

# Virulence Factors and the Effect of Garlic Extract against *Proteus mirabilis* Isolated from Patients with UTI at Thi-Qar Province

Ali Abd Kadhum<sup>1\*</sup>

<sup>1</sup>Department of Community Health Techniques, Al-nasiriyah Technical Institute, Southern Technical University, Thi-Qar Province, Iraq

DOI: [10.36348/sjls.2024.v09i07.003](https://doi.org/10.36348/sjls.2024.v09i07.003)

| Received: 02.06.2024 | Accepted: 05.07.2024 | Published: 12.07.2024

\*Corresponding author: Ali Abd Kadhum

Department of Community Health Techniques, Al-nasiriyah Technical Institute, Southern Technical University, Thi-Qar Province, Iraq

## Abstract

**Background:** *Proteus mirabilis* isolates are nosocomial bacteria that increase the incidence of multidrug-resistant infections in immunocompromised patients. The garlic genus belongs to the Allium family, and its cultivation is widespread throughout the world. The study aimed to evaluate the efficiency of garlic extract against different strains of *P. mirabilis* that can produce biofilms and carry genes such as *blaCTX-M* and *pm1* genes. **Method:** Sixty three specimens were collected from identified patients' UTI from Al-nasiriyah general hospital between September and December 2023. Using microbial culture methods, the samples were cultured, and using Biochemical and VITEK system tests, the *P. mirabilis* isolates were diagnosed and confirmed. **Results:** Out of a total of 63 specimens isolated from UTI patients, there were 11 bacterial isolates, and the results of the garlic extract were against the bacteria. 3 isolates were susceptible, and only two isolates were moderately sensitive at a concentration of 100%, three isolates were sensitive at a concentration of 75%, and only two bacterial isolates, one of which was moderately sensitive and the other resistant, respectively. It was noted that most of the bacterial isolates carried the *blaCTX-M* gene. While all isolates were able to produce biofilms using sterile polystyrene 96-well microtiter plates. In addition, all bacterial isolates carried the *pm1* gene.

**Keywords:** Garlic extract, *Proteus mirabilis*, Biofilm, *blaCTX-M*.

**Copyright © 2024 The Author(s):** This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International License (CC BY-NC 4.0) which permits unrestricted use, distribution, and reproduction in any medium for non-commercial use provided the original author and source are credited.

## INTRODUCTION

Antibiotics, when used repeatedly, can lead to an increase in the resistance of microorganisms in many patients, especially those suffering from urinary tract infections, and this can be affected by the surrounding environment. There has been increasing interest in natural plant extracts because they contain insecticidal activity against pathogenic bacterial organisms, especially urinary tract bacteria, and this is considered an alternative to treatments that are beneficial for patients with urinary tract infection (Vijayalakshmi, 2023) (Yap, P.S. et al., 2014). The UTI that have been studied are often caused by many bacterial species, such as *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. But UTI caused by *Proteus mirabilis* pose major health challenges because the bacteria possess many virulence factors, such as fimbriae, which enable them to colonize the bladder mucosa and interact with epithelial cells. *Proteus mirabilis* bacteria also possess many genetic factors, such as genes important in the formation of biofilms and beta-lactam genes that Antibiotics break

down (Yuan et al., 2021). Garlic extract is the most important type used in traditional medicine to treat many diseases, including, chills, even influenza, and body aches such as muscle and fever. Biofilms can bind to bacteria and are considered one of their most important virulence factors. *P. mirabilis* has the ability to produce biofilms and thus develop resistance against unnatural factors in the environment. *P. mirabilis* requires factors that enable it to live in the environment away from environmental complications (Alyasari et al., 2018) (Wasfi et al., 2020). There are many naturally active mechanisms in garlic that play an important role in treating many bacterial disease. It comprises many sulfur compounds such as ajoene, allylmethyltrisulfide, diallyltrisulfide, diallyldisulphide and others that can give multiple biological properties such as antimicrobials. The antimicrobial effectiveness of garlic extract is achieved by increasing the concentrations of the extract, the area of inhibition of bacteria is larger, and this is a natural phenomenon that leads to its killing (Fufa B. K. 2019). It is difficult to find a recognized and proven method to eliminate biofilm growth without causing

many negative side effects. Increased resistance of biofilms to some antidote drugs. It is necessary to search for useful, safer, and more environmentally friendly alternatives to antibiotics and chemical additives, especially with the development of botanical medicine. Antimicrobials extracted from plants are known and widely studied by researchers because of their long use and proven health benefits in treating many diseases. (Onsare, J. G., & Arora, D. S. 2015).

## METHODS AND MATERIAL

### Specimens collection and bacterial growth:

Sixty three specimens were collected from identified patients' UTI from Al-nasiriyah general hospital between September and December 2023. *P. mirabilis* were also identified by biochemical tests and were confirmed to be *P. mirabilis* using of VITEK system, gram stain, was also used, and the characteristics on the plate were based on bacterial growth on CLED agar, MacConkey agar and characteristic morphologically the non-lactose fermenting growth as well as was feature the swarming, (Shaaban *et al.*, 2020).

### Preparation of crude extract:

Garlic plants were collected from local markets, then washing and clean by water. Then it was dehydrated by temperature at room, and using a blender, the garlic plant was ground into powder. Then, one hundred grams (100 grams) of nitrate powder was extracted using a Soxhlet device for 8 hours with 500 ml of 70% ethanol. The extracts were filtered using filter paper and then the filter was evaporated at 40 degrees Celsius using a low-pressure rotary evaporator. After that, the extracts were stored at 4 degrees Celsius in sterile vials to be used to prepare 150 mg/ml. From each extract (Abaas *et al.*, 2022).

### The agar-diffusion test for extract:

The Okeke agar-well the technique used to test the effectiveness of the extract against bacteria, as

Mueller-Hintens medium was used to grow the bacteria for a period of 24 h. in the incubator 37°C. The bacteria were grown on the medium containing wells with a diameter of 6 mm. We put different concentrations of garlic plant extract in these wells, then left the dishes for 10 min. to give the extract room to spread, and then the dishes planted with bacteria were incubated. There is a negative control, such as distilled water with 20% DMSO, and another positive control 0.2% ciprofloxacin (Jain *et al.*, 2015).

### Formation of biofilm:

For the purpose of determining the ability of bacteria to produce biofilms, the capacity of the wells of a 96-well microtiter plate was used. Polystyrene 96-well microtiter plates. The plate was incubated for 48 hours at 37°C in 200 µl of the broth of bacteria. We removed unbound bacteria with saline solution while washing all wells. After that, we placed 200 µl microliters of 1% crystal violet solution in each well for 15 min. then, all the holes were washed three times with 200 µl of sterile solution. At 540 nm, we measured the absorbance through an ELISA reader after which 200% of alcohol was extracted, the crystal violet color of the films was extracted (Kazim and Khader 2021).

### Molecular methods:

According to the instructions in the Genomic DNA Mini-Kit, DNA was extracted. After that, a PCR was done to amplify the *blaCTX-M* genes and the *pml* gene. The total PCR volume was 20 µl and included 5 µl of the bacterial DNA and 0.5 µl primers. We used a thermal cycler during the reaction and amplification (Burrell *et al.*, 2011). Sequence of primers in a table 1. The volume of reaction for the *blaCTX-M* gene was 20 µl and that for the *pml* gene was 25 µl, and the amplification schedule included three cycles of annealing at 95°C for 45 seconds, extension at 72°C for 50 seconds, and extension. Final. at 72°C for 10 minutes (Hamid *et al.*, 2020) (Allawi & Motaweq 2021).

**Table 1: size and sequence for primers used for genes amplification**

Genes	Sequence of primer	Size of gene bp.	References
<i>blaCTX-M</i>	F: CGC TTT GCG ATG TGC AC R: ACC GCG ATA TCG TTG GT	550	Hamid <i>et al.</i> , 2020
<i>pml</i>	F: GGATCATCTATAATGAACTG R: CTGATAATCAACTTGAAGTT	563	Allawi & Motaweq 2021

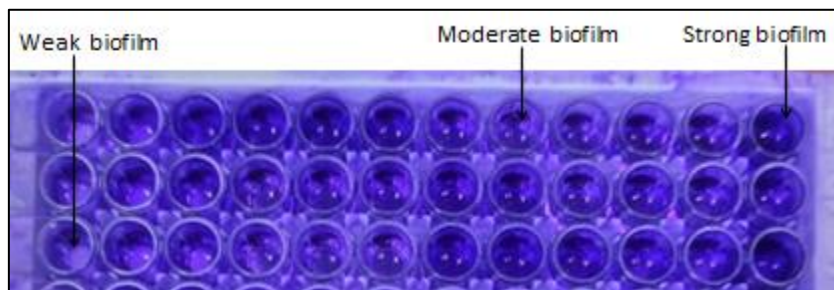
## RESULT

A total of 63 UTI samples were tested, and eleven *Proteus mirabilis* isolates were found. The results revealed 4 of the isolates tested were susceptible. While there were 7 resistant isolates, Plant extracts have been shown to be the effectiveness increases, which leads to inhibition of bacterial growth, noting that microorganisms are affected whenever the higher dose concentration (100 mg/ml) table 2 figure 2. Also, all *P.*

*mirabilis* isolates were able to production biofilms (100%). Using sterile 96-well microtiter plates figure 1, eight *P. mirabilis* produced robust biofilms, while 2 isolates produced intermediate biofilms and one isolation was weak. There were nine isolates that contained the *blaCTX-M* gene and produced MBL, while two isolates produced moderate biofilms and one isolation was weak biofilm production. There were nine isolates containing the *blaCTX-M* gene. All bacterial isolates carried the *pml* gene figure 3.

**Table 2: The effect of garlic extract on *P. mirabilis***

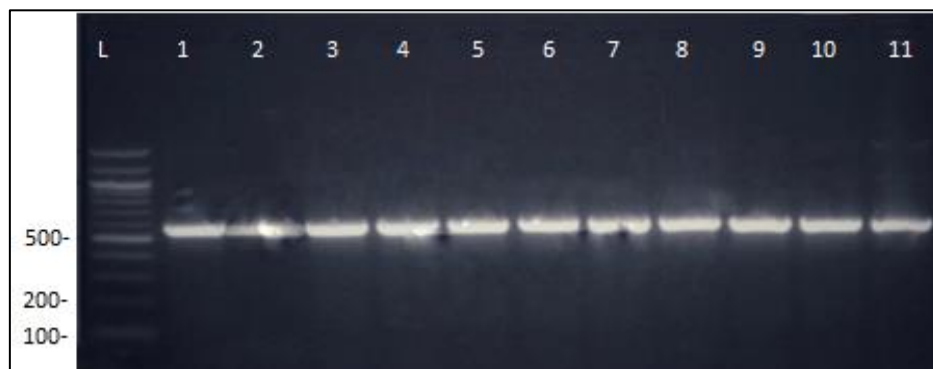
<i>Proteus mirabilis</i>	The concentration of <i>Garlic</i> extract			
	25 mg/ml	50 mg /ml	75 mg/ml	100mg /ml
Number of sensitive / mm	-	-	1 \ 9	3 \ 10
Number of moderate / mm	-	1 / 3	2 \ 6	1 / 11
Number of resistance	-	1 / 0	1 / 0	-
Control	+ Ve: ciprofloxacin		-Ve: DMSO 20%	



**Figure 1: microtiter plate 96-well polystyrene for biofilm formation by *P. mirabilis* tested**



**Figure 2: PCR amplification of *blaCTX-M* gene 550 bp. for *P. mirabilis* (L: 1500 bp) DNA ladder. (lanes: 1- 11) PCR product from *blaCTX-M* gene**



**Figure 3: PCR amplification of *pm1* gene 563 bp. for *P. mirabilis* (L: 1500 bp) DNA ladder. (lanes: 1- 11) PCR product from *pm1* gene**

**DISCUSSION**

The emergence of antibiotic-resistant bacteria is a health problem that must be eliminated because it threatens the public health of society. There must be an alternative, as plants contain substances that fight bacterial infections, and this is considered an alternative source of medicines and antibiotics. This study observed the inefficiency of garlic extract against the biofilm-production *Proteus mirabilis* a identified antibiotics resistant bacteria. Plants play an important

role in continuing the health of a community and improving its quality of life. (Lionel *et al.*, 2020).

The study aimed to investigate the inhibitory effect of garlic extract concentration on *P.* The data showed that the response was dependent on the dose or concentration, with an extract concentration of 100 mg/ml being new helpful. By measuring the zone of inhibition of the plant extract, we determined its effect, and this gave a clear result, as the highest concentration of the extract 100 mg/ml is considered to have the

greatest effect of inhibiting microbial growth, and this reflects the importance of the plant extract in getting rid of disease and killing bacteria (Kubba *et al.*, 2021)

This study showed that garlic extract has an inhibitory effect on *P. mirabilis*. This results from the interaction of garlic extract with important factors for bacterial growth and the production of unfavorable conditions that stopped their growth and led to an increase in the inhibition zone. The result is consistent with some studies recorded by (Al-Defiery *et al.*, 2021) and (Petropoulos *et al.*, 2018), which stated that garlic plant extract meddles with quorum sensing and differ with study reported by (Alyasari *et al.*, 2018).

This study demonstrated that biofilm production using phenotypic examination was 100% of all biofilm-producing bacterial isolates. They also all carried the *pml* gene, and this result is consistent with the investigation conducted by (Allawi and Motawaq 2021) and the percentage was 100%. There was a high level of agreement on the phenotypic and genotypic patterns of biofilm-producing bacteria. The result was also close to a study conducted by (Hussein *et al.*, 2020). This study found that the *blaCTX-M* gene is present in 9 isolates, at a rate of 81%, and these results are close to (Musa *et al.*, 2019). But in contrast to the results recorded by (Shabeeb *et al.*, 2017), where the result was 44.74%. and the result in this study differ with results reported by (Tamma *et al.*, 2021).

## CONCLUSION

The study concluded that the garlic extract presented a significant inhibitory consequence on both the susceptible and moderately resistant *P. mirabilis* associated with specimen isolated from urine sample which could emphasize the role of the dietary supplement of garlic to reduce antibiotic resistant strain in the community acquired infections. In addition, *Proteus mirabilis* biofilms be apt to production high metallo-beta-lactamase on a regular basis, which can further contribute to antibiotic resistance.

## REFERENCES

- Abaas, A. T., Salih, H. A., & Al-Waheeb, A. N. (2022). *Serratia marcescens* isolated from neonate patients with septicemia and study of the inhibition effect of Garlic plant extracts compared to antimicrobial agents in Thi Qar province in Iraq. *International Journal of Health Sciences*, 6(S6), 9900–9908. <https://doi.org/10.53730/ijhs.v6nS6.12557>
- Hamid, S. F., Taha, A. B., & Abdulwahid, M. J. (2020). Distribution of *blaTEM*, *blaSHV*, *blaCTX-M*, *blaOXA*, and *blaDHA* in *Proteus mirabilis* Isolated from Diabetic Foot Infections in Erbil, Iraq. *Cellular and Molecular Biology*, 66(1), 88-94.
- Shaaban, M., Abd El-Rahman, O. A., Al-Qaidi, B., & Ashour, H. M. (2020). Antimicrobial and antibiofilm activities of probiotic *Lactobacilli* on antibiotic-resistant *Proteus mirabilis*. *Microorganisms*, 8(6), 960.
- Bhat, S., Muthunatarajan, S., Mulki, S. S., ArchanaBhat, K., & Kotian, K. H. (2021). Bacterial infection among cancer patients: analysis of isolates and antibiotic sensitivity pattern. *International journal of microbiology*, 2021.
- Jain, I., Jain, P., Bisht, D., Sharma, A., Srivastava, B., & Gupta, N. (2015). Comparative evaluation of antibacterial efficacy of six Indian plant extracts against *Streptococcus mutans*. *Journal of clinical and diagnostic research: JCDR*, 9(2), ZC50.
- Kadhum, A. A., & Khudor, M. H. (2021). Phenotypic and molecular characteristics of biofilm and other virulence genes in *E. coli* and *K. pneumoniae* isolates from healthy dairy cow, human and environmental sources. *Indian Journal of Forensic Medicine & Toxicology*, 15(1), 2452-2458.
- Onsare, J. G., & Arora, D. S. (2015). Antibiofilm potential of flavonoids extracted from *Moringaoleifera* seed coat against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans*. *Journal of applied microbiology*, 118(2), 313-325.
- Alyasari, H. F., Al-khafaji, J. K., & Al-Masoudi, H. K. (2018). Inhibitory effects of garlic extract on uropathogenic *Escherichia coli*; *Proteus mirabilis* and *Trichomonas vaginalis* isolated from urogenital tract cases. *Research Journal of Pharmacy and Technology*, 11(3), 1071-1077.
- Wasfi, R., Hamed, S. M., Amer, M. A., & Fahmy, L. I. (2020). *Proteus mirabilis* biofilm: development and therapeutic strategies. *Frontiers in cellular and infection microbiology*, 10,
- Fufa, B. K. (2019). Anti-bacterial and anti-fungal properties of garlic extract (*Allium sativum*): A review. *Microbiol. Res. J. Int*, 28, 1-5.46552.
- Allawi, F. A., & Motawaq, Z. Y. (2021). Phenotypic and Molecular Correlation Between Biofilm Production and Antibiotic Resistance of *Proteus Mirabilis* Isolated From Different Clinical Sources/Iraq. *Turkish Journal of Physiotherapy and Rehabilitation*, 32(3).
- Lionel, O. O., Adegboyega, I. P., Ezekiel, A. O., & Olufunke, B. C. (2020). Antimicrobial activity of garlic (*Allium sativum*) on selected uropathogens from cases of urinary tract infection. *Annals of Tropical Pathology*, 11(2), 133.
- Kubba, M. A., Hussein, S. M., & Al-Zaidi, O. S. (2021). The Effect *Allium sativum* (Garlic Extract) as Prebiotic Substance on the Activity of Probiotic Bacteria *Lactobacillus acidophilus* Against Some Locally Isolates of Pathogenic Bacteria. *Indian Journal of Forensic Medicine & Toxicology*, 15(2), 387-394.
- Al-Defiery, M. E., Al-Muttairi, A. K., Saeed, H. H., & Hadi, R. K. (2021, April). Antimicrobial activity

of garlic and Pomegranate Peel extracts against some pathogenic bacteria. In *IOP Conference Series: Earth and Environmental Science* (Vol. 722, No. 1, p. 012017). IOP Publishing.

- Petropoulos, S., Fernandes, Â., Barros, L., Ciric, A., Sokovic, M., & Ferreira, I. C. (2018). Antimicrobial and antioxidant properties of various Greek garlic genotypes. *Food chemistry*, 245, 7-12.
- Hussein, E. I., Al-Batayneh, K., Masadeh, M. M., Dahadhah, F. W., Al Zoubi, M. S., Aljabali, A. A., & Alzoubi, K. H. (2020). Assessment of pathogenic potential, virulent genes profile, and antibiotic susceptibility of *Proteus mirabilis* from urinary tract infection. *International journal of microbiology*, 2020(1), 1231807.
- Shabeeb, B. T., Alghanimi, Y. K., Al-Juhaishi, A. M., & Ahmed, M. M. (2017). Detection of CTX-M genes from  $\beta$ -lactam Resistance *Proteus mirabilis* associated with Urinary Tract Infection in Holy Karbala province, Iraq. *International Journal of Pharmaceutical Quality Assurance*, 9(4), 410-15.
- Musa, H. A., Osman, M. A., Abdelaziz, Y. H., Mohamed, S., & Ibrahim-Saeed, M. (2019). Distribution of extended-spectrum beta-lactamase TEM and CTX-M resistance genes among *Proteus* species isolated in Sudan. *VacchiMonitor*, 28(2), 80-84.
- Yap, P.S.; Krishnan, T.; Yiap, B.C.; Hu, C.P.; Chan, K.G.; Lim, S.H. Membrane disruption and anti-quorum sensing effects of synergistic interaction between *Lavandula angustifolia* (lavender oil) in combination with antibiotic against plasmid-conferred multi-drug-resistant *Escherichia coli*. *J. Appl. Microbiol.* 2014, 116, 1119–1128. [CrossRef] [PubMed]
- Vijayalakshmi, S. (2023). Chapter-6 An Overview of Antibiotic Resistance and Herbal Remedies for Urinary Tract Infection. *ISBN: Book DOI: Price:762/-, 87.*
- Yuan, F., Huang, Z., Yang, T., Wang, G., Li, P., Yang, B., & Li, J. (2021). Pathogenesis of *Proteus mirabilis* in catheter-associated urinary tract infections. *Urologia internationalis*, 105(5-6), 354-361.
- Alyasari, H. F., Al-khafaji, J. K., & Al-Masoudi, H. K. (2018). Inhibitory effects of garlic extract on uropathogenic *Escherichia coli*; *Proteus mirabilis* and *Trichomonas vaginalis* isolated from urogenital tract cases. *Research Journal of Pharmacy and Technology*, 11(3), 1071-1077.
- Tamma, P. D., Smith, T. T., Adebayo, A., Karaba, S. M., Jacobs, E., Wakefield, T., ... & Simner, P. J. (2021). Prevalence of bla CTX-M genes in Gram-negative bloodstream isolates across 66 hospitals in the United States. *Journal of clinical microbiology*, 59(6), 10-1128.