 doi:10.20944/preprints202104.0443.v1

*Article*

# Elucidating the antimycobacterial mechanism of action of ciprofloxacin using metabolomics

**Kirsten E. Knoll[1], Zander Lindeque[1], Adetomiwa A. Adeniji[1], Carel B. Oosthuizen[2], Namrita Lall [2], Du Toit Loots[1,*]**

[1] Human Metabolomics, North-West University, Private Bag x6001, Box 269, Potchefstroom, 2531, South Africa; kirsten.e.knoll@gmail.com (K.E.K); adenijiadetomiwa@gmail.com (A.A.A); zander.lindeque@nwu.ac.za (Z.L)

[2] Depart of Plant and Soil Sciences, Faculty of Natural and Agricultural Sciences, University of Pretoria, Pretoria 0002, South Africa; carel.oosthuizen@uct.ac.za (C.O.)

* Correspondence: dutoit.loots@nwu.ac.za; Tel.: +27 (0)18 299 1818

**Abstract:** In the interest of developing more effective and safer anti-Tuberculosis treatment, we aimed for a better understanding of the antimycobacterial action of ciprofloxacin against *Mycobacterium tuberculosis* (*Mtb*). We used GCxGC-TOF-MS and well described metabolomics statistical approaches, to investigate and compare the metabolic profiles of *Mtb* in the presence and absence of the drug. The metabolites that best describe the differences between the compared groups were identified as markers characterizing the changes induced by ciprofloxacin. Malic acid was ranked as the most significantly altered metabolite marker induced by ciprofloxacin, indicative of an inhibition of the tricarboxylic acid (TCA) and glyoxylate cycle of *Mtb*. The altered fatty acid, *myo*-inositol and triacylglycerol metabolism seen in this group, supports the previous observations of ciprofloxacin action on the *Mtb* cell wall. Furthermore, the altered pentose phosphate intermediates, glycerol metabolism markers, glucose accumulation, and the reduction in the glucogenic amino acids specifically, indicates a flux towards DNA (as well as cell wall) repair, also supporting previous findings of DNA damage caused by ciprofloxacin. This study further provides insights useful for designing network whole-system strategies for the identification of possible modes of actions of various drugs and possibly adaptations by *Mtb* resulting in resistance.

**Keywords:** Fluoroquinolones; Ciprofloxacin; Untargeted Metabolomics; *Mycobacterium tuberculosis;* Tuberculosis; GCxGC-TOFMS

## 1. Introduction

Tuberculosis (TB), caused by the *Mycobacterium tuberculosis* (*Mtb*), is the leading cause of death globally from a single infectious agent [1], resulting in a mortality rate of 1.5 million and an infection rate of about 10 million annually [2]. Furthermore, the prevalence of drug resistant TB is also on the rise, which is mainly attributed to poor patient adherence to the drug regimen, [3] as a result of the many side-effects experienced by patients being treated with first-line anti TB medication, accompanied by the long treatment duration required [4,5]. Further contributing factors to developing drug resistant TB include; inaccurate diagnosis, unsupervised treatment, poor economic status [6], and in 2020, was further exacerbated by the COVID-19 pandemic [2,7]. Currently, the WHO's approved first-line therapy for patients with active TB, is a 6-month "directly observed treatment short-course" (DOTS) regimen, and consists of isoniazid, ethambutol, pyrazinamide and rifampicin [8,9]. Infection with multi-drug resistant (MDR)-TB and extensively drug resistant (XDR)-TB requires treatment using various second-line antibiotics, which are expensive, have far more side effects due to their higher toxicity and need to be consumed for even a longer duration [2,10]. The only newly approved drugs for TB over the last 50 years, are the second-line drugs for treating MDR-TB; linezolid, bedaquiline and delamanid, and, not long after, resistance followed [9,11,12]. Consider-

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 16 April 2021 doi:10.20944/preprints202104.0443.v1

ing this, there is an urgent need for well-tolerated and effective treatment for TB, using drugs with novel mode of action against the infectious organism.

A suggested approach for avoiding the long drug trial-phases usually required for approving new drug candidates, is further investigation of already existing drugs, re-purposed for use in treating TB/MDR-TB, [13,14]. In order for a drug to be selected for possible repurposing applications, it should preferably be affordable, easily available and show good pharmacokinetic/pharmacodynamic properties. For these reasons and of late, several fluoroquinolones (FQs) are intensively being investigated for use as anti-TB therapy [15]. FQs, originally used to treat urinary tract infection [16], were first shown to be effective against *Mtb* in 1984, and have since gained continuous interest for such applications [17-19]. Currently, they are among the most frequently prescribed drugs [20] and are considered the backbone of MDR-TB treatment [10,21]. FQs target two *Mtb* topoisomerase deoxyribonucleic acid (DNA) enzymes, DNA gyrase and topoisomerase IV [22]. The first introduces negative super helical twists in the bacterial DNA-double helix and catalyses the separation of daughter chromosomes [23], whereas the latter is responsible for the segregation into two daughter cells at the end of DNA replication [24]. Earlier generations, ciprofloxacin and levofloxacin, exhibit greater activity against gram-negative bacteria (and some gram-positive bacteria), and target mainly DNA gyrase [25-27]. Newer generation FQs, gatifloxacin, ofloxacin and moxifloxacin, show greater activity against gram-positive bacteria and anaerobes, and targets both, DNA gyrase and topoisomerase IV [28]. In *Mtb*, however, only DNA gyrase is present, which is capable of carrying out the action of both topoisomerases [4]. FQs inhibit DNA gyrase by binding to the enzyme and DNA, which leads to double-stranded DNA breaks [29]. When stabilized, replication and transcription cannot happen, leading to slow *Mtb* cell death. If the topoisomerase is removed, the double stranded breaks are processed into single-strand DNA, which, if left unrepaired, leads to lethal chromosomal fragmentation [30]. These are recognized by the DNA damage response regulon, recA/lexA, which in turn induce the bacterial stress (SOS) response. During the SOS response, DNA repair is activated, reactive oxygen species (ROS) are released, and cell growth arrest is initiated [31]. The underlying molecular mechanisms are, however, still largely unclear [32].

The innate resistance mechanism of *Mtb* to many anti-TB drugs can be attributed to its complex cell wall [33], however, the specific, highly lipophilic characteristics of FQs [34], provide great permeability over this [35]. These antibiotics chelate with Mg2+ cations and electrostatically interact with membrane phosphodiesters, subsequently traversing the *Mtb* cell wall [36]. FQ's are not exempt from resistance however, of which the best described are mutations in genes *gyrA* and *gyrB*, encoding subunit GyrA and GyrB of DNA gyrase [37-39]. Furthermore, several resistance-forming proteins have also been identified; the efflux pumps, LfrA [40,41] and MmpL (mycobacterium membrane protein large) [42], the target protection proteins, MfpA and MfpB (*Mycobacterium* fluoroquinolone resistance proteins) [43,44], and the cell-survival promotor, HtrA2 (high temperature requirement A) [45]. DNA repair and mutations by the SOS regulon have also been described [18].

Interactions between DNA gyrase and FQs have been thoroughly investigated [22,38,46]. Still, little is known about the overall biochemical mechanisms of action against *Mtb* specifically, and drug resistance to these [10,19]. The results published thus far are somewhat contradictory. In a study by Verma, *et al.* [47], the macromolecular composition of the *M. smegmatis* cell wall after sub-MIC cipro treatment, indicated a significant decrease in the total lipids, phospholipids and sugars, suggesting ciprofloxacin-induced alterations of the cell wall. In contrast, Halouska, *et al.* [48] indicated ciprofloxacin-induced inhibition of transcription, translation and DNA supercoiling, without effects on the cell wall. While most data suggest cell death due to the inhibition of DNA replication [49], altered DNA biosynthesis could set in motion secondary events contributing to its bacteriostatic or bacteriocidic effects. Furthermore, it is important to remember that stronger target activity does not predict better antimycobacterial activity. This is perfectly demonstrated by ciprofloxacin-dimers, which show enhanced DNA gyrase in-

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 16 April 2021 doi:10.20944/preprints202104.0443.v1

hibition, while less effectively killing *Mtb* [50]. Proposedly, this is a result of stronger cleavage of FQ to the DNA-enzyme complex, which results in less single strand DNA fragments, and subsequently prevents RecA from recognizing damaged DNA and inducing the SOS regulon [32]. The SOS response assists in killing by releasing ROS [51,52], yet simultaneously activates DNA repair and creates a dormancy-state, ultimately leading to resistance. Before the SOS response can be used as an advantage, this phenomenon, and how it connects to the mechanism of ciprofloxacin, still needs to be elaborated on.

The selection of ciprofloxacin as our investigational compound is predominantly based on its safety profile. Although less potent than moxifloxacin (MIC 0.12–0.5 μg/mL), and levofloxacin (MIC 1 μg/mL), ciprofloxacin (MIC 0.5-4.0 μg/mL) [53] has the lowest risk for causing serious ventricular arrhythmia, cardiovascular mortality, and hepatotoxicity [54,55]. Furthermore, ciprofloxacin demonstrates the highest clearance rate of all FQ's [56] and is thus the preferred option for treatment of renally-impaired patients [17]. The levels of ciprofloxacin in cerebrospinal fluid can be as high as 40-90% compared to that of plasma [53], which offers further advantages for its use in the treatment of tuberculous meningitis. Adverse drug reactions (ADRs) are usually minimal (5% or less) and the most common ADRs are usually gastrointestinal in nature (nausea, vomiting, diarrhea, and abdominal pain [57,58]. Previous studies demonstrated that mitochondrial topoisomerases bear less than 30% homology to their prokaryotic counterparts and are not inhibited [23,59], and it has been reported that ciprofloxacin does affect mitochondrial DNA synthesis [60]. These and many other advantages of ciprofloxacin have not gone unnoticed, as stated by the World Health Organization [61], who has included ciprofloxacin as a critically important antibiotic.

Most of the evidence brought to light thus far has been discovered using genomics, transcriptomics or proteomics [62]. Metabolomics, the latest addition to "omics" technologies, identifies the down-stream metabolites of altered pathways and therefor presents a more sensitive level of organization, of which up-stream deductions can be made [8,63]. We identified the metabolite markers best differentiating *Mtb* with and without ciprofloxacin, using a two-dimensional gas chromatography coupled with time-of-flight mass spectrometry (GCxGC-TOF-MS) metabolomics approach, combined with universally connected metabolic libraries and advanced statistical analysis, in order to better elucidate its mechanism of action.

## 2. Materials and Methods

### *2.1. Cell culture*

Antimycobaterial minimum inhibitory concentration (MIC) and sub-MIC (50% inhibitory concentrations ($MIC_{50}$)) of ciprofloxacin was determined via the Alamar Blue Essay [64]. The cell cultures (5 individually cultured samples per group) were prepared as previously described [65], in the presence and absence of ciprofloxacin. All reagents were purchased from Sigma-Aldrich, St. Louis, MO, USA, unless otherwise stated. Briefly, *Mtb* H37Rv ATCC 27294 (kindly obtained from the Medical Research Council, Pretoria, Gauteng, South Africa) was cultured and maintained for 4 weeks on LJ slants. The bacterial inoculum was prepared to a McFarland standard of 1 (approximately 3 x $10^8$ colony-forming units/mL) in Middlebrook 7H9 broth supplemented with 10% OADC (oleic acid, albumin, dextrose, catalase) (Becton, Dickinson, UK) and 2% PANTA (polymyxin B, amphotericin B, nalidixic acid and trimethoprim, azlocillin) (Becton, Dickinson, UK). PANTA was added for the prevention of contamination with negligible impact on *Mtb's* growth [66]. Ciprofloxacin was dissolved in DMSO (150 μM) and diluted into Middlebrook 7H9 broth to a final concentration of 0.3 μM (0.12 μg/mL) (0.2 % DMSO). One milliliter of the prepared inoculum was added to yield a final assay volume of 5 mL, with a bacterial test concentration of 6 x $10^7$ CFU/mL ciprofloxacin. For the untreated *Mtb* control samples, 4 mL of Middlebrook 7H9 broth (0.2 % DMSO) was added to each replicate culture, followed by the addition of the bacterial inoculum as described

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 16 April 2021 doi:10.20944/preprints202104.0443.v1

above. The DMSO solvent was kept constant throughout the assay. After 5 days of incubation at 37 °C, the samples were centrifuged to pellet the bacteria at 4500 rpm for 15 min. The pellets were washed with 1 mL of PBS and pelleted again under the same conditions. Finally, the PBS was aspirated from the samples and the pellets were stored immediately at -80 °C until further testing.

*2.2. Whole metabolome extraction procedure and derivatization*

The metabolites were extracted from the samples and derivatized as previously described by Beukes, *et al.* [67], with slight modifications. Briefly, 8 mg of each of the individually cultured samples were weighed out into an Eppendorf tube, followed by the addition of 50 µL 3-Phenylbutyric acid (0.13 mg/ml $H_2O$) (Sigma-Aldrich, Lot#536478V) as internal standard. One milliliter of a chloroform: methanol: water (1:3:1 ratio) solution was added, after which the Eppendorf tubes were shaken in a vibration mill at 30 Hz for 5 min, with a 3 mm carbide tungsten bead in each. The samples were centrifuged at 12 000 rpm for 5 min and the supernatant was transferred to a GC glass vial. The extracts were dried under a nitrogen stream, followed by the addition of 50 µL methoxamine hydrochloride (Sigma-Aldrich, Lot#BCBP2843V) in pyridine (Lot#S2BC335SV) at a concentration of 15 mg/mL. The glass vials were heated at 50°C for 90 min. Following, methoximation, 40 µL N,O-bis(trimethylsilyl)trifluoroacetamide with 1% trimethylsilyl chloride (Lot#BCBW2670) was added, and vials were heated again for 60 min at 50°C. Each extract was then transferred to a 0.1 mL vial insert in a GC sample vial and injected into GCxGC-TOF-MS.

*2.3. GCxGC-TOFMS analysis*

A 4D Pegasus GCxGC-TOF-MS (LECO Africa (Pty) Ltd, Johannesburg, South Africa), equipped with a Gerstel Multi-Purpose Sampler (Gerstel GmbH and Co. KG, Mülheim an der Ruhr, Germany) and an Agilent 7890 gas chromatograph (Agilent, Atlanta, USA) coupled to TOF-MS (LECO Africa) was used for the analysis. The samples were analyzed in random sequence, in a split-less ratio. To monitor the analytical performance throughout the entire analysis, a quality control (QC) sample was analyzed at regular intervals. The processed samples were injected into Rxi-5Sil MSprimary capillary column (28.8 m x 0.25 mm internal diameter, 0.25 µm film thickness, Restec), and a Rxi-17 secondary capillary column (1.2 m x 0.25 mm internal diameter, 0.25 µm film thickness), for GC compound separation. The primary GC oven temperature was set at 70°C for 2 min, and then increased at a rate of 4°C/min to a final temperature of 300°C, at which it was maintained for an additional 2 min. The secondary oven was set at 85°C for 2 min, increased at 4.5°C/min, to a final temperature of 300°C, at which it was maintained for 4.5 min. Helium, set to a column flow rate of 1 mL/min, was used as a carrier gas, and held at a constant temperature of 270°C. Mass spectrometric data acquisition was carried out at -70 eV, with a solution delay of 350 sec, and a mass range of 50-800 m/z was scanned with a rate of 200 spectra/sec.

*2.4. Data processing, clean-up and statistics*

ChromaTof software (version 4.32) was used for mass spectral deconvolution (at a signal to noise ratio of 20), peak alignment and peak identification on the obtained mass spectra. Metabolites were identified by comparing their mass fragment patterns to that of compounds in commercially available databases containing previously injected standards. For normalization and assessment of data quality, the data were pretreated using a standardized metabolomics data clean-up procedure [67]. Each detected compound was normalized using MS total useful signal (TUS), which is based on a factor calculated from the sum of all metabolites identified in all samples, and by calculating the relative concentration of each by using the internal standard. All missing/zero values were replaced by a value calculated as 20% of the minimum detection limit of the entire dataset, as these are most likely present in sub-minimum concentrations rather than being completely

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 16 April 2021 doi:10.20944/preprints202104.0443.v1

absent [68]. An 80% data filter was then applied to eliminate compounds with more than 80% zero values within both groups [69]. To provide a balanced representation of all metabolites, log transformation and auto-scaling (mean-centered and divided by the standard deviation of each variable) was applied. This prevents compounds with minor concentrations to be overlooked, due to domination of compounds with higher concentrations [70]. Making use of MetaboAnalyst (Version 5.0) [71], multivariate statistical methods in the form of unsupervised principal component analysis (PCA) and supervised partial least squares-discriminant analysis (PLS-DA) were applied [72]. Subsequently, uni-variate analysis was done by calculating t-test and effect size values [73].

Relationships between the selected metabolites were mapped using the KEGG, MetaCyc and BioCyc databases, in addition to intensive research of the previously published literature on the topic.

## 3. Results

### *3.1. Data overview*

When visualising the analytical technique's repeatability graphically (Figure 1), approximately 86% of all the compounds identified (n=260) had a coefficient of variation (CV) value under 50%, of which 70% had CV values under the 20%. The analytical technique used during this analysis thus proves to be highly repeatable and can be trusted to provide reliable results. PCA was initially used to get an overview of the natural grouping of metabolic data (Figure 2). The total variance between the groups, described by the first two principal components (PCs), was 57.1%, of which PC1 and PC2 accounted for 32.1% and 25%, respectively. The PCA scores plot of the metabolite data analyzed by GCxGC-TOF-MS shows clear clustering between *Mtb* in the presence and absence of ciprofloxacin, as represented in Figure 2.

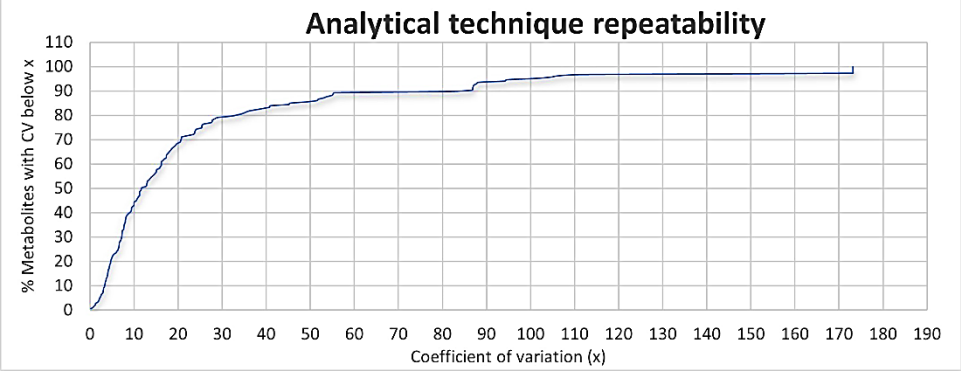



**Figure 1**. Distribution of the coefficients of variation values for the technical repeatability.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 16 April 2021 doi:10.20944/preprints202104.0443.v1

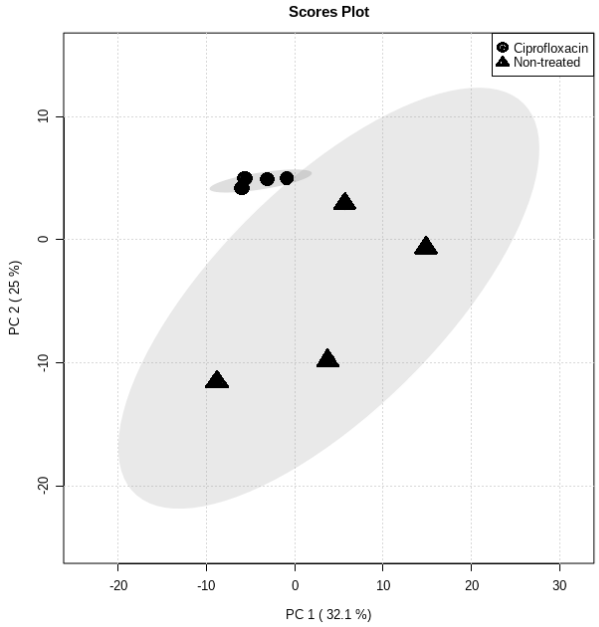



**Figure 2.** PCA scores plot obtained from GCxGC-TOFMS whole metabolome analysis of *Mtb* samples in the presence and absence of ciprofloxacin. The variances accounted for are indicated in parenthesis.

*3.2. Marker selection*

The met metabolite markers (n=26) best describing the differences between the ciprofloxacin and control samples were selected based on compliance with the following criteria: a PLS-DA VIP value > 1 [74], a t-test P-value < 0.05 [75] or an effect size > 0.8 [76] (Figure 3).

The selected metabolites markers are listed according to their PLS-DA VIP values in Table 1, along with their respective average concentrations and univariate test outcomes. Of the total, 61.5 % (16/26) markers were elevated, of which most include fatty acids. The most differentiating marker was malic acid, with an exceptionally high d-value of 6.621 and low p-value of >0.0001.

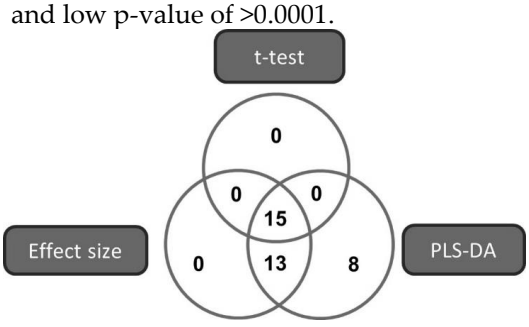



**Figure 3.** Venn diagram illustrating the multi-statistical approach for selecting the metabolites that best describe the variation detected in the metabolome of *Mtb* cultured with and without ciprofloxacin.

**Table 1.** Metabolite markers best describing the variance between the individually cultured *Mtb* samples in the absence (*Mtb* controls) and presence of ciprofloxacin.

| **Metabolite name (ChEBI ID)** | **Average concentration (mg/g cell mass) (standard deviation)** | | **t-test (*P*-value)** | **Effect size (d-value)** | **PLS-DA (VIP)** | **Fold Change ($log_2$)** |
|---|---|---|---|---|---|---|
| | ***Mtb* with ciprofloxacin** | ***Mtb* controls** | | | | |

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 16 April 2021 doi:10.20944/preprints202104.0443.v1

| | | | | | | |
|---|---|---|---|---|---|---|
| Malic acid (6650) | 0.033 (0.002) | 0.054 (0.004) | 0.000 | 6.621 | 2.012 | -0.39 |
| Aspartic acid (17053) | 0.007 (0.001) | 0.013 (0.004) | 0.008 | 2.236 | 1.732 | -0.46 |
| Glycerol (17754) | 0.497 (0.033) | 0.633 (0.031) | 0.013 | 1.932 | 1.678 | -0.21 |
| 5-Oxoproline (17203) | 0.069 (0.014) | 0.099 (0.020) | 0.014 | 1.962 | 1.665 | -0.30 |
| Xylofuranose (46432) | 0.075 (0.037) | 0.034 (0.008) | 0.037 | 1.376 | 1.511 | 1.21 |
| Myo-inositol-1-phosphate (18297) | 0.004 (0.001) | 0.003 (0.001) | 0.051 | 1.637 | 1.445 | 0.33 |
| 9-Hexadecenoic acid (28716) | 0.005 (0.000) | 0.004 (0.001) | 0.091 | 1.329 | 1.299 | 0.25 |
| Nonadecanoic acid | 0.067 (0.011) | 0.055 (0.008) | 0.094 | 1.151 | 1.292 | 0.22 |
| Heptadecanoic acid (32365) | 0.015 (0.003) | 0.011 (0.002) | 0.095 | 1.355 | 1.288 | 0.36 |
| Octadecanoic acid (28842) | 0.582 (0.164) | 0.371 (0.141) | 0.096 | 1.241 | 1.287 | 0.57 |
| Valine (16414) | 0.017 (0.004) | 0.022 (0.005) | 0.106 | 1.117 | 1.255 | -0.23 |
| β-Aminoisobutanoic acid (33094) | 0.011 (0.002) | 0.014 (0.004) | 0.121 | 1.256 | 1.216 | -0.21 |
| Glutamic acid (16015) | 0.010 (0.001) | 0.015 (0.006) | 0.124 | 0.960 | 1.209 | -0.33 |
| Hexadecanoic acid (15756) | 0.575 (0.051) | 0.480 (0.077) | 0.128 | 0.969 | 1.198 | 0.20 |
| 9-Octadecenoic acid (36021) | 0.679 (0.060) | 0.600 (0.041) | 0.132 | 1.089 | 1.187 | 0.13 |
| Tetradecanoic acid (28875) | 0.089 (0.010) | 0.071 (0.017) | 0.141 | 0.934 | 1.167 | 0.25 |
| Eicosanoic acid (28822) | 0.005 (0.001) | 0.004 (0.000) | 0.146 | 0.991 | 1.154 | 0.25 |
| Glucose (17234) | 0.045 (0.006) | 0.037 (0.005) | 0.152 | 0.934 | 1.140 | 0.22 |
| Urea (16199) | 0.005 (0.002) | 0.002 (0.002) | 0.171 | 0.816 | 1.099 | 1.50 |
| N-Acetyl-L-Lysine (64859) | 0.002 (0.001) | 0.004 (0.003) | 0.175 | 0.884 | 1.090 | -0.50 |
| 2-Monopalmitin (75455) | 0.011 (0.002) | 0.009 (0.001) | 0.175 | 0.907 | 1.089 | 0.22 |
| 1-Monomyristin (75562) | 0.007 (0.003) | 0.005 (0.001) | 0.175 | 0.882 | 1.089 | 0.40 |
| Pentadecanoic acid (42504) | 0.005 (0.001) | 0.004 (0.001) | 0.181 | 1.021 | 1.077 | 0.25 |
| Glycerol 3-phosphate (15978) | 0.005 (0.001) | 0.007 (0.002) | 0.186 | 0.908 | 1.067 | -0.29 |
| 1-Monoheptadecanoin (144339) | 0.010 (0.002) | 0.007 (0.001) | 0.186 | 0.947 | 1.066 | 0.43 |
| Erythritol (17113) | 0.021 (0.002) | 0.023 (0.001) | 0.202 | 0.856 | 1.034 | -0.09 |

## 4. Discussion

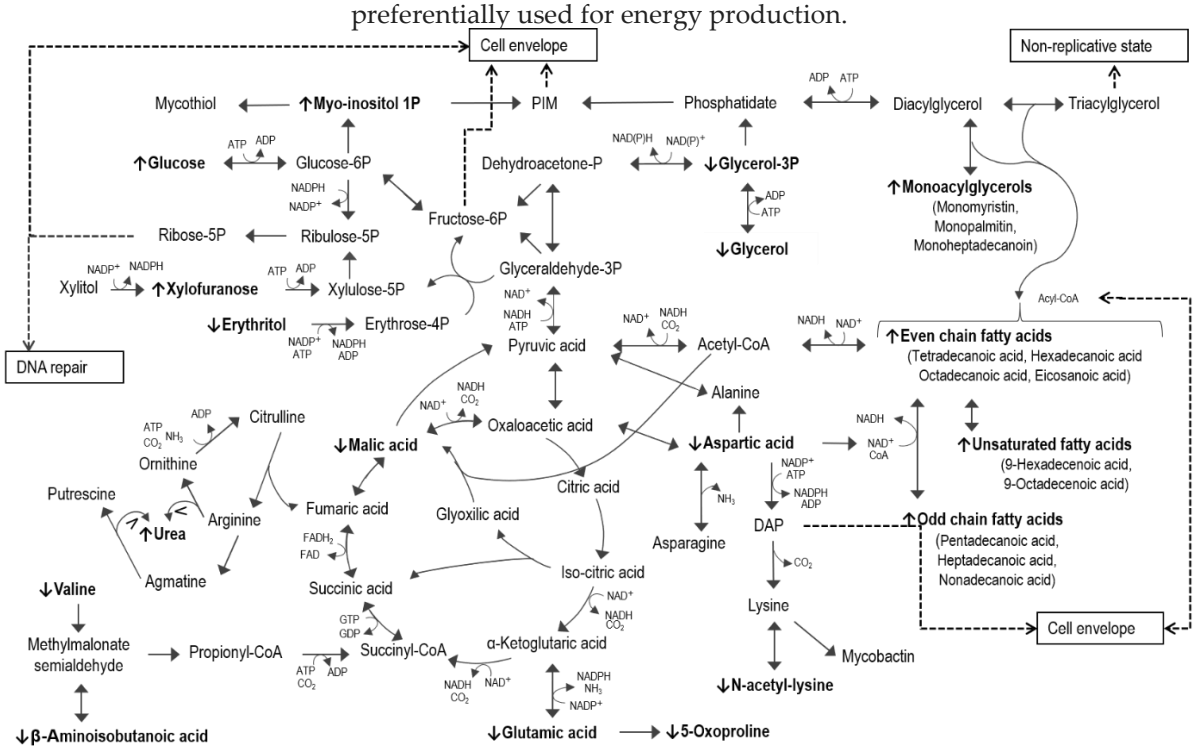
In this study, we identified a number of significantly altered metabolites induced by the administration of ciprofloxacin to *Mtb* culture, which when interpreted, in the light of known metabolism and previous ciprofloxacin findings, better elucidate its mechanisms of action against *Mtb*, as shown in Figure 4. The most prominently altered pathways included gluconeogenesis, fatty acid metabolism, amino acid metabolism, the pentose phosphate pathway (PPP) and the urea cycle.

Of note, was the elevation of many of the even and odd chain saturated fatty acids of between 14 to 20 carbons (C14:0-C20:0) in length, in *Mtb* treated with ciprofloxacin. This was also true for two $\Delta^9$-unsaturated fatty acids; 9-hexadecenoic ($\Delta^9$C16:1) and 9-octadecenoic ($\Delta^9$C18:1) acids. These indicate a strongly upregulated synthesis towards cell wall repair, supporting previous evidence associating ciprofloxacin with cell wall damage [47]. Simplified, lying outside of the cytoplasmic membrane, a peptidoglycan (PG) layer is covalently attached to arabinogalactan (AG), which itself attaches to mycolic acids (MA), to form the MA-AG-PG complex (MAPc) [77,78]. Interspersed within the

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 16 April 2021 doi:10.20944/preprints202104.0443.v1

MAPc, are the glycerolipids, phosphatidyl myo-inositol mannosides (PIM) and lipoarabinomannans (LAM) [79]. PIM is a crucial part of the membrane structure and serves as a precursor of LAM [80]. The saturated fatty acid markers in this study are produced by fatty acid synthase type I (FAS I). FAS I generates 16 to 26 carbon length fatty acyl-coenzyme A's (CoA) [81], which are fed into FAS II for elongation. FAS I and FAS II provide acyl-groups for the synthesis of all cell envelope components, except for AG [82,83]. $\Delta^9$C16:1 and $\Delta^9$C18:1, and their precursors, hexadecanoic (C16:0) and octadecanoic (C18:0) acid, respectively, are considered major fatty acids of glycerolipids and mycolic acids [84-86]. $\Delta^9$C16:1 and $\Delta^9$C18:1 are reduced from C16:0 and C18:0, in the presence of $Fe^{2+}$, a flavin, NADPH and $O_2$ [87,88]. Interestingly, some *mmpL* genes, encoding fatty acid transporter protein MmpL, have been shown to be repressed when their transcriptional regulator proteins bind to C:16 fatty acids and monoacylglycerols (MAG) [89,90]. Even so, further research is needed to establish possible activity of different fatty acids on different MmpL regulator proteins. It is, however, important to note, that the damage that ciprofloxacin administration induces to the cell wall, may be direct, but is most likely indirect, by inhibition of other energy producing mechanisms/or simply by inducing the SOS response in *Mtb*, shifting energy production away from glucose, toward using preferably fatty acids [91], and hence less of these fatty acids are now available to cell wall synthesis.

The dramatically elevated synthesis of intracellular fatty acids would be expected to consume a considerable amount of carbon, which can be supplied from various sources [91]. However, as will be explained, glucose and glycerol seem to be the major suppliers for such, and the various components required for DNA repair. This is supported by previous findings indicating ROS, produced during the FQ-induced SOS-response, causes oxidative stress, which in turn activates utilization of triacylglycerol (TAG) and cell wall lipids for energy [92-94], as is generally the case during the non-replicative phase of *Mtb* [95,96]. These results are also supported by previous findings showing reduced concentrations of phospholipids and mycolic acids in the cell wall macromolecules of sub-MIC ciprofloxacin-treated *M. smegmatis* [47], since these are now being preferentially used for energy production, with glucose supplying the necessary carbon substrates for the continued synthesis of these much-needed fatty acids which are now preferentially used for energy production.



Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 16 April 2021 doi:10.20944/preprints202104.0443.v1

**Figure 4.** Metabolomic pathway map of *Mtb* treated with ciprofloxacin. The metabolite markers best describing the variation in the metabolome compared to that of untreated *Mtb* are represented in bold text with up or down arrows indicating elevated or reduced concentrations, respectively.

A shift in energy supply to β-oxidation of fatty acids, as opposed to the TCA cycle, reserves $NAD^+$, in addition to $CO_2$, for *de novo* synthesis of nucleotides (for DNA repair and fatty acids). NADH and NADPH released during fatty acid metabolism, fuels the upregulated non-oxidative PPP and glycerolipid metabolic pathway. In this study, the downregulated TCA cycle is indicated by a reduction in malic acid and aspartic acid, which according to the uni-and multivariate statistics, were ranked as the two most important metabolite markers (Table 1). Aspartic acid is considered a validated reporter of oxaloacetate (OAA) [97], and together with malic acid, support TCA cycle inhibition. Although oxidative stress is normally associated with an increase in glyoxylate shunt activity, in this study, the glyoxylate shunt is clearly downregulated. This indicates a greater need of carbon flux through gluconeogenesis towards the PPP and glucose, for subsequent fatty acid and nucleotide synthesis [98,99]. This supports the SOS response-induced decrease in oxidative phosphorylation and increase in energy reserves for DNA repair, in response to FQs [32,97]. It is noteworthy to mention that the ATPase activity of DNA gyrase (29), shows reduced adenosine triphosphate (ATP) conversion in the presence of ciprofloxacin [100], which most likely is for the purpose of reserving ATP for other energy consuming pathways, such as gluconeogenesis and DNA repair [101].

Various PPP intermediates were detected to be altered in the ciprofloxacin treated *Mtb*, which contribute to DNA and cell wall repair [94,102,103]. Under normal circumstances, erythrose-4-phosphate and glyceraldehyde-3-phosphate produce xylulose-5-phosphate (xylulose-5P) and fructose-6-phosphate (fructose-6P) [91]. The latter is converted to glucose-6-phosphate, for subsequent PIM synthesis, via the myo-inositol pathway [104], and to glucose-N-acyl 6-phosphate (GlcN6P), for subsequent PG synthesis [105]. Xylose-5P is the precursor of ribose-5-phosphate (ribose-5P), which, in the presence of ATP, is converted to 5-phosphoribosyl-1-pyrophosphate (pRpp) [98,106]. PRpp is the branch point intermediate of decaprenyl-phospho-arabinofuranose (DPA) [107], the only donor of arabinose to AG and LAM [99,108] and nucleotide synthesis. In this investigation, the upregulated non-oxidative PPP in the ciprofloxacin treated *Mtb*, is supported by the elevated concentrations of xylofuranose and reduced erythritol (Figure 4). Furthermore, it is well known, that PPP metabolism is fueled by glucose and the glucogenic amino acids; valine, aspartic acid and glutamic acid [109], all of which were specifically and significantly reduced in the ciprofloxacin-treated *Mtb*, in addition to their degradation products; β-aminoisobutanoic acid [110], 5-oxoproline [111] and N-acetyl-lysine [112], respectively (Figure 4). Furthermore, the reduced levels of glutamic acid and aspartic acid in our investigation, support an oxidative state in the ciprofloxacin treated group [113]. The above-mentioned amino acids also serve as precursors of cell wall related intermediates. Alanine, derived from aspartic acid, and valine combined, produce CoA, which is used for the use of fatty acids during the synthesis of cell wall lipids [114-116]. Valine also serves as a precursor of propionyl-CoA [110,117] for the elongation of odd chain fatty acids [118]. Aspartic acid is the precursor of $NAD^+$, 2,6-diaminopimelate (DAP) [119,120] and S-adenosyl methionine (SAM) [121]. Here, $NAD^+$ serves as a cofactor for FAS I [122], and SAM is required for the methylation of cell-wall fatty acids [123]. DAP, along with alanine and glutamic acid, serve as substrates for PG [124]. The flux through aspartic acid can be supported by the elevated levels of urea in *Mtb* treated with ciprofloxacin [125]. Considering that aspartic acid is one of the top 3 metabolite markers identified (Table 1), it is possibly utilized for many, if not all, of these cell-envelope associated intermediates.

Furthermore, the FQ induced SOS response is known to cause cell growth arrest [126]. In this study, inhibited cell growth is suggested by increased TAG metabolism and reduced TCA activity, in addition to the inhibited protein synthesis. Protein synthesis requires ATP-dependent activation of glutamate and aspartate, followed by amination by

 doi:10.20944/preprints202104.0443.v1

ammonia, to form aminoacyl-tRNA [109]. However, in this study, urea was found elevated in *Mtb* treated with ciprofloxacin, indicating accumulation of ammonia and reduced recycling of nitrogen via glutamine and proline metabolism. This, in addition to a decreased degradation of urea by urease, also linked to the *Mtb* stress-response [127], retains ammonia from protein synthesis [128]. Inhibited protein formation has also been suggested in a proteomics study of *Mtb* treated with ciprofloxacin [129], as well as a metabolomics study of *M. smegmatis* treated with ciprofloxacin [48]. Inhibited protein synthesis would in turn disrupt the functionality of membrane proteins, subsequently inhibiting nutrient uptake as well as fatty acid transport to the cell wall [42]. Additionally, urea acts as an osmolyte preventing dehydration or water loss as a result of the seemingly damaged cell wall [130]. Increased urea could also be indicative of polyamine synthesis (Figure 4) [131]. Polyamines, such as putrescine, have been reported to reduce accumulation of trans-membrane proteins [132] and contribute to the phenotypic drug resistance to FQs [133].

Changes to the monoacylglycerols (MAG); 1-monomyristin, 2-monopalmitin and 1-monoheptadecanoin, are indicative of cell envelope changes via the glycerolipid and triacylglycerol (TAG) pathways. TAG in the cell wall [134] can be metabolized during the stress-induced transition to the non-replicated phase [135] and during infection [136]. *Mtb*'s adaption during these circumstances involves the use of lipids as main energy reserves as previously mentioned [137]. During the deacetylation of TAG to diacylglycerol (DAG), and from DAG to MAG, acyl-CoA's are released, which are either directed towards of glycerolipid synthesis (Figure 4) or towards energy production [138,139]. Glycerolipid and TAG metabolism share an important intermediate at the branch point, 1,2-diacyl-*sn*-glycerol 3-phosphate, commonly known as phosphatidate (PA) (Figure 4) [140]. PA is synthesized via 2 pathways: 1) the phosphorylation of DAG, by DAG kinase [141] or 2) the acylation of glycerol-3-phosphate (glycerol-3P) by glycerol phosphate- and acyl glycerol phosphate acyltransferase [142]. In this study, the concentrations of glycerol-3-phosphate, and its precursor, glycerol, were decreased, supporting a MAG metabolic flux towards TAG and PIM in the ciprofloxacin treated *Mtb* group. Glycerol-3P appears to recycle the transport of membrane lipids [143] and can thus be expected to be the rate limiting step, which would explain the accumulation of the fatty acids and the accompanied reduction in glycerol levels. The synthesis of glycerolipids also requires myo-inositol, which is produced via glucose-6-phosphate (glucose-6P) [144]. Firstly, myo-inositol 3-phosphate synthase converts glucose-6P into myo-inositol 3-phosphate, which in turn is dephosphorylated by several myo-inositol monophosphates to produce myo-inositol [84]. The elevated levels of myo-inositol monophosphates in the ciprofloxacin-treated *Mtb*, hence further supports the aforementioned flux towards glycerolipids [145]. Furthermore, myo-inositol-1-phosphate is converted by a glycosyltransferase, Msh, mycothiol. Mycothiol is also considered an important antioxidant required for balancing the cytosolic $NAD^+$/NADH ratio [146]. In summary, the mycobacterial cell wall metabolism is visibly linked to the SOS response, which has frequently been proposed to cause resistance towards ciprofloxacin and challenge the otherwise impressive bactericidal activity of this drug [18,147-149].

## 5. Conclusions

In this study, we investigated the metabolic changes to *Mtb* induced by sub-MIC of ciprofloxacin, in order to better understand its mechanism of action and perhaps also the adaptations of *Mtb* to this, which may lead to possible drug resistance. Previous studies have identified alterations in transcription, translation, and cell wall synthesis, as part of the mechanism of action of ciprofloxacin against *Mtb* (51, 52, 135). Our metabolomics study identified metabolite markers which support previous results, as indicated by the drastic accumulation of cell wall related fatty acids, metabolites associated with an elevated gluconeogenesis and PPP flux towards DNA repair, further supported by a reduc-

tion in specifically the glycogenic amino acids, and increased urea indicative of protein catabolism, as opposed to protein synthesis.

Moreover, many of these markers indicate that ciprofloxacin induces an SOS-induced non-replicative phase in *Mtb*. The DNA damage-induced SOS response is a key mechanism determining *Mtb* persistence and tolerance to the drug. Differentiating the pathways resulting in ROS from those leading to drug resistance, and subsequently combining FQ with medication inhibiting the mutation-related pathways, could lead to eradication of resistance and more effective killing. This study not only gives a better understanding of ciprofloxacin's mode of action but provides helpful insight for further investigation of antibiotic-induced resistance by *Mtb*, and also perhaps the use of ciprofloxacin, in combination with existing anti-TB drugs.

**Author Contributions:** Du Toit Loots and Adetomiwa A. Adeniji contributed to the conceptualization, project administration and supervision. Zander Lindeque contributed to the data curation and validation. Carel Oosthuizen and Namrita Lall contributed to the resources. Each author contributed to the writing, review and editing of this article.

**Funding:** This research did not receive any specific grant from funding agencies in the public, commercial, or non-profit sectors.

**Data Availability Statement:** Not applicable.

**Conflicts of Interest:** The authors declare no conflict of interest.

## References

1. MacNeil, A.; Glaziou, P.; Sismanidis, C.; Maloney, S.; Floyd, K. Global epidemiology of tuberculosis and progress toward achieving global targets—2017. *Morbidity and Mortality Weekly Report* **2019**, *68*, 263.
2. Harding, E. WHO global progress report on tuberculosis elimination. *The Lancet Respiratory Medicine* **2020**, *8*, 19.
3. Vernon, A.; Fielding, K.; Savic, R.; Dodd, L.; Nahid, P. The importance of adherence in tuberculosis treatment clinical trials and its relevance in explanatory and pragmatic trials. *PLoS Med* **2019**, *16*, e1002884, doi:10.1371/journal.pmed.1002884.
4. Da Silva, P.E.A.; Palomino, J.C. Molecular basis and mechanisms of drug resistance in Mycobacterium tuberculosis: classical and new drugs. *Journal of antimicrobial chemotherapy* **2011**, *66*, 1417-1430.
5. Holmes, K.K.; Bertozzi, S.; Bloom, B.R.; Jha, P. Major infectious diseases. **2017**.
6. Migliori, G.B.; Thong, P.M.; Akkerman, O.; Alffenaar, J.-W.; Álvarez-Navascués, F.; Assao-Neino, M.M.; Bernard, P.V.; Biala, J.S.; Blanc, F.-X.; Bogorodskaya, E.M. Worldwide effects of coronavirus disease pandemic on tuberculosis services, January–April 2020. *Emerging infectious diseases* **2020**, *26*, 2709.
7. McQuaid, C.F.; McCreesh, N.; Read, J.M.; Sumner, T.; Houben, R.M.; White, R.G.; Harris, R.C.; Group, C.C.-W. The potential impact of COVID-19-related disruption on tuberculosis burden. *European Respiratory Journal* **2020**, *56*.
8. Luies, L.; Du Preez, I.; Loots, D.T. The role of metabolomics in tuberculosis treatment research. *Biomarkers in medicine* **2017**, *11*, 1017-1029.
9. WHO. World Health Organizationon, Consolidated guidelines on drug-resistant tuberculosis treatment. *Geneva* **2019**.
10. Ahmad, N.; Javaid, A.; Sulaiman, S.A.S.; Ming, L.C.; Ahmad, I.; Khan, A.H. Resistance patterns, prevalence, and predictors of fluoroquinolones resistance in multidrug resistant tuberculosis patients. *Brazilian Journal of Infectious Diseases* **2016**, *20*, 41-47.
11. Nguyen, T.V.A.; Anthony, R.M.; Bañuls, A.-L.; Nguyen, T.V.A.; Vu, D.H.; Alffenaar, J.-W.C. Bedaquiline resistance: its emergence, mechanism, and prevention. *Clinical Infectious Diseases* **2018**, *66*, 1625-1630.
12. Polsfuss, S.; Hofmann-Thiel, S.; Merker, M.; Krieger, D.; Niemann, S.; Rüssmann, H.; Schönfeld, N.; Hoffmann, H.; Kranzer, K. Emergence of low-level delamanid and bedaquiline resistance during extremely drug-resistant tuberculosis treatment. *Clinical Infectious Diseases* **2019**, *69*, 1229-1231.

13. Adeniji, A.A.; Knoll, K.E. Potential anti-TB investigational compounds and drugs with repurposing potential in TB therapy: A conspectus. *Applied microbiology and biotechnology* **2020**, *104*, 5633-5662.
14. Brown, D. Antibiotic resistance breakers: can repurposed drugs fill the antibiotic discovery void? *Nature reviews Drug discovery* **2015**, *14*, 821-832.
15. Pranger, A.; Van Der Werf, T.; Kosterink, J.; Alffenaar, J. The role of fluoroquinolones in the treatment of tuberculosis in 2019. *Drugs* **2019**, *79*, 161-171.
16. Domagala, J.M. Structure-activity and structure-side-effect relationships for the quinolone antibacterials. *Journal of Antimicrobial Chemotherapy* **1994**, *33*, 685-706.
17. Berning, S.E. The role of fluoroquinolones in tuberculosis today. *Drugs* **2001**, *61*, 9-18.
18. O'Sullivan, D.M.; Hinds, J.; Butcher, P.D.; Gillespie, S.H.; McHugh, T.D. Mycobacterium tuberculosis DNA repair in response to subinhibitory concentrations of ciprofloxacin. *J Antimicrob Chemother* **2008**, *62*, 1199-1202, doi:10.1093/jac/dkn387.
19. Jabeen, K.; Shakoor, S.; Hasan, R. Fluoroquinolone-resistant tuberculosis: implications in settings with weak healthcare systems. *International Journal of Infectious Diseases* **2015**, *32*, 118-123.
20. Chen, T.-C.; Lu, P.-L.; Lin, C.-Y.; Lin, W.-R.; Chen, Y.-H. Fluoroquinolones are associated with delayed treatment and resistance in tuberculosis: a systematic review and meta-analysis. *International Journal of Infectious Diseases* **2011**, *15*, e211-e216.
21. Raman, K.; Rajagopalan, P.; Chandra, N. Flux balance analysis of mycolic acid pathway: targets for anti-tubercular drugs. *PLoS Comput Biol* **2005**, *1*, e46.
22. Drlica, K.; Zhao, X. DNA gyrase, topoisomerase IV, and the 4-quinolones. *Microbiology and molecular biology reviews* **1997**, *61*, 377-392.
23. Blondeau, J.M. Fluoroquinolones: mechanism of action, classification, and development of resistance. *Surv Ophthalmol* **2004**, *49*, S73-S78.
24. Zawadzki, P.; Stracy, M.; Ginda, K.; Zawadzka, K.; Lesterlin, C.; Kapanidis, A.N.; Sherratt, D.J. The localization and action of topoisomerase IV in Escherichia coli chromosome segregation is coordinated by the SMC complex, MukBEF. *Cell reports* **2015**, *13*, 2587-2596.
25. Walker, R.C. The fluoroquinolones. In Proceedings of the Mayo Clinic Proceedings, 1999; pp. 1030-1037.
26. Collin, F.; Karkare, S.; Maxwell, A. Exploiting bacterial DNA gyrase as a drug target: current state and perspectives. *Applied microbiology and biotechnology* **2011**, *92*, 479-497.
27. Chatterji, M.; Unniraman, S.; Mahadevan, S.; Nagaraja, V. Effect of different classes of inhibitors on DNA gyrase from Mycobacterium smegmatis. *Journal of Antimicrobial Chemotherapy* **2001**, *48*, 479-485.
28. Zhanel, G.G.; Ennis, K.; Vercaigne, L.; Walkty, A.; Gin, A.S.; Embil, J.; Smith, H.; Hoban, D.J. A critical review of the fluoroquinolones. *Drugs* **2002**, *62*, 13-59.
29. Aldred, K.J.; Blower, T.R.; Kerns, R.J.; Berger, J.M.; Osheroff, N. Fluoroquinolone interactions with Mycobacterium tuberculosis gyrase: Enhancing drug activity against wild-type and resistant gyrase. *Proceedings of the National Academy of Sciences* **2016**, *113*, E839-E846.
30. Cheng, G.; Hao, H.; Dai, M.; Liu, Z.; Yuan, Z. Antibacterial action of quinolones: from target to network. *Eur J Med Chem* **2013**, *66*, 555-562, doi:10.1016/j.ejmech.2013.01.057.
31. Bush, N.G.; Diez-Santos, I.; Abbott, L.R.; Maxwell, A. Quinolones: Mechanism, Lethality and Their Contributions to Antibiotic Resistance. *Molecules* **2020**, *25*, doi:10.3390/molecules25235662.
32. Pham, T.D.M.; Ziora, Z.M.; Blaskovich, M.A.T. Quinolone antibiotics. *Medchemcomm* **2019**, *10*, 1719-1739, doi:10.1039/c9md00120d.

33. Jarlier, V.; Nikaido, H. Mycobacterial cell wall: structure and role in natural resistance to antibiotics. *FEMS microbiology letters* **1994**, *123*, 11-18.
34. Arjomandzadegan, M.; Titov, L.; Farnia, P.; Owlia, P.; Ranjbar, R.; Sheikholeslami, F.; Surkova, L. Molecular detection of fluoroquinolone resistance-associated gyrA mutations in ofloxacin-resistant clinical isolates of Mycobacterium tuberculosis from Iran and Belarus. *International journal of mycobacteriology* **2016**, *5*, 299-305.
35. Angala, S.K.; Belardinelli, J.M.; Huc-Claustre, E.; Wheat, W.H.; Jackson, M. The cell envelope glycoconjugates of Mycobacterium tuberculosis. *Critical reviews in biochemistry and molecular biology* **2014**, *49*, 361-399.
36. Fresta, M.; Guccione, S.; Beccari, A.R.; Furneri, P.M.; Puglisi, G. Combining molecular modeling with experimental methodologies: mechanism of membrane permeation and accumulation of ofloxacin. *Bioorganic & medicinal chemistry* **2002**, *10*, 3871-3889.
37. Disratthakit, A.; Prammananan, T.; Tribuddharat, C.; Thaipisuttikul, I.; Doi, N.; Leechawengwongs, M.; Chaiprasert, A. Role of gyrB Mutations in Pre-extensively and Extensively Drug-Resistant Tuberculosis in Thai Clinical Isolates. *Antimicrob Agents Chemother* **2016**, *60*, 5189-5197, doi:10.1128/AAC.00539-16.
38. Blower, T.R.; Williamson, B.H.; Kerns, R.J.; Berger, J.M. Crystal structure and stability of gyrase–fluoroquinolone cleaved complexes from Mycobacterium tuberculosis. *Proceedings of the National Academy of Sciences* **2016**, *113*, 1706-1713.
39. Chen, J.; Chen, Z.; Li, Y.; Xia, W.; Chen, X.; Chen, T.; Zhou, L.; Xu, B.; Xu, S. Characterization of gyrA and gyrB mutations and fluoroquinolone resistance in Mycobacterium tuberculosis clinical isolates from Hubei Province, China. *The Brazilian Journal of Infectious Diseases* **2012**, *16*, 136-141.
40. Aldred, K.J.; Kerns, R.J.; Osheroff, N. Mechanism of quinolone action and resistance. *Biochemistry* **2014**, *53*, 1565-1574.
41. Rossi, E.D.; Aínsa, J.A.; Riccardi, G. Role of mycobacterial efflux transporters in drug resistance: an unresolved question. *FEMS microbiology reviews* **2006**, *30*, 36-52.
42. Radhakrishnan, A.; Kumar, N.; Wright, C.C.; Chou, T.-H.; Tringides, M.L.; Bolla, J.R.; Lei, H.-T.; Rajashankar, K.R.; Su, C.-C.; Purdy, G.E. Crystal structure of the transcriptional regulator Rv0678 of Mycobacterium tuberculosis. *Journal of Biological Chemistry* **2014**, *289*, 16526-16540.
43. Redgrave, L.S.; Sutton, S.B.; Webber, M.A.; Piddock, L.J. Fluoroquinolone resistance: mechanisms, impact on bacteria, and role in evolutionary success. *Trends in microbiology* **2014**, *22*, 438-445.
44. Montero, C.; Mateu, G.; Rodriguez, R.; Takiff, H. Intrinsic resistance of Mycobacterium smegmatis to fluoroquinolones may be influenced by new pentapeptide protein MfpA. *Antimicrobial agents and chemotherapy* **2001**, *45*, 3387-3392.
45. Daisy, P.; Vijayalakshmi, P.; Selvaraj, C.; Singh, S.; Saipriya, K. Targeting multidrug resistant Mycobacterium tuberculosis HtrA2 with identical chemical entities of fluoroquinolones. *Indian journal of pharmaceutical sciences* **2012**, *74*, 217.
46. Palomino, J.C.; Martin, A. Drug resistance mechanisms in Mycobacterium tuberculosis. *Antibiotics* **2014**, *3*, 317-340.
47. Verma, I.; Rohilla, A.; Khuller, G.K. Alterations in macromolecular composition and cell wall integrity by ciprofloxacin in Mycobacterium smegmatis. *Lett App Microbiol* **1999**, *29*, 113-117.
48. Halouska, S.; Fenton, R.J.; Barletta, R.G.; Powers, R. Predicting the in vivo mechanism of action for drug leads using NMR metabolomics. *ACS Chem Biol* **2012**, *7*, 166-171, doi:10.1021/cb200348m.
49. Ginsburg, A.S.; Grosset, J.H.; Bishai, W.R. Fluoroquinolones, tuberculosis, and resistance. *The Lancet Infectious Diseases* **2003**, *3*, 432-442, doi:10.1016/s1473-3099(03)00671-6.
50. Ross, A.G.; Benton, B.M.; Chin, D.; De Pascale, G.; Fuller, J.; Leeds, J.A.; Reck, F.; Richie, D.L.; Vo, J.; LaMarche, M.J. Synthesis of ciprofloxacin dimers for evaluation of bacterial permeability in atypical chemical space. *Bioorg Med Chem Lett* **2015**, *25*, 3468-3475, doi:10.1016/j.bmcl.2015.07.010.
51. Tyagi, P.; Dharmaraja, A.T.; Bhaskar, A.; Chakrapani, H.; Singh, A. Mycobacterium tuberculosis has diminished capacity to counteract redox stress induced by elevated levels of endogenous superoxide. *Free Radic Biol Med* **2015**, *84*, 344-354, doi:10.1016/j.freeradbiomed.2015.03.008.

52. Podlesek, Z.; Zgur Bertok, D. The DNA Damage Inducible SOS Response Is a Key Player in the Generation of Bacterial Persister Cells and Population Wide Tolerance. *Front Microbiol* **2020**, *11*, 1785, doi:10.3389/fmicb.2020.01785.
53. Arbex, M.A.; Varella, M.d.C.L.; Siqueira, H.R.d.; Mello, F.A.F.d. Antituberculosis drugs: drug interactions, adverse effects, and use in special situations-part 2: second line drugs. *Jornal Brasileiro de Pneumologia* **2010**, *36*, 641-656.
54. Gorelik, E.; Masarwa, R.; Perlman, A.; Rotshild, V.; Abbasi, M.; Muszkat, M.; Matok, I. Fluoroquinolones and cardiovascular risk: a systematic review, meta-analysis and network meta-analysis. *Drug safety* **2019**, *42*, 529-538.
55. Schloss, M.; Becak, D.; Tosto, S.T.; Velayati, A. A case of Levofloxacin-Induced hepatotoxicity. *The American journal of case reports* **2018**, *19*, 272.
56. Aminimanizani, A.; Beringer, P.; Jelliffe, R. Comparative pharmacokinetics and pharmacodynamics of the newer fluoroquinolone antibacterials. *Clinical pharmacokinetics* **2001**, *40*, 169-187.
57. Xu, Z.; Zhao, S.J.; Deng, J.L.; Wang, Q.; Lv, Z.S. Ciprofloxacin–Isatin Hybrids and Their Antimycobacterial Activities. *Journal of Heterocyclic Chemistry* **2019**, *56*, 319-324.
58. Ball, P. Ciprofloxacin: an overview of adverse experiences. *Journal of antimicrobial chemotherapy* **1986**, *18*, 187-193.
59. Barnhill, A.E.; Brewer, M.T.; Carlson, S.A. Adverse effects of antimicrobials via predictable or idiosyncratic inhibition of host mitochondrial components. *Antimicrobial agents and chemotherapy* **2012**, *56*, 4046-4051.
60. Hangas, A.; Aasumets, K.; Kekäläinen, N.J.; Paloheinä, M.; Pohjoismäki, J.L.; Gerhold, J.M.; Goffart, S. Ciprofloxacin impairs mitochondrial DNA replication initiation through inhibition of Topoisomerase 2. *Nucleic acids research* **2018**, *46*, 9625-9636.
61. WHO. World Health Organization, Critically important antimicrobials for human medicine, 6th revision. *Geneva* **2019**, https://creativecommons.org/licenses/by-nc-sa/3.0/igo.
62. Correia, S.; Poeta, P.; Hébraud, M.; Capelo, J.L.; Igrejas, G. Mechanisms of quinolone action and resistance: where do we stand? *Journal of medical microbiology* **2017**, *66*, 551-559.
63. Lau, S.K.; Lam, C.-W.; Curreem, S.O.; Lee, K.-C.; Lau, C.C.; Chow, W.-N.; Ngan, A.H.; To, K.K.; Chan, J.F.; Hung, I.F. Identification of specific metabolites in culture supernatant of Mycobacterium tuberculosis using metabolomics: exploration of potential biomarkers. *Emerging microbes & infections* **2015**, *4*, 1-10.
64. Franzblau, S.G.; Witzig, R.S.; McLaughlin, J.C.; Torres, P.; Madico, G.; Hernandez, A.; Degnan, M.T.; Cook, M.B.; Quenzer, V.K.; Ferguson, R.M. Rapid, low-technology MIC determination with clinical Mycobacterium tuberculosis isolates by using the microplate Alamar Blue assay. *Journal of clinical microbiology* **1998**, *36*, 362-366.
65. Van Breda, S.V.; Buys, A.; Apostolides, Z.; Nardell, E.A.; Stoltz, A.C. The antimicrobial effect of colistin methanesulfonate on Mycobacterium tuberculosis in vitro. *Tuberculosis* **2015**, *95*, 440-446.
66. Palomino, J.; Portaels, F. Effects of decontamination methods and culture conditions on viability of Mycobacterium ulcerans in the BACTEC system. *Journal of clinical microbiology* **1998**, *36*, 402-408.
67. Beukes, D.; du Preez, I.; Loots, D.T. Total Metabolome Extraction from Mycobacterial Cells for GC-MS Metabolomics Analysis. *Methods Mol Biol* **2019**, *1859*, 121-131, doi:10.1007/978-1-4939-8757-3_6.
68. Gromski, P.S.; Xu, Y.; Kotze, H.L.; Correa, E.; Ellis, D.I.; Armitage, E.G.; Turner, M.L.; Goodacre, R. Influence of missing values substitutes on multivariate analysis of metabolomics data. *Metabolites* **2014**, *4*, 433-452.
69. Wei, R.; Wang, J.; Su, M.; Jia, E.; Chen, S.; Chen, T.; Ni, Y. Missing value imputation approach for mass spectrometry-based metabolomics data. *Scientific reports* **2018**, *8*, 1-10.
70. Shen, X.; Zhu, Z.-J. MetFlow: an interactive and integrated workflow for metabolomics data cleaning and differential metabolite discovery. *Bioinformatics* **2019**, *35*, 2870-2872.
71. Xia, J.; Wishart, D.S. Web-based inference of biological patterns, functions and pathways from metabolomic data using MetaboAnalyst. *Nature protocols* **2011**, *6*, 743-760.

72. Meissner-Roloff, R.J.; Koekemoer, G.; Warren, R.M. A metabolomics investigation of a hyper-and hypo-virulent phenotype of Beijing lineage M. tuberculosis. *Metabolomics* **2012**, *8*, 1194-1203.
73. Sullivan, G.M.; Feinn, R. Using effect size—or why the P value is not enough. *Journal of graduate medical education* **2012**, *4*, 279.
74. Cho, H.-W.; Kim, S.B.; Jeong, M.K.; Park, Y.; Miller, N.; Ziegler, T.; Jones, D. Discovery of metabolite features for the modelling and analysis of high-resolution NMR spectra. *International journal of data mining and bioinformatics* **2008**, *2*, 176-192.
75. Byliński, H.; Dymerski, T.; Gębicki, J.; Namieśnik, J. Complementary use of GCxGC–TOF–MS and statistics for differentiation of variety in biosolid samples. *Monatshefte für Chemie-Chemical Monthly* **2018**, *149*, 1587-1594.
76. Coe, R. It's the effect size, stupid: What effect size is and why it is important. **2002**.
77. Vijay, S.; Vinh, D.N.; Hai, H.T.; Ha, V.T.N.; Dung, V.T.M.; Dinh, T.D.; Nhung, H.N.; Tram, T.T.B.; Aldridge, B.B.; Hanh, N.T.; et al. Influence of Stress and Antibiotic Resistance on Cell-Length Distribution in Mycobacterium tuberculosis Clinical Isolates. *Front Microbiol* **2017**, *8*, 2296, doi:10.3389/fmicb.2017.02296.
78. Chatterjee, D.; Bozic, C.M.; McNeil, M.; Brennan, P.J. Structural features of the arabinan component of the lipoarabinomannan of Mycobacterium tuberculosis. *Journal of Biological Chemistry* **1991**, *266*, 9652-9660, doi:10.1016/s0021-9258(18)92870-x.
79. Jankute, M.; Cox, J.A.; Harrison, J.; Besra, G.S. Assembly of the Mycobacterial Cell Wall. *Annu Rev Microbiol* **2015**, *69*, 405-423, doi:10.1146/annurev-micro-091014-104121.
80. Chiaradia, L.; Lefebvre, C.; Parra, J.; Marcoux, J.; Burlet-Schiltz, O.; Etienne, G.; Tropis, M.; Daffé, M. Dissecting the mycobacterial cell envelope and defining the composition of the native mycomembrane. *Scientific reports* **2017**, *7*, 1-12.
81. Abrahams, K.A.; Besra, G.S. Mycobacterial cell wall biosynthesis: a multifaceted antibiotic target. *Parasitology* **2018**, *145*, 116-133, doi:10.1017/S0031182016002377.
82. Black, P.N.; DiRusso, C.C. Transmembrane movement of exogenous long-chain fatty acids: proteins, enzymes, and vectorial esterification. *Microbiology and Molecular Biology Reviews* **2003**, *67*, 454-472.
83. Santangelo, M.P.; Heuberger, A.; Blanco, F.; Forrellad, M.; Taibo, C.; Klepp, L.; García, J.S.; Nikel, P.I.; Jackson, M.; Bigi, F. Metabolic profile of Mycobacterium smegmatis reveals Mce4 proteins are relevant for cell wall lipid homeostasis. *Metabolomics* **2016**, *12*, 97.
84. Nigou, J.; Gilleron, M.; Puzo, G. Lipoarabinomannans: from structure to biosynthesis. *Biochimie* **2003**, *85*, 153-166.
85. Škovierová, H.; Larrouy-Maumus, G.; Zhang, J.; Kaur, D.; Barilone, N.; Korduláková, J.; Gilleron, M.; Guadagnini, S.; Belanová, M.; Prevost, M.-C. AftD, a novel essential arabinofuranosyltransferase from mycobacteria. *Glycobiology* **2009**, *19*, 1235-1247.
86. Vilchèze, C.; Jacobs, W.R. Isolation and analysis of Mycobacterium tuberculosis mycolic acids. *Current protocols in microbiology* **2007**, *5*, 10A. 13.11-10A. 12.11.
87. Fulco, A.J.; Bloch, K. Cofactor requirements for the formation of Δ9-unsaturated fatty acids in Mycobacterium phlei. *Journal of Biological Chemistry* **1964**, *239*, 993-997.
88. Fulco, A.J.; Levy, R.; Bloch, K. The Biosynthesis of Δ9-and Δ5-Monosaturated Fatty Acids by Bacteria. *Journal of Biological Chemistry* **1964**, *239*, 998-1003.
89. Delmar, J.A.; Chou, T.H.; Wright, C.C.; Licon, M.H.; Doh, J.K.; Radhakrishnan, A.; Kumar, N.; Lei, H.T.; Bolla, J.R.; Rajashankar, K.R.; et al. Structural Basis for the Regulation of the MmpL Transporters of Mycobacterium tuberculosis. *J Biol Chem* **2015**, *290*, 28559-28574, doi:10.1074/jbc.M115.683797.
90. Melly, G.; Purdy, G.E. MmpL Proteins in Physiology and Pathogenesis of M. tuberculosis. *Microorganisms* **2019**, *7*, 70.

91. Rhee, K.Y.; de Carvalho, L.P.; Bryk, R.; Ehrt, S.; Marrero, J.; Park, S.W.; Schnappinger, D.; Venugopal, A.; Nathan, C. Central carbon metabolism in Mycobacterium tuberculosis: an unexpected frontier. *Trends Microbiol* **2011**, *19*, 307-314, doi:10.1016/j.tim.2011.03.008.
92. Bothra, A.; Arumugam, P.; Panchal, V.; Menon, D.; Srivastava, S.; Shankaran, D.; Nandy, A.; Jaisinghani, N.; Singh, A.; Gokhale, R.S.; et al. Phospholipid homeostasis, membrane tenacity and survival of Mtb in lipid rich conditions is determined by MmpL11 function. *Sci Rep* **2018**, *8*, 8317, doi:10.1038/s41598-018-26710-z.
93. Zerbini, E.; Cardoso, M.; Sequeira, M.; Taher, H.; Santi, N.; Larpin, D.; Latini, O.; Tonarelli, G. Characterization of fatty acids and mycolic acid degradation products in mycobacterial species of major incidence in Argentina. *Revista Argentina de microbiologia* **1997**, *29*, 184-194.
94. Crellin, P.K.; Brammananth, R.; Coppel, R.L. Decaprenylphosphoryl-beta-D-ribose 2'-epimerase, the target of benzothiazinones and dinitrobenzamides, is an essential enzyme in Mycobacterium smegmatis. *PLoS One* **2011**, *6*, e16869, doi:10.1371/journal.pone.0016869.
95. Kumar, R.; Sanyal, S. Mycobacterium tuberculosis: Dormancy, Persistence and Survival in the Light of Protein Synthesis. In *Understanding Tuberculosis - Deciphering the Secret Life of the Bacilli*; 2012.
96. Kumar, A.; Farhana, A.; Guidry, L.; Saini, V.; Hondalus, M.; Steyn, A.J. Redox homeostasis in mycobacteria: the key to tuberculosis control? *Expert Rev Mol Med* **2011**, *13*, e39, doi:10.1017/S1462399411002079.
97. Erental, A.; Kalderon, Z.; Saada, A.; Smith, Y.; Engelberg-Kulka, H. Apoptosis-like death, an extreme SOS response in Escherichia coli. *mBio* **2014**, *5*, e01426-01414, doi:10.1128/mBio.01426-14.
98. Horecker, B.L. The pentose phosphate pathway. *J Biol Chem* **2002**, *277*, 47965-47971, doi:10.1074/jbc.X200007200.
99. Lucarelli, A.P.; Buroni, S.; Pasca, M.R.; Rizzi, M.; Cavagnino, A.; Valentini, G.; Riccardi, G.; Chiarelli, L.R. Mycobacterium tuberculosis phosphoribosylpyrophosphate synthetase: biochemical features of a crucial enzyme for mycobacterial cell wall biosynthesis. *PLoS One* **2010**, *5*, e15494, doi:10.1371/journal.pone.0015494.
100. Kampranis, S.C.; Maxwell, A. The DNA gyrase-quinolone complex. ATP hydrolysis and the mechanism of DNA cleavage. *J Biol Chem* **1998**, *273*, 22615-22626, doi:10.1074/jbc.273.35.22615.
101. Villela, A.D.; Sanchez-Quitian, Z.A.; Ducati, R.G.; Santos, D.S.; Basso, L.A. Pyrimidine salvage pathway in Mycobacterium tuberculosis. *Curr Med Chem* **2011**, *18*, 1286-1298, doi:10.2174/092986711795029555.
102. Maitra, A.; Munshi, T.; Healy, J.; Martin, L.T.; Vollmer, W.; Keep, N.H.; Bhakta, S. Cell wall peptidoglycan in Mycobacterium tuberculosis: An Achilles' heel for the TB-causing pathogen. *FEMS Microbiol Rev* **2019**, *43*, 548-575, doi:10.1093/femsre/fuz016.
103. Miggiano, R.; Morrone, C.; Rossi, F.; Rizzi, M. Targeting Genome Integrity in Mycobacterium Tuberculosis: From Nucleotide Synthesis to DNA Replication and Repair. *Molecules* **2020**, *25*, doi:10.3390/molecules25051205.
104. Haites, R.E.; Morita, Y.S.; McConville, M.J.; Billman-Jacobe, H. Function of phosphatidylinositol in mycobacteria. *Journal of Biological Chemistry* **2005**, *280*, 10981-10987.
105. Heijenoort, J.v. Formation of the glycan chains in the synthesis of bacterial peptidoglycan. *Glycobiology* **2001**, *11*, 25R-36R.
106. Titgemeyer, F.; Amon, J.; Parche, S.; Mahfoud, M.; Bail, J.; Schlicht, M.; Rehm, N.; Hillmann, D.; Stephan, J.; Walter, B.; et al. A genomic view of sugar transport in Mycobacterium smegmatis and Mycobacterium tuberculosis. *J Bacteriol* **2007**, *189*, 5903-5915, doi:10.1128/JB.00257-07.
107. Alderwick, L.J.; Lloyd, G.S.; Lloyd, A.J.; Lovering, A.L.; Eggeling, L.; Besra, G.S. Biochemical characterization of the Mycobacterium tuberculosis phosphoribosyl-1-pyrophosphate synthetase. *Glycobiology* **2011**, *21*, 410-425, doi:10.1093/glycob/cwq173.
108. Klutts, J.S.; Hatanaka, K.; Pan, Y.T.; Elbein, A.D. Biosynthesis of d-arabinose in Mycobacterium smegmatis: specific labeling from d-glucose. *Arch Biochem Biophys* **2002**, *398*, 229-239, doi:10.1006/abbi.2001.2723.

109. Sheppard, K.; Yuan, J.; Hohn, M.J.; Jester, B.; Devine, K.M.; Soll, D. From one amino acid to another: tRNA-dependent amino acid biosynthesis. *Nucleic Acids Res* **2008**, *36*, 1813-1825, doi:10.1093/nar/gkn015.

110. Kudo, F.; Miyanaga, A.; Eguchi, T. Biosynthesis of natural products containing beta-amino acids. *Nat Prod Rep* **2014**, *31*, 1056-1073, doi:10.1039/c4np00007b.

111. Rieck, B.; Degiacomi, G.; Zimmermann, M.; Cascioferro, A.; Boldrin, F.; Lazar-Adler, N.R.; Bottrill, A.R.; le Chevalier, F.; Frigui, W.; Bellinzoni, M.; et al. PknG senses amino acid availability to control metabolism and virulence of Mycobacterium tuberculosis. *PLoS Pathog* **2017**, *13*, e1006399, doi:10.1371/journal.ppat.1006399.

112. Hasenoehrl, E.J.; Rae Sajorda, D.; Berney-Meyer, L.; Johnson, S.; Tufariello, J.M.; Fuhrer, T.; Cook, G.M.; Jacobs, W.R., Jr.; Berney, M. Derailing the aspartate pathway of Mycobacterium tuberculosis to eradicate persistent infection. *Nat Commun* **2019**, *10*, 4215, doi:10.1038/s41467-019-12224-3.

113. Rizvi, A.; Yousf, S.; Balakrishnan, K.; Dubey, H.K.; Mande, S.C.; Chugh, J.; Banerjee, S. Metabolomics Studies To Decipher Stress Responses in Mycobacterium smegmatis Point to a Putative Pathway of Methylated Amine Biosynthesis. *J Bacteriol* **2019**, *201*, doi:10.1128/JB.00707-18.

114. Genschel, U. Coenzyme A biosynthesis: reconstruction of the pathway in archaea and an evolutionary scenario based on comparative genomics. *Molecular biology and evolution* **2004**, *21*, 1242-1251.

115. Leonardi, R.; Jackowski, S. Biosynthesis of pantothenic acid and coenzyme A. *EcoSal Plus* **2007**, *2*.

116. Daniel, J.; Sirakova, T.; Kolattukudy, P. An acyl-CoA synthetase in Mycobacterium tuberculosis involved in triacylglycerol accumulation during dormancy. *PLoS One* **2014**, *9*, e114877, doi:10.1371/journal.pone.0114877.

117. Massey, L.K.; Sokatch, J.R.; Conrad, R.S. Branched-chain amino acid catabolism in bacteria. *Bacteriological reviews* **1976**, *40*, 42.

118. Surger, M.J.; Angelov, A.; Stier, P.; Ubelacker, M.; Liebl, W. Impact of Branched-Chain Amino Acid Catabolism on Fatty Acid and Alkene Biosynthesis in Micrococcus luteus. *Front Microbiol* **2018**, *9*, 374, doi:10.3389/fmicb.2018.00374.

119. Zhao, H.; Roistacher, D.M.; Helmann, J.D. Aspartate deficiency limits peptidoglycan synthesis and sensitizes cells to antibiotics targeting cell wall synthesis in Bacillus subtilis. *Mol Microbiol* **2018**, *109*, 826-844, doi:10.1111/mmi.14078.

120. Rodionova, I.A.; Schuster, B.M.; Guinn, K.M.; Sorci, L.; Scott, D.A.; Li, X.; Kheterpal, I.; Shoen, C.; Cynamon, M.; Locher, C. Metabolic and bactericidal effects of targeted suppression of NadD and NadE enzymes in mycobacteria. *MBio* **2014**, *5*.

121. Ramakrishnan, T.; Murthy, P.S.; Gobinathan, K.P. Intermediary Metabolism of Mycobacteria. *Bacteriol Rev* **1972**, *36*, 65-108.

122. Heath, R.J.; Rubin, J.R.; Holland, D.R.; Zhang, E.; Snow, M.E.; Rock, C.O. Mechanism of triclosan inhibition of bacterial fatty acid synthesis. *Journal of Biological Chemistry* **1999**, *274*, 11110-11114.

123. Martin, J.L.; McMillan, F.M. SAM (dependent) I AM: the S-adenosylmethionine-dependent methyltransferase fold. *Current opinion in structural biology* **2002**, *12*, 783-793.

124. Crick, D.C.; Mahapatra, S.; Brennan, P.J. Biosynthesis of the arabinogalactan-peptidoglycan complex of Mycobacterium tuberculosis. *Glycobiology* **2001**, *11*, 107R-118R, doi:10.1093/glycob/11.9.107r.

125. Agapova, A.; Serafini, A.; Petridis, M.; Hunt, D.M.; Garza-Garcia, A.; Sohaskey, C.D.; de Carvalho, L.P.S. Flexible nitrogen utilisation by the metabolic generalist pathogen Mycobacterium tuberculosis. *Elife* **2019**, *8*, doi:10.7554/eLife.41129.

126. Iacobino, A.; Piccaro, G.; Pardini, M.; Fattorini, L.; Giannoni, F. Moxifloxacin Activates the SOS Response in Mycobacterium tuberculosis in a Dose- and Time-Dependent Manner. *Microorganisms* **2021**, *9*, doi:10.3390/microorganisms9020255.

127. Petridis, M.; Vickers, C.; Robson, J.; McKenzie, J.L.; Bereza, M.; Sharrock, A.; Aung, H.L.; Arcus, V.L.; Cook, G.M. Structure and Function of AmtR in Mycobacterium smegmatis: Implications for Post-Transcriptional Regulation of Urea Metabolism through a Small Antisense RNA. *J Mol Biol* **2016**, *428*, 4315-4329, doi:10.1016/j.jmb.2016.09.009.

128. Gouzy, A.; Poquet, Y.; Neyrolles, O. Nitrogen metabolism in Mycobacterium tuberculosis physiology and virulence. *Nat Rev Microbiol* **2014**, *12*, 729-737, doi:10.1038/nrmicro3349.

129. Stallings, C.L.; Stephanou, N.C.; Chu, L.; Hochschild, A.; Nickels, B.E.; Glickman, M.S. CarD is an essential regulator of rRNA transcription required for Mycobacterium tuberculosis persistence. *Cell* **2009**, *138*, 146-159.

130. Yancey, P.H. Water Stress, Osmolytes and Proteins. *Amer. Zool.* **2001**, *41*, 699-709.

131. Majumdar, R.; Barchi, B.; Turlapati, S.A.; Gagne, M.; Minocha, R.; Long, S.; Minocha, S.C. Glutamate, Ornithine, Arginine, Proline, and Polyamine Metabolic Interactions: The Pathway Is Regulated at the Post-Transcriptional Level. *Front Plant Sci* **2016**, *7*, 78, doi:10.3389/fpls.2016.00078.

132. Jain, A.; Tyagi, A.K. Role of polyamines in the synthesis of RNA in mycobacteria. *Mol Cell Biochem* **1987**, *78*, 3-8.

133. Sarathy, J.P.; Lee, E.; Dartois, V. Polyamines inhibit porin-mediated fluoroquinolone uptake in mycobacteria. *PLoS One* **2013**, *8*, e65806, doi:10.1371/journal.pone.0065806.

134. Ortalo-Magne, A.; Lemassu, A.; Laneelle, M.-A.; Bardou, F.; Silve, G.; Gounon, P.; Marchal, G.; Daffé, M. Identification of the surface-exposed lipids on the cell envelopes of Mycobacterium tuberculosis and other mycobacterial species. *Journal of bacteriology* **1996**, *178*, 456-461.

135. Singh, A.; Crossman, D.K.; Mai, D.; Guidry, L.; Voskuil, M.I.; Renfrow, M.B.; Steyn, A.J. Mycobacterium tuberculosis WhiB3 maintains redox homeostasis by regulating virulence lipid anabolism to modulate macrophage response. *PLoS Pathog* **2009**, *5*, e1000545, doi:10.1371/journal.ppat.1000545.

136. Muñoz-Elías, E.J.; Upton, A.M.; Cherian, J.; McKinney, J.D. Role of the methylcitrate cycle in Mycobacterium tuberculosis metabolism, intracellular growth, and virulence. *Molecular microbiology* **2006**, *60*, 1109-1122.

137. Daniel, J.; Deb, C.; Dubey, V.S.; Sirakova, T.D.; Abomoelak, B.; Morbidoni, H.R.; Kolattukudy, P.E. Induction of a novel class of diacylglycerol acyltransferases and triacylglycerol accumulation in Mycobacterium tuberculosis as it goes into a dormancy-like state in culture. *Journal of bacteriology* **2004**, *186*, 5017-5030.

138. Crellin, P.K.; Luo, C.-Y.; Morita, Y.S. Metabolism of Plasma Membrane Lipids in Mycobacteria and Corynebacteria. In *Lipid Metabolism*; 2013.

139. Deb, C.; Daniel, J.; Sirakova, T.D.; Abomoelak, B.; Dubey, V.S.; Kolattukudy, P.E. A novel lipase belonging to the hormone-sensitive lipase family induced under starvation to utilize stored triacylglycerol in Mycobacterium tuberculosis. *Journal of Biological Chemistry* **2006**, *281*, 3866-3875.

140. Barksdale, L.; Kim, K.-S. Mycobacterium. *Bacteriological reviews* **1977**, *41*, 217.

141. Athenstaedt, K.; Daum, G. Phosphatidic acid, a key intermediate in lipid metabolism. *Eur J Biochem* **1999**, *266*, 1-16.

142. Rottig, A.; Steinbuchel, A. Acyltransferases in bacteria. *Microbiol Mol Biol Rev* **2013**, *77*, 277-321, doi:10.1128/MMBR.00010-13.

143. Aschauer, P.; Zimmermann, R.; Breinbauer, R.; Pavkov-Keller, T.; Oberer, M. The crystal structure of monoacylglycerol lipase from M. tuberculosis reveals the basis for specific inhibition. *Sci Rep* **2018**, *8*, 8948, doi:10.1038/s41598-018-27051-7.

144. Clarke, O.B.; Tomasek, D.; Jorge, C.D.; Dufrisne, M.B.; Kim, M.; Banerjee, S.; Rajashankar, K.R.; Shapiro, L.; Hendrickson, W.A.; Santos, H.; et al. Structural basis for phosphatidylinositol-phosphate biosynthesis. *Nat Commun* **2015**, *6*, 8505, doi:10.1038/ncomms9505.

145. Salman, M.; Lonsdale, J.T.; Besra, G.S.; Brenna, P.J. Phosphatidylinositol synthesis in mycobacteria. *Biochimica et Biophysica Acta* **1999**, *1436*, 437-450.

146. Newton, G.L.; Ta, P.; Bzymek, K.P.; Fahey, R.C. Biochemistry of the initial steps of mycothiol biosynthesis. *J Biol Chem* **2006**, *281*, 33910-33920, doi:10.1074/jbc.M604724200.

147. Qin, T.T.; Kang, H.Q.; Ma, P.; Li, P.P.; Huang, L.Y.; Gu, B. SOS response and its regulation on the fluoroquinolone resistance. *Ann Transl Med* **2015**, *3*, 358, doi:10.3978/j.issn.2305-5839.2015.12.09.

148. Tian, J.; Bryk, R.J.; Itoh, M.; Suematsu, M.; Nathan, C. Variant tricarboxylic acid cycle in Mycobacterium tuberculosis: Identification of a-ketoglutarate decarboxylase. *PNAS* **2005**, *102*, 10670–10675, doi:10.1073pnas.0501605102.

 doi:10.20944/preprints202104.0443.v1

149. Torres-Barceló, C.; Kojadinovic, M.; Moxon, R.; MacLean, R.C. The SOS response increases bacterial fitness, but not evolvability, under a sublethal dose of antibiotic. *Proceedings of the Royal Society B: Biological Sciences* **2015**, *282*, 20150885.