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Computational Analysis of Acquired Antimicrobial Resistance Genes in Mycobacterium Plasmid

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ABSTRACT

Mycobacterium species are becoming an increasing health challenge worldwide due to antimicrobial resistance (AMR). Although chromosomal mutations are well characterized, not much is known about the contribution of acquired AMR genes acquired via plasmids in this genus. In this study, a computational pipeline was used to examine acquired AMR genes in plasmids in both pathogenic Mycobacterium species. Out of 195 species, 51 species were identified to be pathogenic; 18 of which harbored a total of 301 plasmids that were obtained in NCBI nucleotide database. The acquired AMR genes in all plasmids were screened with ResFinder 4.0, which was designed to screen 15 antibiotic classes. The resistance hits of plasmids were additionally analysed with KmerResistance and ResFinderFG, and such basic genomic characteristics as size and GC content were analysed. The detection of acquired AMR genes was done by comparative sequence analysis with the help of BLAST and MEGA X by detecting three plasmids, which were parts of the Mycobacterium abscessus complex, and contained a total of 15 resistance genes. These plasmids contained a high GC content (64.25-64.3 percent), which was quite similar to that of their host genome, hence indicating adaptation. Comparison of sequences showed that there was close relatedness between the resistance plasmids, which was in line with recent divergence and horizontal gene transfer in the complex. Even though a small proportion of Mycobacterium plasmids already harbor acquired AMR genes, the genes are in clinically relevant, hard-to-treat species, and thus, genomic surveillance and wise antibiotic intake are still necessary.

INTRODUCTION

Antimicrobial resistance (AMR) has become an urgent and vital health issue worldwide, and recent reports indicate that in 2019, bacterial AMR was linked to about five million deaths worldwide (Murray et al., 2022). The given burden is especially acute within low and middle-income environments, where the access to effective antibiotics and up-to-date diagnostics is low. Among such broad picture, *Mycobacterium* species are of special interest since they are known to cause a range of chronic and opportunistic infections, such as pulmonary disease, skin and soft tissue infections and disseminated disease in immunocompromised hosts. The resistance in Mycobacterium has been mainly associated with mutation in the chromosomes of the target drug, efflux pumps or components of the cell wall particularly in *Mycobacterium tuberculosis* complex. Nevertheless, the transmission of resistance can be increased by plasmids and other mobile genetic factors that make it possible to transfer the gene horizontally (HGT) across and between species of bacteria (Harrison and Brockhurst, 2012). The plasmids may include several resistance genes that can produce multidrug-resistant (MDR) and extensively drug-resistant (XDR) phenotypes and their spread may make it hard to control an infection in the community and healthcare settings. Computational analyses of plasmid sequences in other bacterial genera have demonstrated large amounts of acquired AMR genes and have been applied to estimate possible multiple-antibiotic-resistance (p-MAR) indices (Nwaiwu & Aduba, 2020). In silico investigations of

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Mycobacterium plasmids remain comparatively scarce, yet plasmid-mediated resistance has been reported in the *Mycobacterium* abscessus complex and in some of the members of the *M. avium* complex (Uchiya et al., 2015). The purpose of the study was to use a computational method on the plasmids of *Mycobacterium* species that were pathogenic to: 1. Determine species that contain pathogenic species and determine the number of plasmids per species. 2. Identify AMR genes in such plasmids by curated resistance gene databases. 3. Investigate plasmid genomic characteristics (GC content) and relatedness of the sequences of resistance plasmids. This study is a complement to chromosomal analyses and adds to a more thorough perspective on how mobile genetic elements influence the development of resistance in *Mycobacterium* through the identification of AMR genes on the plasmids.

MATERIALS AND METHODS

The selection of plasmids and the collection of the data will be performed as follows: Out of 195 known species of the genus *Mycobacterium*, 51 species were considered to be pathogenic or of clinical interest according to the literature sources and database annotations. Out of these 51 species 18 species had been identified as having plasmids with a total of 301 plasmids. In the case of these 18 species all the available plasmid sequences were downloaded through the NCBI nucleotide database. Species that featured prominently were *Mycobacterium fortuitum*, *Mycobacteroides abscessus*, *M. kansasii*, *M. marinum*, *M. scrofulaceum*, *M. frederiksbergense*, *M. gordonae*, *M. avium*, *M. chimaera*, *M. massiliense*, *M. punctualis*, *M. pseudoshottsii*, *M. boenickei*, *M. branderi*, *M. intracellulare*, *M. ulcerans*, and *M. liflandii*. Each plasmid was associated with its species of origin, which was extracted in metadata of the submission. The length of the sequence and the GC content were extracted. Further investigation is needed on the acquisition of AMR genes screening. The 301 plasmid sequences were analysed with the ResFinder 4.0 web tool (Bortolaia et al., 2020) that recognises acquired AMR genes and predicts AMR resistance phenotypes using curated sequences databases (Florensa et al., 2022). It analyzed 15 classes of antibiotics. The identity cut-off was adjusted to 90 percent and the minimum alignment length was adjusted to 60 base pairs so as to narrow down to the confidently acquired AMR genes and to eliminate misidentification of the chromosomal mutations. The detection of each plasmid was recorded by the number and type of identified resistance genes, the group of the antibiotic and the predicted resistance phenotype. The potential multiple-antibiotic-resistance (p-MAR) index was a ratio of the number of antibiotic classes that had AMR genes identified to the number of classes being screened. The verification with KmerResistance and ResFinderFG was made at 2.3. The acquired AMR genes detected by ResFinder 4.0 were re-examined with the KmerResistance tool that employs a k-mer-based methodology to identify the resistance genes (Bortolaia et al., 2020). The default settings were applied, where Mycobacterium was chosen as a host organism and resistance genes were chosen as a target database. The identity threshold was adjusted to 70 percent and depth of 10. The comparison between ResFinder and KmerResistance was done to verify the existence of AMR genes and possible new or discordant hits. In order to investigate AMR determinants discovered in functional metagenomics, AMR-positive plasmids were screened with ResFinderFG (percentage identity threshold: 98% and minimum query length: 60 base pairs) in 13 antibiotic resistance determinant (ARD) classes. XLSTAT was used to conduct principal component analysis (PCA) based on the plasmid size and GC content to graphically visualise patterns in the plasmid characteristics as well as compare the AMR-positive plasmids with the overall plasmid set. The similarity between AMR-positive plasmid sequences and the other sequences at the NCBI nucleotide collection was studied by BLAST. The MEGA X was used to estimate pairwise genetic distances by applying maximum composite likelihood model. The gaps or missing data in positions were not counted in calculating distances.

RESULTS

Distribution of *Mycobacterium* species in pathogens plasmids are distributed among the species of *Mycobacterium* by way of a horizontal transmission mechanism. Out of the 51 pathogenic *Mycobacterium* species taken into consideration, 18 of them were identified to harbour plasmids, which totaled to 301 plasmids. The incidence of the plasmids was extremely skewed among the species (Table 1). *Mycobacterium avium* (108 plasmids), *Mycobacterium intracellulare* (97 plasmids) and *Mycobacterium chimaera* (82 plasmids) had the highest counts (Figure 1). Conversely, a number of species such as *M. scrofulaceum*, *M. gordonae*, *M. boenickei*, *M. branderi*, *M. celatum* had one plasmid each.

Table 1. Distribution of plasmids among pathogenic Mycobacterium species

No.	Species	Number of plasmids
1	*Mycobacterium fortuitum*	7
2	*Mycobacteroides abscessus*	68
3	*Mycobacterium kansasii*	13
4	*Mycobacterium marinum*	12
5	*Mycobacterium scrofulaceum*	1
6	*Mycobacterium frederiksbergense*	6
7	*Mycobacterium gordonae*	1
8	*Mycobacterium avium*	108
9	*Mycobacterium chimaera*	82
10	*Mycobacterium massiliense*	10
11	*Mycacterium pseudoshottsii*	2
12	*Mycobacterium boenickei*	1
13	*Mycobacterium branderi*	1
14	*Mycobacterium intracellulare*	97
15	*Mycobacterium celatum*	1
16	*Mycobacterium ulcerans*	50
17	*Mycobacterium liflandii*	3
18	*Mycobacterium yongonense*	4

This distribution suggests that only a small group of pathogenic species, in particular, *M. avium* members, *M. intracellulare* and *M. chimaera* complex, are large plasmid reservoirs, and others contain only a few known plasmids. Bar chart of number of plasmids in each of the species, highest numbers of which were in *M. avium*, *M. intracellulare*, and *M. chimaera*.

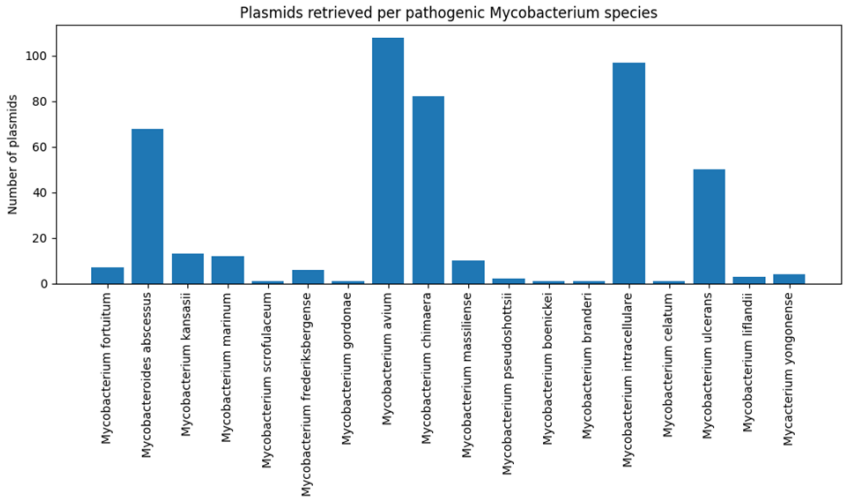

Figure 1: Plasmids recovered in each pathogenic Mycobacterium species

The content of AMR-positive plasmid DNA in the samples was determined through the GC method, including the analysis of the GC content of all DNA samples<|human|>3.2 GC value of AMR-positive plasmid DNA in the samples The GC values of all plasmid DNA samples were measured by the GC method, which involves analysis of GC content of the AMR-positive plasmid DNA. The AMR genes were discovered in three plasmids as a result of ResFinder 4.0 screening and all pertained to Mycobacterium abscessus complex: *M. abscessus subsp. bolletii* F1725 plasmid BRA100. CRM-0020 plasmid CRM0020-0044. *M. abscessus subsp. bolletii* CRM-0020. *M. abscessus subsp. bolletii* INCQS 00594 pMAB01 plasmid. The three plasmids were found to have a high GC content ranging between 64.2-64.3% (Table 2).

Table 2. GC content of Mycobacterium chromosomes

No.	Name of Plasmid	Species / Strain	GC Content (%)
1	BRA100	*M. abscessus subsp. bolletii F1725*	64.3%
2	CRM0020 0044	*M. abscessus subsp. bolletii CRM-0020*	64.2%
3	pMAB01	*M. abscessus subsp. bolletii INCQS 00594*	64.3%

The fact that plasmid and host GC content closely match one another implies a long-term interaction and evolution between these plasmids and their *M. abscessus* hosts (Figure 2), which is consistent with the findings indicated in other large Mycobacterium plasmids (Uchiya et al., 2015).

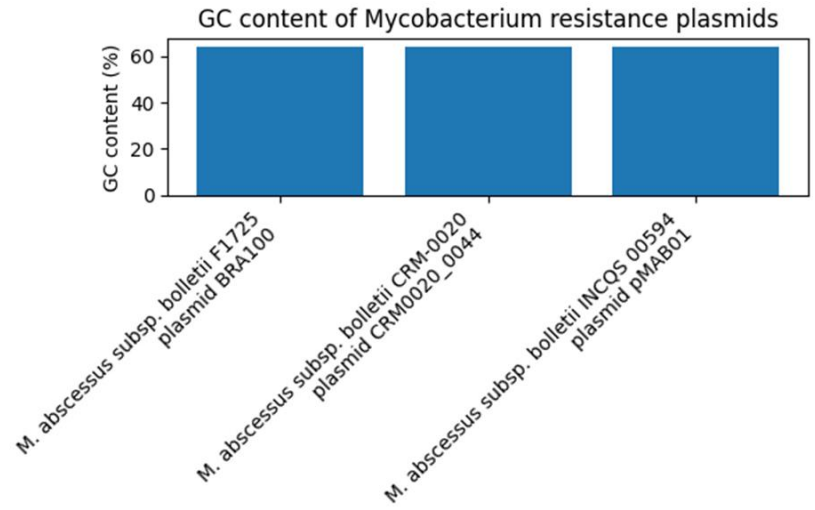

Figure 2: Bar chart of GC Content of the three Mycobacterium abscessus complex plasmids which contain acquired AMR genes (BRA 100, CRM0020_0044 and pMAB01)

The plasmids have high GC content (64.2-64.3%) which is similar to the GC content of the host genomes. In the three *M. abscessus* complex plasmids, 15 different acquired AMR genes were identified. Such genes were predicted to confer resistance to a variety of antibiotic classes, including the macrolides, aminoglycosides, 0-lactam, sulphonamide and tetracyclines. Relying on the quantity of classes of affected drugs, each plasmid met the multidrug resistance threshold and at least one plasmid was close to meeting extensively drug-resistant (XDR) criteria. The rest of the 298 plasmids had no acquired AMR genes found at the selected thresholds, suggesting that plasmid-mediated acquired resistance is only currently limited to a small group of plasmids in the genus, but is highly concentrated in the *M. abscessus* complex. The testing and sequence relatedness is checked here. Re-examination of the three AMR-positive plasmids by KmerResistance did not identify any new resistance genes than those identified by ResFinder 4.0 and no contradictory findings were found.

This congruence justifies the strength of the bottle-based pipeline, which involves ResFinder, in identifying acquired AMR genes (Bortolaia et al., 2020; Florensa et al., 2022). Further functional metagenomics-derived resistance determinants were not found upon screening with ResFinderFG at stringent thresholds, implying that the major plasmid-mediated resistance burden in this data set is represented by classical acquired AMR gene families. The sequence similarity was high in the three *M. abscessus* complex plasmids and to the similar plasmids in *M. abscessus* previously reported in the literature in BLAST and MEGA x analyses. The distances between the pair-wise were low and were conformable to the recent evolutionary split and the current circulations of closely related strains.

DISCUSSION

This in Silico paper has shown that currently AMR gene acquisition in *Mycobacterium* is limited to few plasmids but these plasmids are correlated to species of clinical interest. The three AMR-positive plasmids were *M. abscessus* complex plasmids and also possessed multiple acquired AMR genes, and thus they would be resistant to various classes of antibiotics. This is in line with the identified challenge of treating *M. abscessus* infections and the few therapeutic options that are effective (Nguyen et al., 2024). The presence of plasmids in pathogenic *Mycobacterium* species in uneven distribution indicates that only a part of the taxa, especially those belonging to the *M. avium–intracellulare–chimaera* complex are significant reservoirs of mobile genetic elements. Such species can be sources of ecological hotspots of horizontal gene transfer especially in biofilms and other environmental niches where stress and cell density are high (Srensen et al., 2005). Conversely, other species of pathogens with minimal plasmids would most probably depend on the resistance to be primarily by chromosome mutations. It is believed that, according to the high GC content of AMR-carrying plasmids, which is in close relationship with the GC content of their *Mycobacterium* hosts, these plasmids could be well adapted and have survived, over evolutionary time, in the population of their hosts. According to the co-evolutionary theories, the plasmids with fitness costs may be still preserved in cases when they offer necessary adaptive characteristics in the face of significant selection pressures, including exposure to antibiotics (Harrison & Brockhurst, 2012). Findings of this paper are consistent with such models: resistance plasmids in *M. abscessus* are found not only functionally important, but also to be genetically embedded in the host lineage. Compared to other species of other genera, including *Aeromonas*, where multiple plasmids carry many AMR genes (Nwaiwu & Aduba, 2020), the extent of plasmid-mediated resistance in *Mycobacterium* does not seem so big. Nevertheless, the clinical effect of resistance in *Mycobacterium* may be disproportionately significant given that infections are frequently chronic, need to be treated with multiple drugs, and happen in patients that are not resistant. This paper has a number of limitations. To start with, it is limited to plasmids that are in the

public databases and may not be a complete representation of the variety of plasmids being circulated in the clinics and in the environment. Second, acquired AMR genes may be overlooked by existing databases since they concentrate on the previously described genes. Third, the predictions of genotype cannot substitute phenotypic susceptibility testing, but they present useful early-warning information and prioritisation to be further developed (Papp et al., 2022). In spite of these shortcomings, these findings highlight the significance of the ongoing surveillance and database-based analysis of the genome to identify the spreading of new resistance plasmids in time before they become widespread.

CONCLUSION

The current computational work demonstrates that the AMR genes which have been gained in *Mycobacterium* plasmids are presently restricted to few plasmids of *Mycobacterium abscessus* complex. These plasmids contain several resistance genes, cause multidrug-resistant phenotypes, and are highly GC content which is reflective of their host genomes implying long-term adaptation. Although the concentration of acquired AMRs in the plasmids of most *Mycobacterium* plasmids analysed could not be detected, the presence of plasmid-borne resistance in clinically challenging species indicates a very high likelihood of further growth in the face of persistent antibiotic pressures. It will be crucial to increase the use of genomic surveillance, rational use of antibiotics and combine the results of genomic analysis with clinical data to avoid the further dissemination of plasmid-mediated resistance in *Mycobacterium* and other pathogens.

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DECLARATIONS

Ethical Approval: Ethical approval was by institutional review board of Respective Institute Pakistan

Informed Consent: Informed Consent was taken from participants.

Authors' Contributions:

Concept: SS; Design: FR; Data Collection: ZF & AA; Analysis and Drafting: HN & MF

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