Ic
	SRR18593229	Aminoglycoside (gentamicin, tobramycin, dibekacin, netilmicin)	aac(2')-Ic
NIGERIA	ERR4829978	Aminoglycoside (gentamicin, tobramycin, dibekacin, netilmicin)	aac(2')-Ic
	ERR4829979	Aminoglycoside (gentamicin, tobramycin, dibekacin, netilmicin)	aac(2')-Ic
	ERR4829980	Aminoglycoside (gentamicin, tobramycin, dibekacin, netilmicin)	aac(2')-Ic
	ERR4829981	Aminoglycoside (gentamicin, tobramycin, dibekacin, netilmicin)	aac(2')-Ic
	ERR4829982	Aminoglycoside (gentamicin, tobramycin, dibekacin, netilmicin)	aac(2')-Ic
AUSTRALIA	SRR14520035	Aminoglycoside (gentamicin, tobramycin, dibekacin, netilmicin)	aac(2')-Ic
	SRR14520036	Aminoglycoside (gentamicin, tobramycin, dibekacin, netilmicin)	aac(2')-Ic
	SRR14520037	Aminoglycoside (gentamicin, tobramycin, dibekacin, netilmicin)	aac(2')-Ic
	SRR14520038	Aminoglycoside (gentamicin, tobramycin, dibekacin, netilmicin)	aac(2')-Ic
	SRR14520039	Aminoglycoside (gentamicin, tobramycin, dibekacin, netilmicin)	aac(2')-Ic
GERMANY	ERR2704675	Aminoglycoside (gentamicin, tobramycin, dibekacin, netilmicin)	aac(2')-Ic
	ERR2704676	Aminoglycoside (gentamicin, tobramycin, dibekacin, netilmicin)	aac(2')-Ic
	ERR2704677	Aminoglycoside (gentamicin, tobramycin, dibekacin, netilmicin)	aac(2')-Ic

	ERR2704678	Aminoglycoside (gentamicin, tobramycin, dibekacin, netilmicin)	aac(2')-Ic
	ERR2704679	Aminoglycoside (gentamicin, tobramycin, dibekacin, netilmicin)	aac(2')-Ic
USA	SRR18913746	Aminoglycoside (gentamicin, tobramycin, dibekacin, netilmicin) & Tetracycline (doxycycline, tetracycline, and minocycline)	aac(2')-Ic & tet(W)
	SRR18913747	Aminoglycoside (gentamicin, tobramycin, dibekacin, netilmicin) & Tetracycline (doxycycline, tetracycline, and minocycline)	aac(2')-Ic & tet(W)
	SRR18913748	Aminoglycoside (gentamicin, tobramycin, dibekacin, netilmicin) & Tetracycline (doxycycline, tetracycline, and minocycline)	aac(2')-Ic & tet(W)
	SRR18913749	Aminoglycoside (gentamicin, tobramycin, dibekacin, netilmicin) & Tetracycline (doxycycline, tetracycline, and minocycline)	aac(2')-Ic & tet(W)
	SRR18913750	Aminoglycoside (gentamicin, tobramycin, dibekacin, netilmicin) & Tetracycline (doxycycline, tetracycline, and minocycline)	aac(2')-Ic & tet(W)

The analysis through the ResFinder 4.1 finds out that all the ten *Mycobacterium tuberculosis* samples of India, Nigeria, Australia, Germany & USA are resistant to only Aminoglycoside class of antibiotics among the tested three classes of Beta-lactam, Tetracycline, and Aminoglycoside. The only exception found in the two study runs in the USA samples i.e., SRR18913746 and SRR18913750 which are also resistant to the Tetracycline class of antibiotics along with Aminoglycoside.

Among a vast collection of aminoglycosides, only gentamicin, tobramycin, dibekacin, and netilmicin of class Aminoglycoside are found to be resistant to the gene aac(2')-Ic. In reference to those two Tetracycline resistant samples, only specific antibiotics such as doxycycline, tetracycline, and minocycline are resistant and the gene responsible is tet(W). All the other country-wise information regarding the analysis in the elucidated form is [here](#).

Conclusion

The downloaded sequences from the SRA explorer went through assembling with the help of the SPAdes assembling tool. The contigs.fasta are thus generated, and exported in the system for further analysis through ResFinder 4.1. The results obtained, figured out the antibiotic resistance class in the *Mycobacterium tuberculosis* samples along with its resistance gene. It is now been clear that in the countries like India, Nigeria, Germany, and Australia, a medical practitioner should not use an aminoglycoside class of antibiotics while treating Tuberculosis patients. While in the USA, a doctor should watch over Aminoglycoside as well as Tetracycline class of antibiotics. It is important to note that the use of antibiotics particularly gentamicin, tobramycin, dibekacin, netilmicin, doxycycline, tetracycline, and minocycline should be prohibited to reduce the rise of antibiotic resistance in the respective countries. Along with a medical practitioner, it is also a patient's duty to take only the prescribed medicines through a qualified medical practitioner only.