In Vitro Activity of Tetracycline Analogs against Multidrug-resistant and Extensive drug resistance Clinical Isolates of *Mycobacterium tuberculosis*

Qi Ouyang

Shenzhen University

Dachuan Lin

Shenzhen University

Guofang Deng

The Third People's Hospital of Shenzhen

Zhihua Wen

Yuebei Second People's Hospital

Houming Liu

The Third People's Hospital of Shenzhen

Hongzhen Li

Yuebei Second People's Hospital

Yi Cai

Shenzhen University

Xinchun Chen (✉ chenxinchun@szu.edu.cn)

Shenzhen University <https://orcid.org/0000-0002-2101-9013>

Research

Keywords: Tetracycline, Multiple-drug resistance, Doxycycline, *Mycobacterium tuberculosis*

Posted Date: November 27th, 2019

DOI: <https://doi.org/10.21203/rs.2.17754/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Background Multidrug-resistant tuberculosis (MDR-TB) has become a big threaten to global health . The current strategy for treatment of MDR-TB and extensive drug resistant tuberculosis (XDR-TB) is with low efficacy and high side effect. While new drug is fundamental for cure MDR-TB, repurposing the Food and Drug Administration (FDA)-approved drugs represents an alternative soluation with less cost.

Methods The activity of 8 tetracycline-class antibiotics against mycobacterium tuberculosis (*M.tb*) were determined by Minimum Inhibitory Concentration (MIC) in vitro. A transposon *M.smeg* libraries was generated by using the Harm phage and then used to isolate the conditional growth mutants in doxycycline containing plate. 11 mutants were isolated and genomic DNAs were extracted using the cetyltrimethyl ammonium bromide (CTAB) method and analyzed by whole genome sequencing.

Results We found that three of eight drugs efficiently inhibited mycobacteria growth under the peak plasma concentration in the human body. Further tests showed these three tetracycline analogs (demeclocycline, doxycycline and methacycline) had antimicrobial activity against seven clinical isolates, including MDR and XDR strains. Among them, Doxycycline had the lowest MICs in all mycobacteria strains tested in this study. By using a transposon library, we identify the insertion of transposon in two genes, porin and MshA, associate with the resistant to doxycycline.

Conclusions Our findings show that tetracycline analogs such as doxycycline, has bactericidal activity against not only drug sensitive *M.tb* , but also clinical MDR and XDR strains, provided proof of concept to repurpose doxycycline to fight MDR-TB and XDR-TB. Further investigations are warranted to clarify the underlying mechanism and optimize the strategy in combination with other anti-TB drugs.

Introduction

Tuberculosis (TB), a chronic infectious diseases caused by *Mycobacterium tuberculosis* (*M.tb*), remains a serious health problem worldwide. Millions of people continue to fall sick with TB each year. In 2018, TB caused about 1.5million deaths[1]. According to WHO, an estimated 500,000 people worldwide developed MDR-TB in 2017, and an additional 186,772 people contracted rifampicin-resistant TB. It is estimated that about 6.2% of these cases were XDR-TB[1]. This is the real challenge in the management and [treatment of TB](#), especially MDR-TB and XDR-TB. The treatment of drug susceptible TB requires multiple antibiotics to be taken daily at least 6 months, while the treatment of MDR- and XDR-TB entails second line drugs that are usually toxic for a longer period[2, 3]. Patients often fail to complete their treatment or do not adhere strictly to the regimen, due to the length of the treatment and the toxicity of second line drugs[2, 3]. Therefore, new drugs with novel mechanisms of action or repurposing the Food and Drug Administration (FDA)-approved drugs are urgently needed to shorten the treatment period and efficiency cure MDR/XDR-TB.

Tetracycline antibiotic was first reported in 1948 and found widespread clinical use shortly thereafter [4–8]. Tetracyclines were known as broad-spectrum antibacterial agents which have a good inhibitory effect

on gram-positive and gram-negative bacteria, rickettsia, filtered virus, spirochetes and even protozoa [9, 10]. Tetracyclines inhibit bacterial protein synthesis by binding to the 16S rRNA of the 30S bacterial ribosome subunit, preventing accommodation of incoming aminoacyl-tRNAs at the acceptor site [11, 12]. Both synthetic and semisynthetic tetracycline analogs have been shown effective against wide ranges of pathogens and different inflammatory diseases and conditions [10], which are the most prescribed oral antibiotics, with a long satisfactory track record of efficacy, low price and safety [13]. Previous studies have showed that doxycycline, a second-generation tetracycline, could efficacy reduce *M.tb* growth both in macrophage and guinea pig TB model via inhibiting host MMPs activity [14]. Doxycycline has been used in vitro against *M.tuberculosis*[15], and combination with amikacin significantly inhibited 18 of the 29 MDR and XDR TB strains replications [16]. Some of the older members of the tetracycline family also showed activity against *M. tuberculosis* strains in animal models[17].

However, very few studies have been carried out to assess all three generation of tetracyclines activity in mycobacteria. Furthermore, the underlying mechanism of doxycycline direct killing activity is still unclear. Here, we investigated the potential anti-mycobacterium activity of several tetracyclines including demeclocycline, tetracycline, chlortetracycline, tigecycline, oxytetracycline, methacycline, tetracycline and doxycycline in vitro. We further identified two mutants which were resistant to doxycycline by using a transposon *M.smeg* library.

Materials And Methods

Bacterial Strains and Culture Conditions

M.smegmatis mc² 155 (*M.smeg*), *M.tb* strains H37Ra, H37Rv, and seven clinical isolates: ER17, ER22, 400–13, 400–86, 400–89, 400–123, 200–28 were used in this study. The bacteria were grown in 7H9 Middlebrook broth supplemented with 10% oleic acid–albumin–dextrose–catalase (OADC, BD), 0.05% Tween 80, and 0.2% glycerol. The culture was incubated at 37°C, with shaking for 4 to 5 days to achieve midlogarithmic phase (OD₆₀₀ = 0.3–0.8).

Drugs

Demeclocycline (hydrochloride), Tetracycline, Chlortetracycline (hydrochloride), Tigecycline, Oxytetracycline, Methacycline (hydrochloride), Tetracycline (hydrochloride) and Doxycycline (hyclate) were purchased from Med Chem Express (MCE, USA). Drugs were diluted and stored according to the manufacturer's instructions.

MIC determination

The anti-mycobacterium activity was tested using *M.smeg*, H37Ra, H37Rv and 7 clinical isolates. The bacterial culture was diluted with 7H9-OADC to an OD = 0.01 and transferred to a 96-well microtiter plate

for the drug sensitivity assay. Serial dilutions of tetracyclines were dispensed into 96-well microtiter plates containing bacteria strains. The 96-well microtiter plates were incubated at 37°C with slowly horizontal shaking. The OD600 was measured in a microplate spectrophotometer (BioTek) in indicated times and the growth rate was calculated. The MIC is defined as the lowest concentration of the antimicrobial agent that prevents visible growth of a microorganism under the compound-containing plates [18].

Whole Genome Sequencing of Transposon Mutants

The transposon *M.smeg* library was constructed with donor phagemid ϕ MycoMarT7 which includes a transposon that encodes a kanamycin resistance gene[19]. The entire sequence of the ϕ MycoMarT7 transposon has been deposited in GenBank (GenBank accession no. AF411123). The transposon library were collected from plates and frozen at -80°C. To find strains resistant to doxycycline among the transposon mutants, the transposon *M.smeg* library were grown to OD600 of 0.8–1.0 in liquid culture. Resistant mutants were isolated by plating 10^8 to 10^9 colony forming units (CFU) of transposon *M.smeg* library [20] on 7H10-OADC doxycycline plates containing $1\times$ MICs. Surviving colonies were collected by scraping colonies off plates and restreaked on 7H10-OADC containing Kanamycin and $1\times$ MIC of doxycycline. Genomic DNAs were extracted using the cetyltrimethyl ammonium bromide (CTAB) method[21]. The extracted DNAs from surviving strains were further analyzed by whole genome sequencing (Genewiz, China).

Statistical analysis

Statistical analysis was performed using GraphPad Prism 7 software. One-way ANOVA was used to assess the effects between more than two groups. Student's t-test was used to assess the effects of only one parameter.

Results

Tetracycline analogs showed bactericidal activity against mycobacteria in vitro

8 tetracycline analogs were selected to investigate the antimicrobial effect against Mycobacterium. We first test the effect of these analogs on *M.smeg* and results showed that seven of the tested tetracycline analogs significantly inhibited *M.smeg* growth (Fig 1 A). Previously studies have showed that tetracycline [22] and tigecycline[23] could significantly inhibited mycobacterial strains growth, which is consistent with our results. We further determined the MIC of four tetracyclines, demeclocycline, chlortetracycline, methacycline and doxycycline. We found that the MICs of *M.smeg* in 7H9 broth were 0.067 μ g/mL, 0.34 μ g/mL, 0.051 μ g/mL and 0.064 μ g/mL for demeclocycline, chlortetracycline, doxycycline and methacycline (Fig 1 B and Table 2), respectively. The MICs of the *M.tb* strain H37Ra were 1.25 μ g/mL,

5.15 µg/mL, 1.03 µg/mL, 2.39µg/mL for demeclocycline, chlortetracycline, doxycycline and methacycline (Fig 1 C and Table 2), respectively. We noted that the MICs of those tetracyclines in *M.tb* strains were about 20-fold higher than that in *M.smeg*. The results above demonstrated that some of tetracycline analogs could significantly inhibited mycobacteria growth in vitro.

Activity of Tetracyclines against drug-resistant clinical isolates

The MICs of demeclocycline, doxycycline and methacycline are lower than that in the peak plasma concentration [24, 25]. We next assessed the activities of these tetracyclines in clinical isolates.

All the clinical isolates were collected from Shenzhen third people's hospital, China. The isolates including 2 ethambutol resistance strains (ER17, ER22), 1 MDR strain (400–89) and 4 XDR strains (400–13,400–86,400–123,200–28). The details of those clinical isolates were showed in Table3.

Three analogs shown anti-Mycobacterium activity for most of the isolates (Table3). The MIC result of three drugs was from 1.28 to 5.13 µg/mL for doxycycline, from 0.25 to 25.06 µg/mL for demeclocycline and 0.59 to 23.9 µg/mL for methacycline (Fig 2 and Table 3). Among these Tetracycline analogs, doxycycline had the lowest MIC value of all the isolates.

Mutation in Porin or MshA showed resistant to doxycycline

Doxycycline showed better anti-mycobacteria activity among all the tetracyclines used in this study. To study the anti-tuberculosis activity of doxycycline, we used a transposon insertion sequencing-based strategy to identify loci that were required for *M.smeg* resistance to doxycycline. Mutants were selected for resistance to doxycycline on plates with a concentration of 1xMIC. We isolated 11 resistant clones. Whole genome sequencing identified two genes which exhibited insertions of transposon were candidates for mediating resistance. The less frequently insertion gene was (2 of 11 clones) MSMEG_0933, encoding MshA. Most of the resistant clones (9 of 11) exhibited the insertion of transposon was MSMEG_0965 (Table 4). MshA, a gene encoding the first enzyme involved in the biosynthesis of mycothiol [26], which has been reported to essential for drug susceptibility in *M.tb* and *M.smeg* [27–29]. MSMEG_0965 is a porin, which locates in the waxy outer layer of mycobacteria [30] and provides a sieving function. Deletion of porin dramatically increased the resistance of *M.smeg* to multiple drugs [31–33].

We further tested the MIC of doxycycline with these two mutants. Our results showed both mutants showed increasing resistant to doxycycline and the MICs were 0.51µg/mL and 0.25µg/mL for MSMEG_0965 and MSMEG_0933 mutant strains, respectively (Table 5). However, both MshA and porin are non-essential genes [28, 34, 35], it was unlikely to encode the target. The mechanism underlying these two mutants resistant to doxycycline needs to be further investigated.

Discussion

With rising levels of antibiotic resistance eliminating the drug classes available for treating MDR- and XDR-TB, identifying new drug classes serves a critical need. Additionally, concerning the rising of heritable resistance to PZA [36], novel molecules that are bactericidal against *M.tb* will be important components of much-needed new cocktails for shorter treatment without relapse. In this study, we examined the activity of tetracycline analogs, including three generations of tetracyclines, against mycobacteria. We identified 6 tetracycline analogs (demeclocycline, tetracycline, chlortetracycline, methacycline, tigecycline and doxycycline) that were efficacious against Mycobacteria while oxytetracycline showed poor activity. Our results is consistent with the previously reports on doxycycline and tigecycline [14, 23, 37, 38]. We have found that demeclocycline, doxycycline and methacycline were effective against clinical MDR- or XDR- isolates. When comparing the MICs of efficacious tetracyclines, doxycycline had lower MICs among all tetracyclines and is a pre-existing drug approved for the treatment of various infections by gram-positive and gram-negative bacteria, aerobes and anaerobes [39].

Doxycycline is a well-tolerated broad-spectrum oral antibiotic that is used in therapy for many infections. In this study, 6 of 7 clinical isolates were susceptible to doxycycline. The result is similar as previously reported doxycycline resistant rate in Russia clinical isolates [15].

Tetracycline-class antibiotics inhibit protein synthesis by preventing attachment of aminoacyl-tRNA to the ribosomal acceptor (A) site that is different with all first line anti-TB drugs [40–42]. Mutations in porin locus show decreased susceptibility to tetracyclines have been described in Gram-negative cells such as *E. coli* and *Helicobacter pylori* in previous studies [43–46]. But there is less knowledge of specific genetic requirements for tetracycline inhibitory activity in *M.tb*. Here, we took advantage of the power of transposon insertion sequencing to identify genetic factors that contribute to the *M.tb* against doxycycline. In consistent with the previous reports, we found a transposon insertion in MSMEG_0965 which encoding porin [32]. Next we examined the role of the porin in the sensitivity of *M. smeg* to doxycycline by using the mutant of *M. smeg* lacking the porin. The MIC for this mutant increased tenfold. Thus, these results indicate further that doxycycline mainly diffuses through the porin in *M. smeg*. In addition, we characterized the molecular basis for doxycycline resistance in mycobacteria, which has not been reported previously. An insertions located in MSMEG_0933, which encodes a glycosyltransferase involved in the first step of mycothiol biosynthesis. Previous study has found that the altered thiol-disulfide status can restricts mutant *M. smeg* ability to grow on plates and at low pH and to resist challenge by hydrogen peroxide, but it still can grow normally in liquid culture [47]. However, our result found that the MIC for this transposon insertion increased fivefold, which still showed increasing resistant to doxycycline in liquid culture. To our knowledge, this study is the first to demonstrate that doxycycline resistance in *mycobacteria* can be associated with the loss of mycothiol function. However, it remains unclear precisely how the altered expression levels of mycothiol drive doxycycline resistance. Elucidating this precise mechanism of action should be the focus of future studies.

Conclusion

There is a lack of effective drugs to cure MDR/XDR-TB. Our study demonstrated that Tetracycline analogs, especially doxycycline has anti-tuberculosis bactericidal activity. Further studies found a transposon insertion in MSMEG_0933 caused the loss of mycothiol function, which resulted in increased resistant of mutant *Mycobacterium tuberculosis* to doxycycline. Future studies will address whether mycothiol play a role in other tetracycline resistance. If so, the development of agents that inactivate the activity of mycothiol could enable the use of tetracycline antibiotics in M.tuberculosis.. Finally, this study highlights the potential clinical application of tetracycline analogs, especially doxycycline as adjunct anti-tuberculosis drugs.

Abbreviations

MDR-TB: multidrug-resistant tuberculosis; FDA: Food and Drug Administration (FDA); MIC: minimum inhibitory concentration (MIC); CTAB: cetyltrimethyl ammonium bromide (CTAB); CFU: colony forming units (CFU); XDR-TB: extensively drug-resistant tuberculosis; OADC: acid–albumin-dextrose–catalase; OD: optical density

Declarations

Acknowledgements

The authors gratefully acknowledge all the study participants and study staff for the help and cooperation during the study in Shenzhen Third peoples' hospital.

Authors' contributions

Authors' contributions XC, YC and QO participated in the study design, analysis of data and writing of the manuscript. OQ performed the laboratory examination. DL, GD, and HL collected clinical isolates and organize strains resistant data. All authors read and approved the final version of manuscript.

Funding

The study was supported by Natural Science Foundation of China (81770013, 81525016 and 81772145), National Science and Technology Major Project (2017ZX10201301), Science and Technology Project of Shenzhen (JCYJ20170412101048337). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Availability of data and materials

All data and materials associated with this study are available from the main text or the additional file.

Ethics approval and consent to participate

This study was approved by the Institutional Review Board of the Shenzhen University School of Medicine, China, and informed written consent was obtained from each participant. All experiments and samplings were carried out in accordance with ethical and biosafety protocols approved by the Institutional guidelines.

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests

References

1. *Global tuberculosis report 2018*: World Health Organization; 2019.
2. Seung KJ, Salmaan K, Rich ML: *Multidrug-Resistant Tuberculosis and Extensively Drug-Resistant Tuberculosis. Cold Spring Harbor Perspectives in Medicine* 2015, 5(9):a017863.
3. Falzon D., Jaramillo E., Schünemann HJ, Arentz M., Bauer M., Bayona J., Blanc L., Caminero JA, Daley CL, Duncombe C., *WHO guidelines for the programmatic management of drug-resistant tuberculosis: 2011 update*. 2011, 38(3):516–528.
4. Duggar BM: *Aureomycin: a product of the continuing search for new antibiotics. Annals of the New York Academy of Sciences* 1948, 51(2):177–181.
5. FINLAY AC, Hobby G, P'an S, Regna P, Routien J, Seeley D, Shull G, Sobin B, Solomons I, Kane J: *Terramycin, a new antibiotic. Science (Washington)* 1950:85–87.
6. KING EQ, LEWIS CN, WELCH H, CLARK EA, JOHNSON JB, LYONS JB, SCOTT RB, CORNELLY PB: *Clinical observations on the use of Terramycin hydrochloride. Journal of the American Medical Association* 1950, 143(1):1–4.
7. Roberts MC: *Tetracycline resistance determinants: mechanisms of action, regulation of expression, genetic mobility, and distribution. FEMS microbiology reviews* 1996, 19(1):1–24.
8. Nelson ML, Levy SB: *The history of the tetracyclines. Annals of the New York Academy of Sciences* 2011, 1241(1):17–32.

- 9.Sum PE, Lee VJ, Testa RT, Hlavka JJ, Ellestad GA, Bloom JD, Gluzman Y., Tally FP: *Glycylcyclines. 1. A new generation of potent antibacterial agents through modification of 9-aminotetracyclines. Journal of Medicinal Chemistry* 1994, 37(1):184.
- 10.Klein NC, Cunha BA: *Tetracyclines. Medical Clinics of North America* 1995, 79(4):789–801.
- 11.Brodersen DE, Clemons Jr WM, Carter AP, Morgan-Warren RJ, Wimberly BT, Ramakrishnan V: *The structural basis for the action of the antibiotics tetracycline, pactamycin, and hygromycin B on the 30S ribosomal subunit. Cell* 2000, 103(7):1143–1154.
- 12.Wilson DN: *The A–Z of bacterial translation inhibitors. Critical reviews in biochemistry and molecular biology* 2009, 44(6):393–433.
- 13.Griffin MO, Fricovsky E, Ceballos G, Villarreal F: *Tetracyclines: a pleiotropic family of compounds with promising therapeutic properties. Review of the literature. American Journal of Physiology-Cell Physiology* 2010, 299(3):C539-C548.
- 14.Walker NF, Clark SO, Tolu O, Nuria A, Liku T, Shivani S, Luísa S, Bernadette P, Kelly DL, Tree JA: *Doxycycline and HIV infection suppress tuberculosis-induced matrix metalloproteinases. American Journal of Respiratory & Critical Care Medicine* 2012, 185(9):989–997.
- 15.Balabanova Y, Ruddy M, Hubb J, Yates M, Malomanova N, Fedorin I, Drobniewski F: *Multidrug-resistant tuberculosis in Russia: clinical characteristics, analysis of second-line drug resistance and development of standardized therapy. European Journal of Clinical Microbiology and Infectious Diseases* 2005, 24(2):136–139.
- 16.Gonzalo X, Casali N, Broda A, Pardieu C, Drobniewski F: *Combination of amikacin and doxycycline against multidrug-resistant and extensively drug-resistant tuberculosis. International Journal of Antimicrobial Agents* 2015, 45(4):406–412.
- 17.Hobby GL, Lenert TF: *Antituberculous activity of tetracycline and related compounds. American Review of Tuberculosis and Pulmonary Diseases* 1955, 72(3):367–372.
- 18.Irith W, Kai H, Hancock REW: *Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. Nature Protocols* 2008, 3(2):163–175.
- 19.Sasseti CM, Boyd DH, Rubin EJ: *Comprehensive identification of conditionally essential genes in mycobacteria. Proceedings of the National Academy of Sciences* 2001, 98(22):12712–12717.
- 20.Guilhot C, Otal I, Van Rompaey I, Martin C, Gicquel B: *Efficient transposition in mycobacteria: construction of Mycobacterium smegmatis insertional mutant libraries. Journal of bacteriology* 1994, 176(2):535–539.
- 21.Parish T, Brown AC: *Mycobacteria protocols*: Springer; 2008.

22. Paul C, Muttucumaru DGN, Tanya P: *Use of a tetracycline-inducible system for conditional expression in Mycobacterium tuberculosis and Mycobacterium smegmatis*. *Applied & Environmental Microbiology* 2005, 71(6):3077–3084.
23. Coban AY, Deveci A, Cayci YT, Uzun M, Akgunes A, Durupinar B: *In vitro effect of tigecycline against Mycobacterium tuberculosis and a review of the available drugs for tuberculosis*. *African Journal of Microbiology Research* 2011, 5(3):311–315.
24. Agwuh KN, MacGowan A: *Pharmacokinetics and pharmacodynamics of the tetracyclines including glycylcyclines*. *Journal of Antimicrobial Chemotherapy* 2006, 58(2):256–265.
25. Newton PN, Chaulet J-F, Brockman A, Chierakul W, Dondorp A, Ruangveerayuth R, Looareesuwan S, Mounier C, White NJ: *Pharmacokinetics of oral doxycycline during combination treatment of severe falciparum malaria*. *Antimicrobial agents and chemotherapy* 2005, 49(4):1622–1625.
26. Newton GL, Ta P, Bzymek KP, Fahey RC: *Biochemistry of the initial steps of mycothiol biosynthesis*. *Journal of Biological Chemistry* 2006, 281(45):33910–33920.
27. Vilcheze C, Av-Gay Y, Barnes SW, Larsen MH, Walker JR, Glynn RJ, Jacobs WR: *Coresistance to Isoniazid and Ethionamide Maps to Mycothiol Biosynthetic Genes in Mycobacterium bovis*. *Antimicrobial Agents and Chemotherapy* 2011, 55(9):4422–4423.
28. Xia X, Catherine V, Yossef AG, Anaximandro GV, Jacobs WR: *Precise null deletion mutations of the mycothiol synthesis genes reveal their role in isoniazid and ethionamide resistance in Mycobacterium smegmatis*. *Antimicrobial Agents & Chemotherapy* 2011, 55(7):3133–3139.
29. Vilchèze C, Av-Gay Y, Attarian R, Liu Z, Hazbón MH, Colangeli R, Chen B, Liu W, Alland D, Sacchettini JC: *Mycothiol biosynthesis is essential for ethionamide susceptibility in Mycobacterium tuberculosis*. *Molecular microbiology* 2008, 69(5):1316–1329.
30. Michael F, Michael N, Schulz GE: *The structure of a mycobacterial outer-membrane channel*. *Science* 2004, 303(5661):1189–1192.
31. Joachim S, Claudia M, Gilles E, Mamadou D, Michael N: *Multidrug resistance of a porin deletion mutant of Mycobacterium smegmatis*. *Antimicrobial Agents & Chemotherapy* 2004, 48(11):4163.
32. Danilchanka O, Pavlenok M, M: *Role of porins for uptake of antibiotics by Mycobacterium smegmatis*. *Antimicrobial Agents & Chemotherapy* 2008, 52(9):3127.
33. Frenzel E, Schmidt S, Niederweis M, Steinhauer K: *Importance of Porins for Biocide Efficacy against Mycobacterium smegmatis*. *Applied and Environmental Microbiology*.
34. Dietmar H, Iris E, Anja T, Michael N: *Expression of the major porin gene mspA is regulated in Mycobacterium smegmatis*. *Journal of Bacteriology* 2007, 189(3):958.

- 35.Pavlenok M, Niederweis M: *Hetero-oligomeric MspA pores in Mycobacterium smegmatis*. *Fems Microbiology Letters* 2016, 363(7):fnw046.
- 36.Njire M, Tan Y, Mugweru J, Wang C, Guo J, Yew W, Tan S, Zhang T: *Pyrazinamide resistance in Mycobacterium tuberculosis: Review and update*. *Advances in medical sciences* 2016, 61(1):63–71.
- 37.Rubinstein E, Vaughan D: *Tigecycline*. *Drugs* 2005, 65(10):1317–1336.
- 38.Wallace RJ, Brown-Elliott BA, Crist CJ, Mann L, Wilson RW: *Comparison of the in vitro activity of the glycylcycline tigecycline (formerly GAR-936) with those of tetracycline, minocycline, and doxycycline against isolates of nontuberculous mycobacteria*. *Antimicrobial Agents and Chemotherapy* 2002, 46(10):3164–3167.
- 39.Joshi N, Miller DQ: *Doxycycline Revisited*. *JAMA Internal Medicine* 1997, 157(13):1421–1428.
- 40.Sarkar S, Thach RE: *Inhibition of formylmethionyl-transfer RNA binding to ribosomes by tetracycline*. *Proceedings of the National Academy of Sciences of the United States of America* 1968, 60(4):1479.
- 41.Janin YL: *Antituberculosis drugs: ten years of research*. *Bioorganic & medicinal chemistry* 2007, 15(7):2479–2513.
- 42.Arbex MA, Varella MdCL, Siqueira HRd, Mello FAFd: *Antituberculosis drugs: drug interactions, adverse effects, and use in special situations-part 1: first-line drugs*. *Jornal Brasileiro de Pneumologia* 2010, 36(5):626–640.
- 43.Mortimer PG, Piddok LJ: *The accumulation of five antibacterial agents in porin-deficient mutants of Escherichia coli*. *Journal of Antimicrobial Chemotherapy* 1993, 32(2):195–213.
- 44.Thanassi DG, Suh G, Nikaido H: *Role of outer membrane barrier in efflux-mediated tetracycline resistance of Escherichia coli*. *Journal of bacteriology* 1995, 177(4):998–1007.
- 45.Pugsley AP, Schnaitman CA: *Outer membrane proteins of Escherichia coli VII. Evidence that bacteriophage-directed protein 2 functions as a pore*. *Journal of bacteriology* 1978, 133(3):1181–1189.
- 46.Trieber CA, Taylor DE: *Mutations in the 16S rRNA genes of Helicobacter pylori mediate resistance to tetracycline*. *Journal of bacteriology* 2002, 184(8):2131–2140.
- 47.Buchmeier NA, Newton GL, Fahey RC: *A mycothiol synthase mutant of Mycobacterium tuberculosis has an altered thiol-disulfide content and limited tolerance to stress*. *Journal of bacteriology* 2006, 188(17):6245–6252.

Tables 1, 2, 5

Table 1 Tetracycline analogs test concentration

Drug	Concentration
	(µg/mL)
Deme	100.3
Tetr	88.9
Chlo	103.1
Tige	117.1
Doxy	102.7
Oxy	92.1
Meth	95.8
Tetr(hy)	96.2

Deme, Demeclocycline (hydrochloride); Tetr, Tetracycline; Chlo, Chlortetracycline (hydrochloride); Tige, Tigecycline; Doxy, Doxycycline (hyclate) (hyclate); Oxy, Oxytetracycline; Meth, Methacycline (hydrochloride); Tetr(hy), Tetracycline (hydrochloride);

Table 2 MIC of *M.smeg* and *M.tb* H37Ra

Drug(µg/mL)	<i>M.smeg</i>	<i>M.tb</i> H37Ra
Deme	0.067	1.25
Chlo	0.34	5.15
Doxy	0.051	1.03
Meth	0.064	2.39

Deme, Demeclocycline (hydrochloride); Chlo, Chlortetracycline (hydrochloride); Doxy, Doxycycline (hyclate) (hyclate); Meth, Methacycline (hydrochloride);

Strain	Concentration
	($\mu\text{g/mL}$)
mc ² 155 mutant MSMEG_0965	0.51
mc ² 155 mutant MSMEG_0933	0.25
mc ² 155 wild type <i>M.smeg</i>	0.051

Table 5 MIC of Transposon *M.smeg*

Figures

Figure 1

Determination of antimicrobial activity of tetracycline analogs against *M.smeg* and *M.tb* H37Ra strains. (A) *M.smeg* was grown in liquid cultures with eight tetracyclines directly effected on it. Deme, Demeclocycline; Tetr, Tetracycline; Chlo, Chlortetracycline; Tige, Tigecycline; Doxy, Doxycycline; Oxy, Oxytetracycline; Meth, Methacycline; Tetr(hy), Tetracycline (hydrochloride); Mock, none drug. (B) *M.smeg* was grown below the two fold serially diluted of four tetracyclines; The final test concentration for each tetracyclines ranged from 0.00052 to 0.0667 $\mu\text{g/mL}$ for demeclocycline, 0.0054 to 0.685 $\mu\text{g/mL}$ for chlortetracycline, 0.00129 to 0.103 $\mu\text{g/mL}$ for doxycycline and 0.000498 to 0.0637 $\mu\text{g/mL}$ for methacycline. (C) *M.tb* H37Ra was grown below the two fold serially diluted of four tetracyclines; The final test concentration for each tetracyclines ranged from 0.16 to 10.02 $\mu\text{g/mL}$ for demeclocycline, 0.16 to 10.31 $\mu\text{g/mL}$ for chlortetracycline, 0.16 to 10.26 $\mu\text{g/mL}$ for doxycycline and 0.15 to 9.58 $\mu\text{g/mL}$ for methacycline.

Figure 2

The MIC result of *M. tb* H37Rv and MDR-TB strain were measured. (A-H) were *M. tb* H37Rv, Clinical strain ER17, ER22, 400-13, 400-86, 400-89, 400-123, 200-28, respectively. The test concentration range was from 0.00128 to 5.1294 $\mu\text{g/mL}$ for doxycycline, from 0.00627 to 25.066 $\mu\text{g/mL}$ for demeclocycline and 0.00599 to 23.944 $\mu\text{g/mL}$ for methacycline.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Table3.xlsx](#)
- [Table4.xlsx](#)