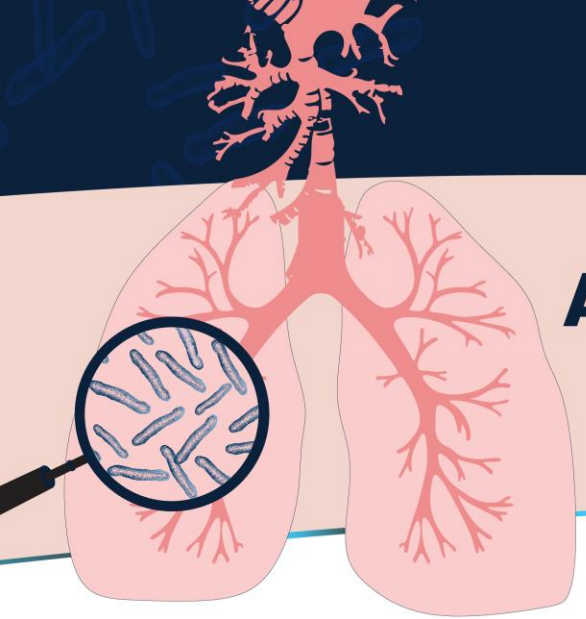

Accelerating progress to end TB

8th SA TB Conference
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Durban ICC

Using the CRISPR/Cas9 System to verify the role of mutations in the *pncA* gene

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INTRODUCTION

- Tuberculosis affects approximately 10 million people globally
- Approximately, **25% of the global population** has been infected with TB
- South Africa is in the 30 high burden TB countries
- **280,000** people fell ill due to TB in South Africa in 2022
- South Africa is on the **high burden list** for TB, TB-associated HIV and MDR/RR-TB

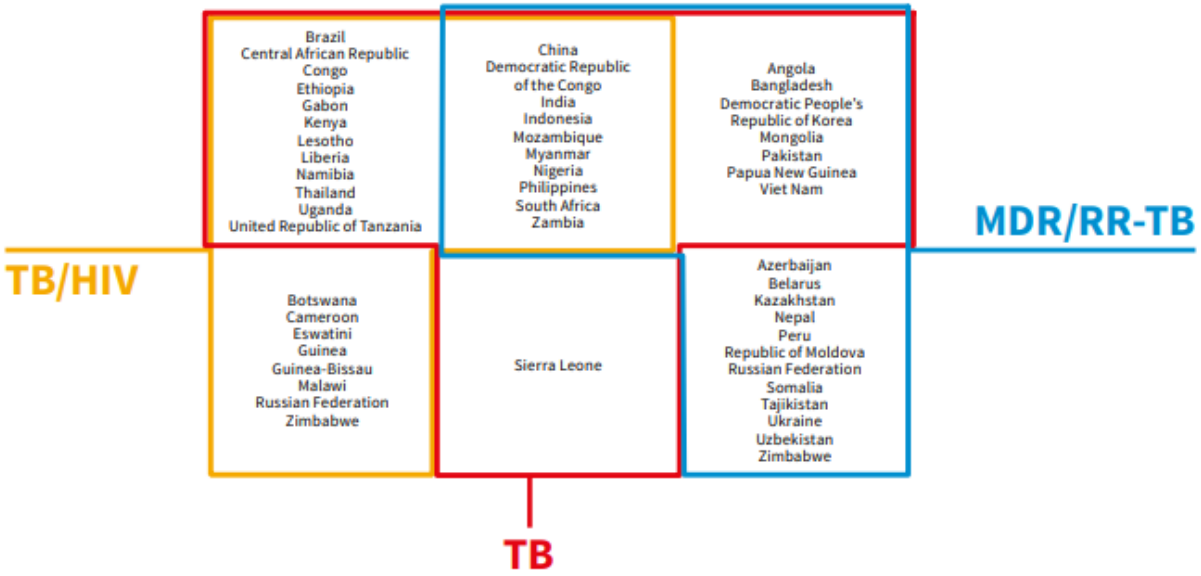


Fig 1. Global lists for countries associated with high burden of TB, HIV-associated TB and MDR/RR-TB to be used by WHO between 2021-2025

INTRODUCTION

- Pyrazinamide (PZA) is a first line drug in the treatment of *M.tb*
- Pyrazinamide is taken in as a pro-drug and converted to its active form, pyrazinoic acid by the *pnc*A gene
- Mutations in the *pnc*A gene have been shown to lead to failure to produce pyrazinoic acid
- This in turn leads to PZA drug resistance

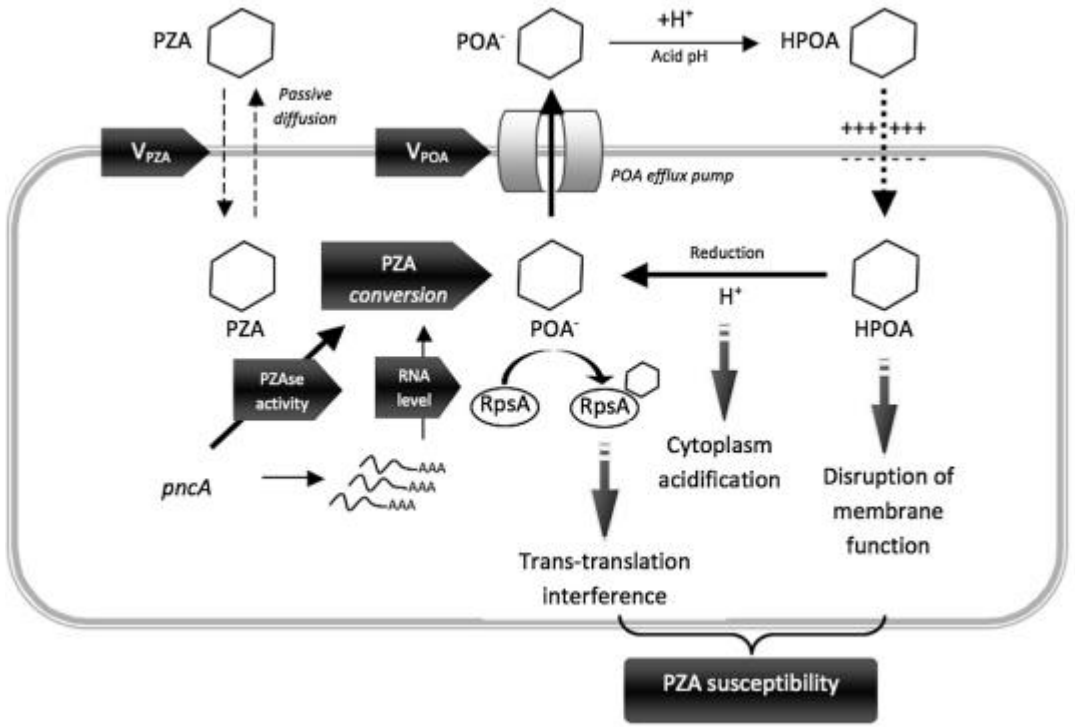


Fig 2. Schematic of the action of Pyrazinamide in *Mycobacterium tuberculosis*

INTRODUCTION

- The CRISPR system is a natural adaptive immunity that was first described in 1987
- It has been found in approximately 90% of sequenced bacteria
- The CRISPR system has been modified by scientists to enable **genome editing** in different organisms

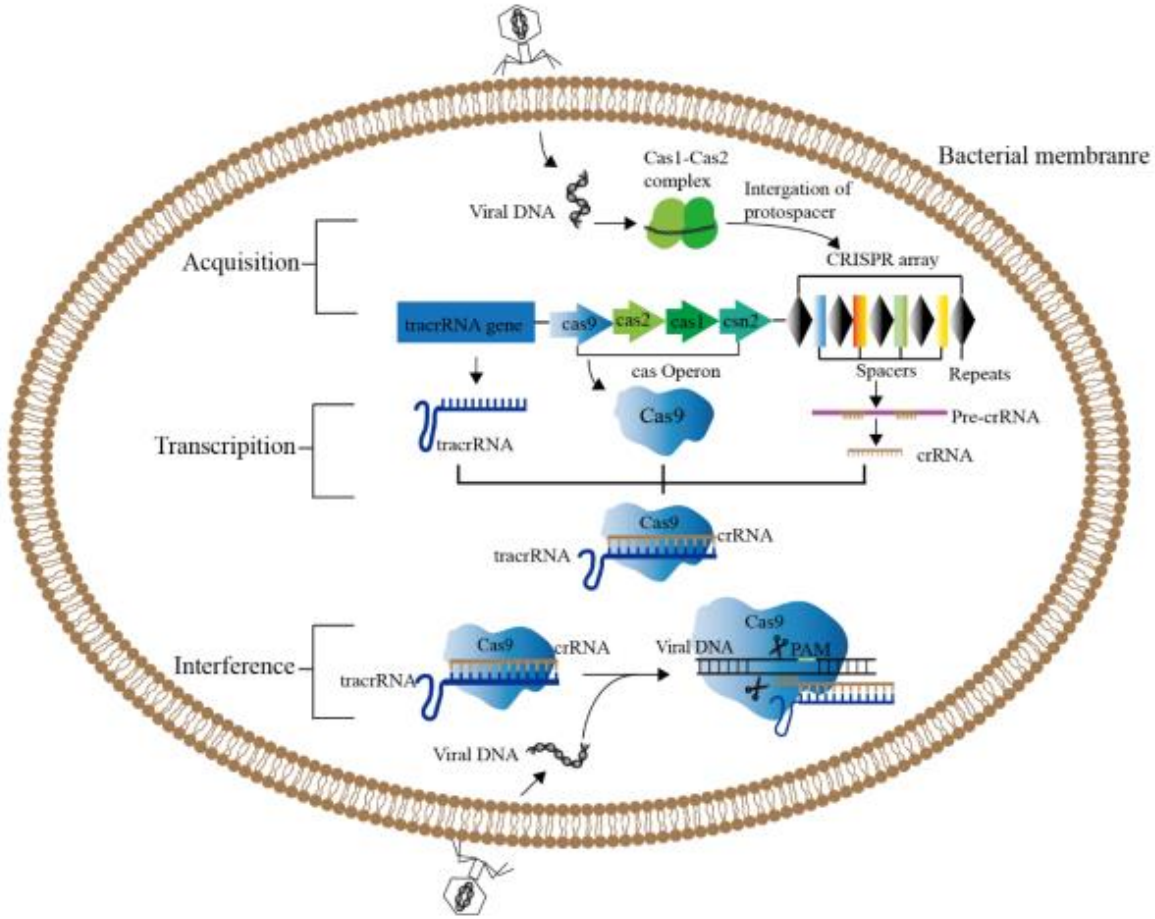
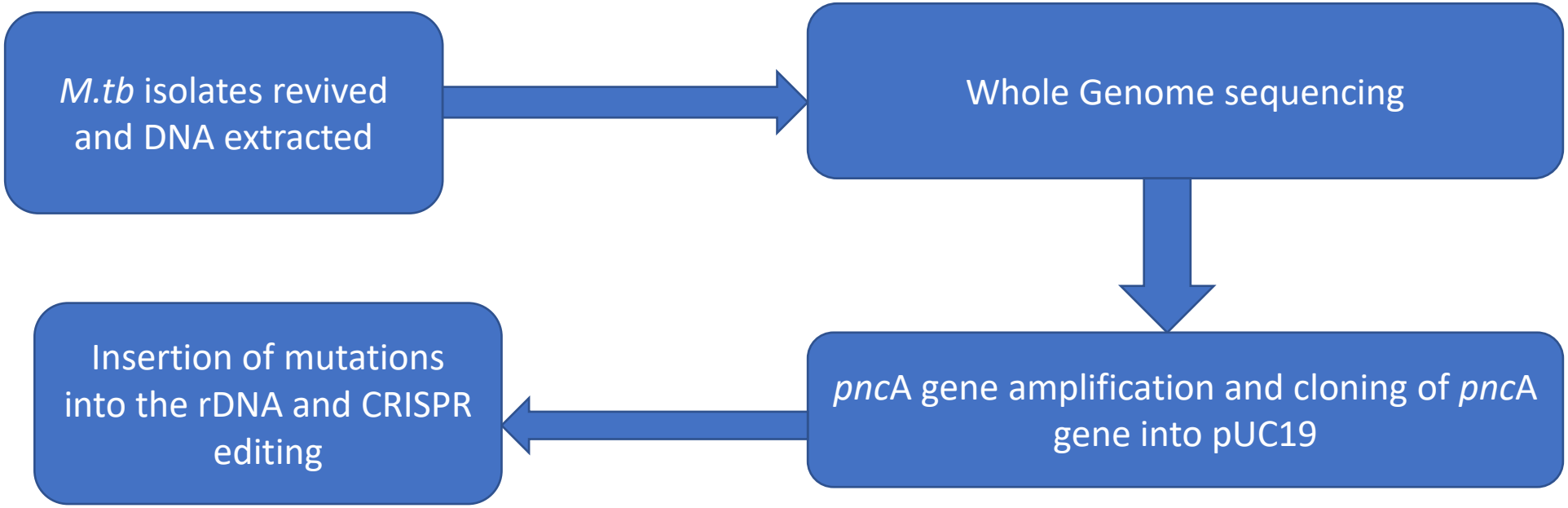


Fig 3. Mechanism of action of the CRISPR system

AIM

- The aim of the study is therefore to identify mutations in the *pnc*A gene that lead to drug resistance against Pyrazinamide.

MATERIALS AND METHODS



RESULTS

Resistance type	Description of Mutation by Whole Genome Sequencing					
	katG	*rpoB*	*embB*	*pncA*	*rpsL*	*gyrA*
MDR-TB (RIF + INH)	S315T	D435V	M306L	L151S		
Pre-XDR TB	S315T	D435V	M306L	L151S	K43R	E21Q
Susceptible	WT	WT	WT	WT	WT	WT

Table 1. Results of TB drug resistance Mutations detected on the Illumina Miseq using Whole Genome sequencing

RESULTS

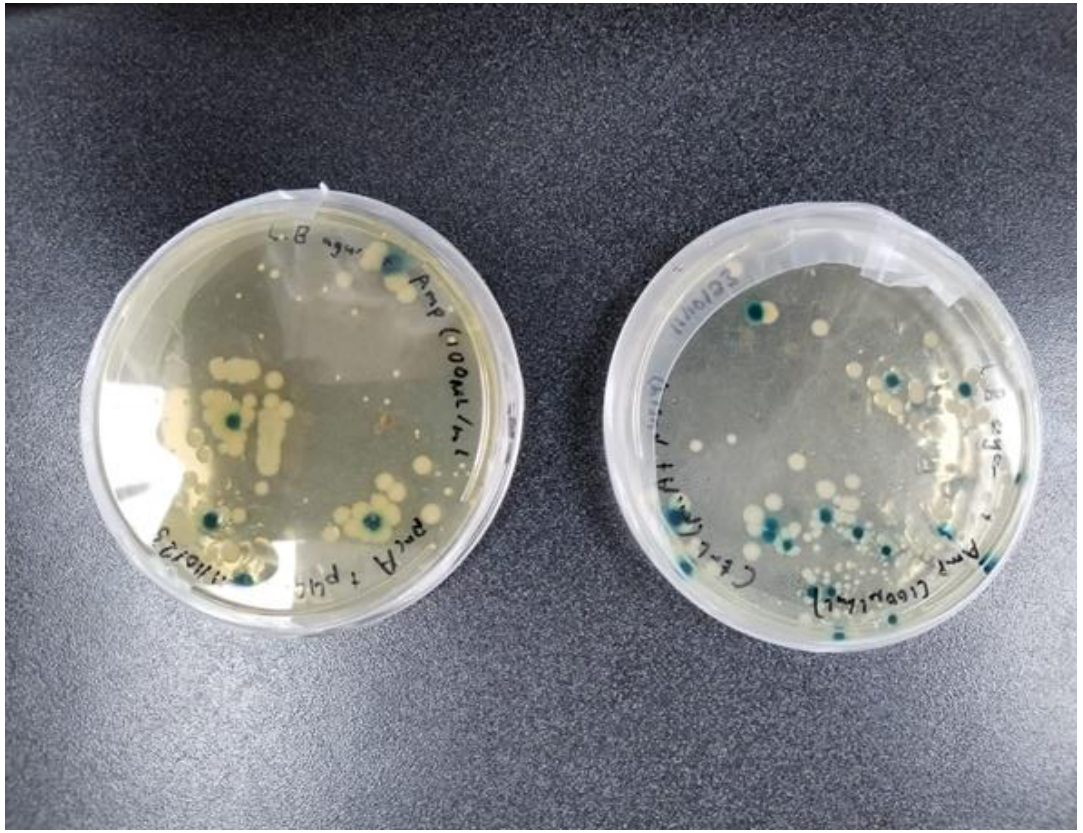


Fig 4. Results of transformed plates after Gibson Assembly cloning

DISCUSSION

- From the Whole genome sequencing results, one of the TB strains is possibly a pre-XDR-TB, indicating the possibility of XDR-TB strains in South Africa

- The G132R mutation was successfully inserted into the *pnc*A gene

- Varying levels of gene expression where observed during gene expression

Acknowledgements

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