Gene Cell Tissue. 2021 October; 8(4):e113202. doi: 10.5812/gct.113202.

Published online 2021 August 1. **Research Article**

Identification of Antibiotic-Resistant Genes and Effect of Garlic Ethanolic Extract on *Mycobacterium tuberculosis* Isolated from Patients in Zabol, Iran

Bahman Fazeli-Nasab [1,*], Moharram Valizadeh [2], Maryam Beigomi [3] and Saeide Saeidi [4]

[1] Research Department of Agronomy and Plant Breeding, Agricultural Research Institute, University of Zabol, Zabol, Iran
[2] Research Center of Medicinal Plants, University of Sistan and Baluchestan, Zahedan, Iran
[3] epartment of Food Science and Technology, Zahedan University of Medical Sciences, Zahedan, Iran
[4] Agricultural Biotechnology Research Institute, University of Zabol, Zabol, Iran

[*] *Corresponding author*: Research Department of Agronomy and Plant Breeding, Agricultural Research Institute, University of Zabol, Zabol, Iran. Email: bfazeli@uoz.ac.ir

Received 2021 January 27; **Revised** 2021 February 26; **Accepted** 2021 March 08.

Abstract

Background: Mycobacterium is a genus of Actinobacteriaceae and the Mycobacterium family, including important pathogens, such as *Mycobacterium tuberculosis* (i.e., the cause of tuberculosis) and Mycobacterium leprae (i.e., the cause of leprosy). Tuberculosis is still a major cause of death in human societies.

Objectives: The current study aimed to evaluate the effect of ethanolic extract of garlic on *Mycobacterium tuberculosis* isolated from patients in Zabol, Iran, and investigate the presence of antibiotic-resistant genes in *Mycobacterium tuberculosis*.

Methods: Garlic (*Allium sativum*) was collected from Zabol, and the ethanolic extract of garlic leaf was obtained. In this study, 50 strains of *Mycobacterium tuberculosis* were obtained from the patients in Zabol. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined. Some antibiotics, such as isoniazid, pyrazinamide, ethambutol, amikacin, streptomycin, and rifampicin, were used for positive control. Genomic deoxyribonucleic acid was extracted by the sodium dodecyl sulfate method. Furthermore, the presence of antibiotic-resistant genes, namely *KatG*, *PncA*, *embC*, *embA1*, *embA2*, *embB1*, *embB2*, *rrs*, *rpsL*, and *ropB*, in *Mycobacterium tuberculosis* was investigated using polymerase chain reaction.

Results: The lowest MIC and MBC of garlic ethanolic extract against *Mycobacterium tuberculosis* were 3.25 and 7.5 ppm, respectively. The highest MIC and MBC were 60 and 120 ppm, respectively. Following the investigation of the presence of antibiotic-resistant genes in *Mycobacterium tuberculosis*, it was determined that it contains *KatG*, *PncA*, *embC*, *embA1*, *embA2*, *ropB*, *rpsL*, *rrs*, *embB2*, and *embB1* genes. The highest resistance of *Mycobacterium tuberculosis* was against rifampin (81%) and then amikacin (76.6%) belonging to *ropB* and *rrs* genes, respectively.

Conclusions: The results of the present study showed that the ethanolic extract of garlic was very effective in *Mycobacterium tuberculosis* and the most effective genes in mycobacteria were *ropB* and *rrs*. Although garlic is very effective in *Mycobacterium tuberculosis*, it is not recommended to directly use the results of this study. Therefore, it is required to perform clinical trials to confirm the results.

Keywords: *Allium sativum*, Antibiotic-Resistant Genes, MIC, MBC, PCR

1. Background

The varieties of garlic have been used since many centuries ago as spices, food flavors, and medications in traditional medicine for treating various types of diseases (1). Garlic has antibacterial, anti-cancer, antioxidant, and anti-inflammatory properties, reduces blood sugar, and protects the cardiovascular system. The antibacterial effects of garlic on different bacteria have also been reported (2).

Tuberculosis is one of the global worst diseases, which is ranked seventh in the world of diseases in 1990 and is expected to remain at the seventh place in the world in 2020, according to the Disability-Adjusted-Life Year indicator. According to the World Health Organization, one-third of the world population is infected with tuberculosis, and 8 million and eight hundred thousand new cases are infected each year. Annually, about 203 million individuals die from tuberculosis. The cause of this disease is basil *Mycobacterium tuberculosis*, intruding into the airborne contaminated with this basil in the lungs and causing tuberculosis.

Multidrug-resistant (MDR) tuberculosis is a type of tuberculosis in which *Mycobacterium tuberculosis* is at least resistant to rifampin and isoniazid. Rifampin and isoniazid

are two drugs together, killing more than 99% of tuberculosis bacilli in the first two months of treatment (3, 4).

2. Objectives

Therefore, the treatment of MDR tuberculosis is associated with many problems, and the identification of MDR patients is essential in accelerating the treatment process. With this background in mind, the current study aimed to evaluate the effect of garlic extract and isolation of resistant genes to antibiotics in *Mycobacterium tuberculosis* isolated from patients in Zabol, Iran.

3. Methods

3.1. Plant Material and Ethanolic Extract

Garlic (Figure 1) was collected from Zabol (coordinates: 31°01'43"N 61°30'04"E), Sistan and Baluchestan, and the species was described in the botanical laboratory of Zabol University. In this study, 10 g of Ggarlic medicinal plant leaf tissue (Figure 1) in the shade and vicinity of dry air, milling, then soaked and shaked in 100 cc of ethanol at room temperature for 48 h. After the desired time, the extracts were refined; then, the solvent was evaporated at a temperature below 40°C by rotary evaporation, and the remainder after drying was stored in a refrigerator at 4°C for experiments (5).

Allium sativum was properly dried and pulverized into a coarse powder (6). Each 20 g ground powder was soaked in 60 mL ethanol 95%, separately for 1 day. After 1 day of the dissolving process, the materials were filtered (Whatman grade 1 filter paper). Subsequently, the filtrates were evaporated using a rotary evaporator. Finally, 0.97 g of the dried extract was obtained and then stored at 4°C in an air-tight screw-cap tube.

For this study, 30 patients were selected referring to University of Zabolin 2020, and then the rest of the experiments were performed at Zabol University of Medical Sciences. In this experimental study, 30 patients with tuberculosis participated who recovered after 3 months of treatment with anti-tuberculosis drugs. We received their phlegm after obtaining consent. After the collection of Sputum from patients having TB, a clinical examination was performed. Tuberculin test was performed for the patient, and sputum sample was performed on Loonstein Johnson solid medium. The lamellas were examined by light microscopy after the Ziehl-Neelsen method. The samples of acid-resistant red bacilli were reported positive, and each sample was cultured in two Lyons-Stein Johnson tubes. After 6-8 weeks, the culture media were examined for the presence of colonies of cream color (i.e., cauliflower of *Mycobacterium tuberculosis*). After preparing the lam from these colonies, the coloring was performed by the Ziehl-Neelsen method. Again, the laminae were investigated in terms of the presence of basil red acid-resistant bacteria. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined. In this study, some antibiotics, such as isoniazid, pyrazinamide, ethambutol, amikacin, streptomycin, and rifampicin, were used for positive control.

3.2. DNA Extraction and PCR

The deoxyribonucleic acid (DNA) of *Mycobacterium tuberculosis* was extracted by the sedimentation method (7). After DNA deposition in 50 μL of solvent buffer, it was dissolved, and a confirmatory polymerase chain reaction was used (7). The current study also investigated the presence of antibiotic-resistant genes (i.e., *KatG*, *PncA*, *embC*, *embA1*, *embA2*, *embB1*, *embB2*, *rrs*, *rpsL*, and *ropB*) in *Mycobacterium tuberculosis* (Table 1). Genomic DNA extraction was performed based on the sodium dodecyl sulfate (8) method, and three samples were extracted from each bacterium.

4. Results

The lowest MIC of garlic ethanolic extract against *Mycobacterium tuberculosis* was 3.25 ppm, and two strains were inhibited at this concentration; however, the highest MIC was 60 ppm, and five strains were inhibited at this concentration. The lowest MBC of garlic ethanolic extract against *Mycobacterium tuberculosis* was 7.5 ppm, and four strains were inhibited at this concentration; nevertheless, the highest MBC was 120 ppm, and three strains were inhibited at this concentration (Table 2).

Following the investigation of the presence of antibiotic-resistant genes in *Mycobacterium tuberculosis*, it was determined that it contains *KatG*, *PncA*, *embC*, *embA1*, *embA2*, *ropB*, *rpsL*, *rrs*, *embB2*, and *embB1* genes (Figures 2 and 3). The resistance of *Mycobacterium tuberculosis* against antibiotics is based on the presence of resistant genes, and each of *KatG*, *PncA*, *embC*, *embA1*, *embA2*, *embB1*, *embB2*, *rrs*, *rpsL*, and *ropB* genes causes bacterial resistance to isoniazid, pyrazinamide, ethambutol, amikacin, streptomycin, and rifampicin, respectively; therefore, in this study, it was determined that the bacterial levels of resistance to these antibiotics were 40%, 23.3%, 33.3%, 26.6%, 63.3%, 40%, 76.6%, 56.6%, and 81%, respectively (Table 3).

The results showed that the highest resistance of *Mycobacterium tuberculosis* was against rifampin (81%) and then amikacin (76.6%) belonging to *ropB* and *rrs* genes, respectively. Therefore, it can be concluded that the most important genes in *Mycobacterium tuberculosis* are *ropB* and *rrs*.

Figure 1. Garlic characteristics (Source: http://www.sfim.ir)

Table 1. Separation of Various Antibiotic-Resistant Genes

Gene Name	F/R	Primer Sequence	Resistant Antibiotic	Size	Reference
KatG	F	GTCGCGACCATCGACGTTGA	Isoniazid	1710	(9)
	R	GACGTCGTTCATGGCCATGC			
PncA	F	GTCGGTCATGTTCGCGATCG	Pyrazinamide	558	(10, 11)
	R	GCTTTGCGGCGAGCGCTCCA			
embC	F	GATACCCGCTACAGCAGCA	Ethambutol	334	(10)
	R	GGTCGTAGTACCAGCCGAAA			
embA1	F	GCCGGCTATGTAGCCAACTA	Ethambutol	338	(12)
	R	GACCGTTCCACCAACACC			
embA2	F	GCGCGCTGGACATCTCGAT	Ethambutol	704	(12)
	R	CGCCTCCGTCGTGCCGAAATA			
embB1	F	CCGACCACGCTGAAACTGC	Ethambutol	364	(12)
	R	GTAATACCAGCCGAAGGGATCCT			
embB2	F	GACGGCTACATCCTGGGCATG	Ethambutol	525	(12)
	R	TGCCGACCAGGCGATGACG			
rrs	F	AAACCTCTTTCACCATCGAC	Amikacin	552	(10, 11)
	R	CAGGTAAGGTTCTTCGCGTTG			
rpsL	F	GTCAAGACCGCGGCTCTGAA	Streptomycin	272	(10)
	R	TTCTTGACACCCTGCGTATC			
ropB	F	TACGGTCGGCGAGCTGATCC	Rifampicin	81	(10)
	R	TACGGCGTTTCGATGAACC			

5. Discussion

Based on the literature, it was concluded that *Streptococcus pyogenes* was the most sensitive bacterium to the inhibitory effect of garlic aqueous extract. In addition, *Pseudomonas aeruginosa* showed the least sensitivity. However, in general, fungal microorganisms have shown the least sensitivity to the aqueous extract of garlic. Furthermore, the MIC of the extract (except for two cases) was highest only in 60% of dilutions (13). In the present study, the ethanolic extract of garlic was effective in *Mycobacterium tuberculosis* and even the MIC was equal to 3.25 ppm.

A laboratory study investigated the inhibitory effect of garlic extract on Aeromonas sobria and concluded that at concentrations of 200 and 400 mg/μL, ethanolic garlic ex-

Table 2. Minimum Inhibitory Concentration and Minimum Bactericidal Concentration of Garlic Extract Against Bacteria

Bacterial Code	MIC	MBC
1	30	60
2	15	30
3	15	30
4	60	60
5	30	60
6	7.5	15
7	15	30
8	3.25	7.5
9	15	30
10	60	120
11	60	60
12	30	30
13	15	30
14	15	30
15	15	30
16	3.25	7.5
17	7.5	15
18	30	60
19	15	30
20	15	30
21	30	60
22	15	30
23	30	30
24	7.5	15
25	15	30
26	60	120
27	7.5	15
28	30	60
29	60	120
30	30	60

Abbreviations: MBC, minimum bactericidal concentration; MIC, minimum inhibitory concentration.

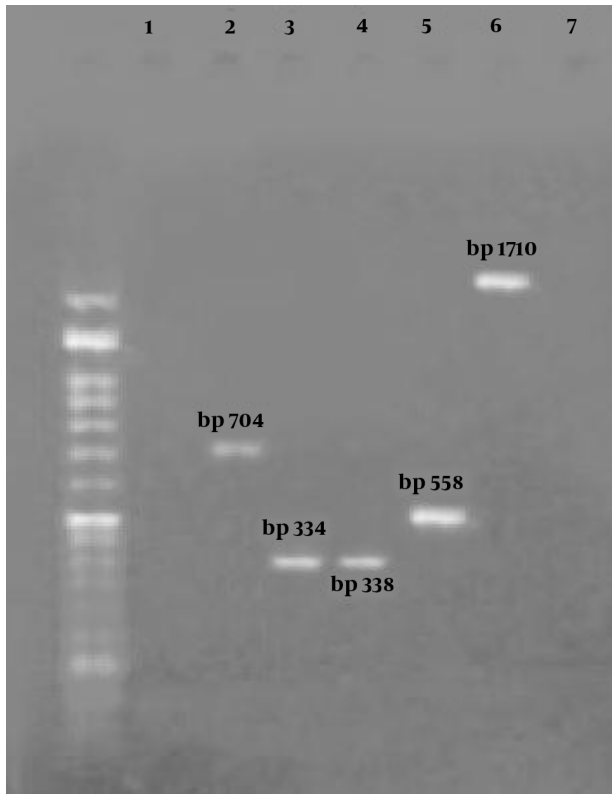

Figure 2. Gel for *KatG* (2), *PncA* (3), *embC* (4), *embA1* (5), and *embA2* (6) Genes

Table 3. Prevalence of Genes Resistant to Antibiotics

Antibiotic-Resistant Genes	Antibiotic	Resistance, %
KatG	Isoniazid	40
PncA	Pyrazinamide	23.3
embA1	Ethambutol	33.3
embA2	Ethambutol	26.6
embB1	Ethambutol	63.3
embB2	Ethambutol	40
rrs	Amikacin	76.6
rpsL	Streptomycin	56.6
ropB	Rifampicin	81

tract had growth inhibition zones of 7 and 10 mm, respectively. No growth aura was observed for all the methanolic concentrations of garlic extract. In aqueous garlic extract, the growth inhibition zones were 8, 10, and 14 mm for concentrations of 100, 200, and 400 mg/μL, respectively. For crude garlic extract at concentrations of 50% and 100%, the growth inhibition zones were 8 and 27 mm, respectively. The MIC for Aeromonas sobria in ethanolic and aqueous extracts were 200 and 400 mg/μL, respectively. Moreover, the MIC for crude garlic extract was estimated at 10%.

The MBC is estimated to be 100 and 300 mg/μL for crude garlic extract, respectively. In general, they concluded that crude and aqueous extracts of garlic have the highest antibacterial effects. The ethanolic extract has the least antibacterial effect, and methanolic extract has no antibacterial effect (14). In the present study, the lowest MIC and MBC of garlic ethanolic extract in *Mycobacterium tuber-*

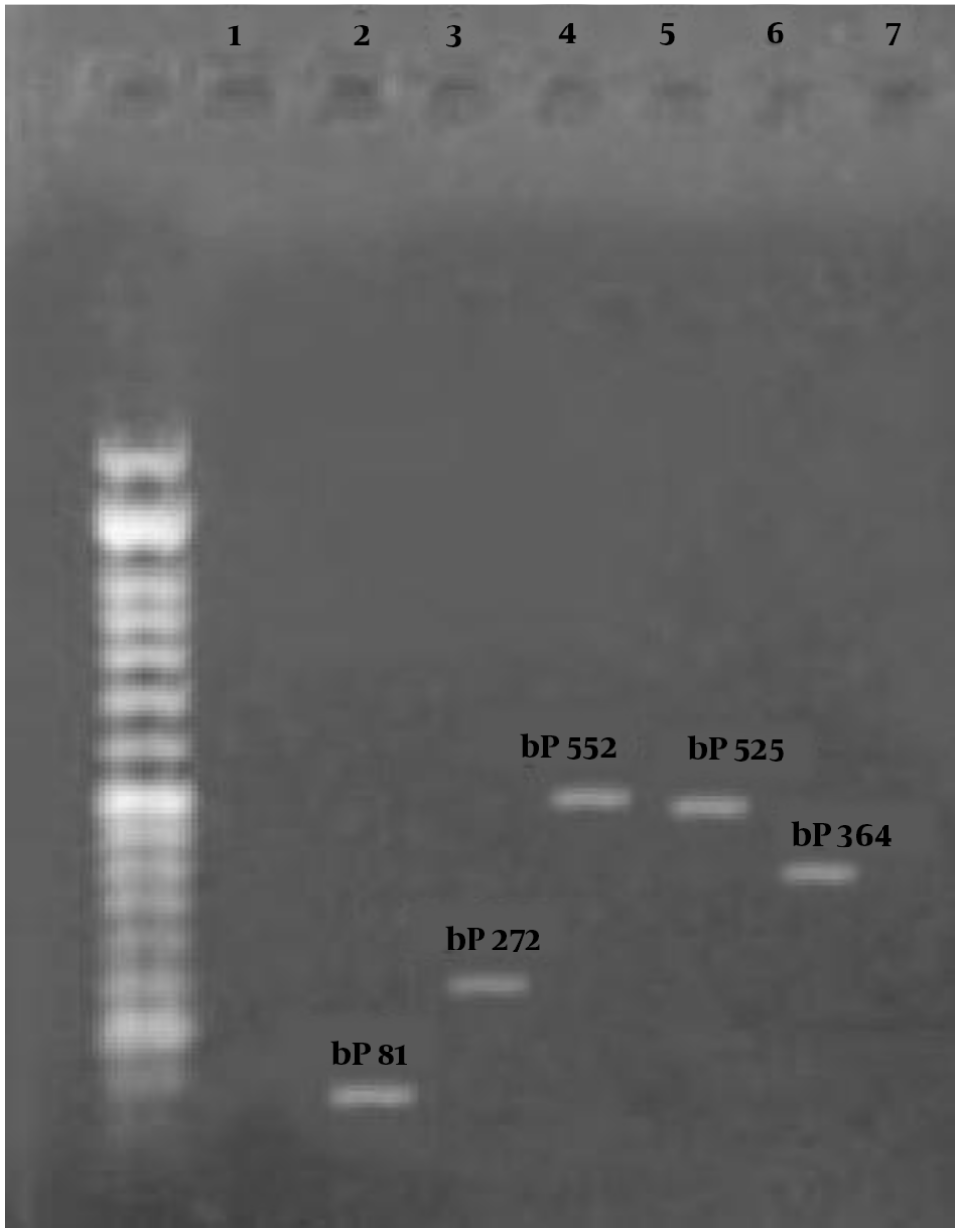

Figure 3. Gel for *ropB* (2), *rpsL* (3), *rrs* (4), *embB2* (5), and *embB1* (6) Genes

culosis were 25.3 and 5.7 ppm, respectively. Based on a previous study (14) and the results of the current study, it can be concluded that ethanol is more effective in extracting antimicrobial substances in garlic than other solvent materials, such as methanol.

Bacteria are thought to have acquired multidrug resistance via the horizontal transfer of resistance genes through mobile genetic elements, such as integrons (15). The evidence that drug resistance may contribute to the global predominance of Beijing strains was demonstrated in a study (16). The aforementioned investigation showed the highest levels of mutations in *rpoB*, *katG315*, and *embB306* among *M. tuberculosis* Beijing strains. The present study also showed an association between phenotypic drug resistance and resistant genes. Resistance to one medicine increases the danger of obtaining protection

from another medicine. Longitudinal studies with consecutive isolates from single patients may help to determine which mutations generally occur first.

Although another study (17) did not show the associations of the Beijing genotype with other drug-resistant genes, such as *pncA*, *gyrA*, and *rpsL*/*rrs*, some studies and the present study demonstrated the associations of the Iranian genotype with drug-resistant genes, such as *pncA*.

Evaluation of the effect of second-line drugs on mycobacterial strains has become very important due to the prevalence of drug resistance, especially multidrug resistance among *Mycobacterium tuberculosis* strains in recent years (18). Therefore, further studies are needed to examine this important issue.

Another study examined the antibacterial effects of some plant extracts on *Yersinia ruckeri* in vitro. Based on the results, it was concluded that the minimum growth inhibitory concentrations of angelica, fennel, pomegranate, green tea, nettle, and garlic extracts for *Yersinia ruckeri* 400, 75, respectively. 250, 250, 75 and 150 μg/mL. The minimum lethal concentrations of the aforementioned extracts were 610, 100, 500, 250, 150, and 250 μg/mL and the diameter of the growth inhibition bacterium were 17.6 $\pm$ 0.6 (mm), respectively, 23.6 $\pm$ 1.2, 20.4 $\pm$ 0.9, 18.8 $\pm$ 0.7, 21.2 $\pm$ 1.3 and 22.6 $\pm$ 1.1 (mm) were obtained. In this study, fennel, nettle, and garlic extracts showed good antibacterial effects on *Yersinia ruckeri*. Overall, it was concluded that the extracts of fennel, nettle, and garlic, after further studies, could be good alternatives to common commercial antibiotics for the treatment of systemic infections caused by *Yersinia ruckeri* (19). Based on the previous results (19) and results of this study, it can be said that the ethanolic extract of garlic is more effective than those of angelica, pomegranate, green tea, and nettle.

A study investigated the antibacterial effect of garlic and thyme essential oils on some of the main species of mastitis in dairy cows. Based on the results, it was concluded that all the concentrations of these essential oils (i.e., 10%, 30%, and 50%) had antimicrobial effects, and the effect of essential oils with lowering their concentration in the disk also decreased. No significant difference was observed between the MIC and MBC of garlic and thyme essential oils. Furthermore, the comparison of the mean growth inhibition zone between the studied antibiotics (e.g., penicillin, bacitracin, and erythromycin) and essential oils at a concentration of 10% showed that there was a significant difference between antibiotics and essential oils. Overall, they concluded that due to their antibacterial effects on the main bacteria that cause mastitis, garlic and thyme essential oils could be suitable alternatives to antibiotics for the treatment of mastitis in cattle (20). It can also be concluded that the antimicrobial properties of garlic are equal to those of thyme.

Another study examined the antibacterial effects of some essential oils of native plants on *Streptococcus iniae* in vitro. Based on the results, it was concluded that the MBC of essential oils of zolang, pomegranate, thyme, black cumin, and garlic on *Streptococcus iniae* 1, 1 <, 0.25 respectively, were 1, 1 <, 0.25, 0.12 and 0.5 μg/mL, respectively. The MIC values for these essential oils were 0.5, 0.5, 0.06, 0.06 and 0.12 μg/mL, respectively. The diameter of inhibitor zone were 27.3 $\pm$ 1.7, 22.8 $\pm$ 1.1, 32.8 $\pm$ 1.3, 17 $\pm$ 0.4 and 18.5 $\pm$ 0.7 (mm), respectively (21). It can also be concluded that the antimicrobial properties of garlic against *Streptococcus iniae* are lower than those reported for thyme, indicating the different effects of medicinal plants and even solvents on different types of bacteria and fungi (22, 23). Therefore, it is recommended to use medicinal plants against bacteria and fungi and pay attention to the type of solvent and plant and not only to the results of similar studies.

Another investigation studied the effect of garlic extract on Staphylococcus aureus. The results showed that the antimicrobial effect in chloroform extract with a mean diameter of 27 $\pm$ 3% mm was significantly higher than that of aqueous extract with a mean diameter inhibition zone of 17 $\pm$ 2 mm. The highest sensitivity of antibiotics to fancicin was observed as 58.199% (24) indicating the different effects of solvents on antimicrobial properties.

5.1. Conclusions

The results of the present study showed that the ethanolic extract of garlic was very effective in *Mycobacterium tuberculosis*. Moreover, the most effective genes in *Mycobacterium tuberculosis* were *ropB* and *rrs*. As a result, for the development of drugs effective in *Mycobacterium tuberculosis*, it is necessary to pay more attention to the aforementioned genes and develop drugs on their basis. The measurement of drug resistance in clinical settings is time-consuming and prone to errors. Therefore, further studies are needed to examine this important issue. Although garlic is very effective in *Mycobacterium tuberculosis*, it is not recommended to directly use the results of this study. Consequently, it is required to perform further clinical trials to confirm the results.

Despite the large number of synthetic or natural inhibitors derived from plant extracts, none have been approved for clinical use. According to reports, there is an inhibitory effect on the reduction of MIC of antibiotics; therefore, it seems necessary to perform further studies in this regard. It is also necessary to identify resistant genes and find a solution to prevent and reduce resistance. This can be achieved by reducing the use of common antibiotics and performing further studies on the effects and side effects of using inhibitors instead of antibiotics.

Footnotes

Authors' Contribution: All the authors approved the final manuscript for publication.

Conflict of Interests: The authors declare that there is no conflict of interest.

Ethical Approval: No humans or animals were used in the present study. Ethical considerations were observed in accordance with the principles of the Helsinki Declaration. In addition, the study protocol was approved by the Ethics Committee of University of Zabol for Reproductive Biomedicine (code: IR.UOZ.REC.1399.003).

Funding/Support: The authors declared nothing regarding funding.

References

1. Hacıseferoğulları H, Özcan M, Demir F, Çalışır S. Some nutritional and technological properties of garlic (Allium sativum L.). *J Food Engin*. 2005;**68**(4):463–9. doi: 10.1016/j.jfoodeng.2004.06.024.
2. Hosseini J N, Shahram Shahabi, Ahya Abdi Ali, Sahar Zarrin, Nasim Ali Daie. In vitro Antibacterial Activity of Garlic Against Burn Isolated Strains of Acinetobacter spp. *J Biol Sci*. 2007;**7**(5):819–22. doi: 10.3923/jbs.2007.819.822.
3. Havlicek J, Dachsel B, Slickers P, Andres S, Beckert P, Feuerriegel S, et al. Rapid Microarray-Based Detection of Rifampin, Isoniazid, and Fluoroquinolone Resistance in Mycobacterium tuberculosis by Use of a Single Cartridge. *J Clin Microbiol*. 2018;**56**(2). doi: 10.1128/JCM.01249-17. [PubMed: 29212699]. [PubMed Central: PMC5786735].
4. Dookie N, Rambaran S, Padayatchi N, Mahomed S, Naidoo K. Evolution of drug resistance in Mycobacterium tuberculosis: a review on the molecular determinants of resistance and implications for personalized care. *J Antimicrob Chemother*. 2018;**73**(5):1138–51. doi: 10.1093/jac/dkx506. [PubMed: 29360989]. [PubMed Central: PMC5909630].
5. Rahnama M, Fazeli Nasab B, Mazarei A, Shahriari A. [Evaluation of antimicrobial activity hydro alcoholic extract of some medicinal herbs against a range of Gram-positive and gram-negative bacteria]. *New Findings Vet Microbiol*. 2018;**1**(1):1–18. Persian.
6. Petrujkic BT, Beier RC, He H, Genovese KJ, Swaggerty CL, Hume ME, et al. Nigella sativa L. as an alternative antibiotic feed supplement and effect on growth performance in weanling pigs. *J Sci Food Agric*. 2018;**98**(8):3175–81. doi: 10.1002/jsfa.8823. [PubMed: 29230814].
7. Forbes BA, Hall GS, Miller MB, Novak SM, Rowlinson MC, Salfinger M, et al. Practice Guidelines for Clinical Microbiology Laboratories: Mycobacteria. *Clin Microbiol Rev*. 2018;**31**(2). doi: 10.1128/CMR.00038-17. [PubMed: 29386234]. [PubMed Central: PMC5967691].
8. Dellaporta SL, Wood J, Hicks JB. A plant DNA minipreparation: Version II. *Plant Mol Biol Rep*. 1983;**1**(4):19–21. doi: 10.1007/bf02712670.
9. Tseng ST, Tai CH, Li CR, Lin CF, Shi ZY. The mutations of katG and inhA genes of isoniazid-resistant Mycobacterium tuberculosis isolates in Taiwan. *J Microbiol Immunol Infect*. 2015;**48**(3):249–55. doi: 10.1016/j.jmii.2013.08.018. [PubMed: 24184004].
10. Park J, Jang W, Kim M, Kim Y, Shin SY, Park K, et al. Molecular drug resistance profiles of Mycobacterium tuberculosis from sputum specimens using ion semiconductor sequencing. *J Microbiol Methods*. 2018;**145**:1–6. doi: 10.1016/j.mimet.2017.12.003. [PubMed: 29242075].
11. Feuerriegel S, Oberhauser B, George AG, Dafae F, Richter E, Rusch-Gerdes S, et al. Sequence analysis for detection of first-line drug resistance in Mycobacterium tuberculosis strains from a high-incidence setting. *BMC Microbiol*. 2012;**12**:90. doi: 10.1186/1471-2180-12-90. [PubMed: 22646308]. [PubMed Central: PMC3404943].
12. Xu Y, Jia H, Huang H, Sun Z, Zhang Z. Mutations Found in embCAB, embR, and ubiA Genes of Ethambutol-Sensitive and -Resistant Mycobacterium tuberculosis Clinical Isolates from China. *Biomed Res Int*. 2015;**2015**:951706. doi: 10.1155/2015/951706. [PubMed: 26417605]. [PubMed Central: PMC4568347].
13. Tajik H, Shokuhi Sabet Jalali F. [In vitro assessment of antimicrobial efficacy of aqueous extract of garlic against wound-infecting microorganisms]. *J Med Plants*. 2008;**7**(26):10–5. Persian.
14. Amiri Farahani M, Peyghan R, Motamedi H. In-vitro study of inhibitory effect of garlic extract on Aeromonas sobria. *Iran J Vet Med*. 2012;**6**(4):213–7.
15. Fazeli Nasab B, Yazdanpour Z. [Antimicrobial effects of extract of Citrullus colocynthis and Teucrium polium on some Bacteria]. *New Findings Vet Microbiol*. 2020;**3**(1):1–10. Persian.
16. Parwati I, van Crevel R, de Beer J, Kusumadewi I, van Soolingen D, Alisjahbana B, et al. Chapter 4- Mycobacterium tuberculosis Beijing genotype in Indonesia is associated with drug resistance gene mutations in katG, rpoB and embB. *Factors Underlying the Success of the Mycobacterium tuberculosis Beijing genotype in Indonesia [dissertation]*. Bandung, Indonesia: Pustaka Billah; 2013. p. 31–43.
17. Parwati I, van Crevel R, van Soolingen D. Chapter 8- The successful emergence of the Mycobacterium tuberculosis Beijing genotype strains; a literature review on underlying mechanisms. *Factors Underlying the Success of the Mycobacterium tuberculosis Beijing genotype in Indonesia [dissertation]*. Bandung, Indonesia: Pustaka Billah; 2013. p. 81–100.
18. Sultana R. *High throughput genotyping and fingerprinting of Mycobacterium tuberculosis multi-drug resistant strains [dissertation]*. Boston University; 2012.
19. Adel M, Safari R, Zorriehzahra MJ, Elahi R. Study the antibacterial effect some of the herbal extracts on Yersinia ruckeri in invitro. *Iran Sci Fisheries J*. 2016;**25**(3):41–50.
20. Hosseinizadeh-ghasemi A, Seiahi A, Korbandeh M, Emmadi M. [Evaluation of antibacterial effect of garlic and thyme essential oil on some of the main causes of mastitis in dairy cows]. *J Vet Lab Res*. 2018;**10**(S1):120. Special Issue No. 1 of the Twelfth Iranian Veterinary Congress. Persian. doi: 10.22075/jvlr.2018.3201.
21. Safari R, Adel M, Monji H, Riyahi Cholicheh H, Nematolahi A. [Evaluation of antibacterial effect of some of the endemic herbal essential oils on Streptococcus iniae in in vitro]. *J Aqua Ecol*. 2015;**4**(4):40–33. Persian.
22. Fazeli-Nasab B, Rahnama M, Mazarei A. [Correlation between antioxidant activity and antibacterial activity of nine medicinal plant extracts]. *J Mazandaran Univ Med Sci*. 2017;**27**(149):63–78. Persian.
23. Fazeli-Nasab B, Moshtaghi N, Forouzandeh M. [Effect of Solvent Extraction on Phenol, Flavonoids and Antioxidant Activity of some Iranian Native Herbs]. *J Ilam Univ Med Sci*. 2019;**27**(3):14–26. Persian. doi: 10.29252/sjimu.27.3.14.
24. Moradkhani S, Hajizadeh N, Molaabaszadeh H. [Anti-bacterial Effects of Aquatic and Chloroformed Eextract of Allium Sativum on E. coli]. *Iran J Infect Dis Trop Med*. 2014;**66**:45–50. Persian.