UMP. 047

Oxazolidinones are essential in resistance-proof drug combinations in M. tuberculosis selected under in vitro conditions

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Background: Drug resistance in *Mycobacterium tuberculosis* is primarily attributed to mutations in target genes. These mutations incur a fitness cost and result in bacterial generations that are less fit, which subsequently acquire compensatory mutations to restore fitness. We hypothesize that mutations in specific drug target genes influence bacterial metabolism and cellular function, which affects its ability to develop subsequent resistance to additional agents. We aim to determine whether the sequential acquisition of drug resistance and specific mutations in a well-defined clinical *M. tuberculosis* strain promotes or limits the development of additional resistance.

Methods & Materials: *In vitro* mutants resistant to pretomanid, linezolid, moxifloxacin, rifampicin and kanamycin were generated from a pan-susceptible clinical strain from the Beijing lineage. The resistant phenotypes to the anti-TB agents were confirmed by the broth microdilution assay and genetic mutations were identified by targeted gene sequencing. Growth of mono-resistant mutants was done in enriched medium for 14 days to assess *in vitro* fitness. Double resistant mutants were generated against anti-TB drug combinations at concentrations 5x and 10x the MIC. Subsequently, mutation frequencies for these anti-TB drugs in the different mono-resistant backgrounds were determined.

Results: The initial level of resistance and the mutation frequencies observed for the mono-resistant mutants were comparable to those previously reported. Targeted gene sequencing revealed the presence of known and clinically relevant mutations in the mutants resistant to linezolid, rifampicin, kanamycin and moxifloxacin. Significant growth defects were observed for mutants grown under *in vitro* conditions compared to the sensitive progenitor. Mutation frequencies determination in the mono-resistant mutants revealed a significant increase in mutation frequency against rifampicin and kanamycin, but a significant decrease in mutation frequency against linezolid and sutezolid. This suggests that these mono-resistant mutants are more prone to develop resistance to rifampicin and kanamycin, but less prone to develop resistance against linezolid and sutezolid. Even though kanamycin and linezolid both inhibit protein synthesis, these compounds target different subunits of the ribosome, thereby leading to different outcomes in terms of fitness in the mutants with impaired cellular function.

Conclusion: These observations showed that oxazolidinone treatment is instrumental in limiting the development of multi-drug resistance in *M. tuberculosis in vitro.*

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Multilocus sequence analysis and synergy tests of Burkholderia species isolated from cystic fibrosis and non-cystic fibrosis patients

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Background: *Burkholderia* spp. are problematic infectious agents for cystic fibrosis (CF) or chronic granulomatous disease patients. Due to the failure of identification with conventional techniques and high intrinsic resistance to most antibiotics, these infections very difficult to treat and can cause a marked reduction in life expectancy. The aim of this study was to identify *Burkholderia* spp. strains isolated from CF and non-CF patients with multilocus sequence analysis (MLSA). Also, the antibiotic resistance and synergies of antibiotics were evaluated.

Methods & Materials: A total of 38 *Burkholderia* spp. (25 CF, 13 non-CF) isolates were analyzed. All the *Burkholderia* spp. were identified by MLSA. Antibiotic susceptibilities of ceftazidime, meropenem, trimethoprim/sulfamethoxazole (TMP-SXT) and levofloxacin were determined by broth microdilution method according to CLSI guidelines. Synergy tests are performed by checkerboard method.

Results: Multilocus sequence analysis identified clinical isolates as *B. cenocepacia* (n = 16), *B. contaminans* (n = 11), *B. gladioli* (n = 4), *B. dolosa* (n = 4), *B. multivorans* (n = 2) and *B. seminalis* (n = 1). Among the 38 *Burkholderia* spp. isolates, overall rates of resistance to ceftazidime, meropenem, TMP-SXT, and levofloxacin was 34.2%, 65.8%, 50% and 39.5%, respectively. No significant difference in antibiotic resistance between cystic fibrosis and non-cystic fibrosis patients were detected. Synergy has shown between meropenem and TMP-SXT at two isolates. Antagonism has detected at 15 isolates (between meropenem and ceftazidime (n = 12); between ceftazidime and TMP-SXT (n = 3))

Conclusion: A variety of molecular approaches have been developed for the identification of closely related species and MLSA has successfully provided a reliable advanced discrimination method for *Burkholderia* isolates. Expression of several resistance mechanisms in species may lead to higher resistance, whereas antagonism between meropenem and ceftazidime might be attributed to the expression of the beta-lactamases.

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