JOURNAL OF NATURAL REMEDIES
DOI: 10.18311/jnr/2023/31916
SHORT COMMUNICATION

Detection of Antibacterial Activity of Garlic Against Acinetobacter Species Isolated from Patients in Tertiary Care Hospital

Sareena Rao and Deepashri Naik*
Department of Microbiology, MGM Medical College and Hospital, Kamothe, Navi Mumbai – 410209, Maharashtra, India; deepashri82@gmail.com

Abstract

The aim of the present study evaluates the antibacterial activity of garlic extracts on isolates of *Acinetobacter.* A total of 100 strains were collected from various samples at MGM hospital in Kamothe, Navi Mumbai. The susceptibilities of isolates to different antibiotics were tested by using the Kirby Bauer agar disk diffusion method. Seventy-nine per cent of isolated strains showed resistance to at least 14 or more antibiotics and all strains are multidrug resistant. Antibacterial activity of different concentrations of Garlic Extract was measured by well diffusion and the disk diffusion method. Garlic extract was used in the range of 5% to 100%. The present study suggests that multidrug-resistant *Acinetobacter* bacteria with significant antibiotic resistance show significant susceptibility to modest amounts of garlic extracts.

Keywords: *Acinetobacter species, Allium sativum* (Garlic), Antibacterial Activity

1. Introduction

The genus *Acinetobacter* belongs to the Moraxellaceae family. They are Gram-negative strictly aerobic, non-motile, non-fermentative organisms[1]. Despite this, it has received more attention as a nosocomial pathogen in the last two decades[2]. Around the world, *Acinetobacter* is the most common cause of hospital-acquired infections. They cause a wide range of clinical complications, such as Ventilator-associated pneumonia isolates accounting for 12.8% of all isolates. Isolates with central line-associated bloodstream infection accounted for 8.8% of all isolates. Isolates of catheter-associated urinary tract infection (1.3%). Surgical site infection isolates[1]. Due to multidrug resistance, treatment failure has become more common with *Acinetobacter* strains because therapeutic choices for infections caused by them are restricted[3]. Garlic is one such botanical plant. Garlic has been traditionally used as a dietary as well as medicine for thousands of years. Throughout history, garlic has played an important dietary and therapeutic function[4].

Garlic (*Allium sativum L.*) belongs to the arc family of plants (*Amaryllidaceae*)[5]. Garlic was traditionally utilized by Indian and Chinese to aid breathing and digestion, as well as to treat leprosy and parasitic infestations. Garlic has received a lot of attention in modern medicine because of widespread belief in its health-promoting properties[6].

Alliin is a chemical substance found in fresh garlic. When the cloves are diced or crushed, an enzyme called alliinase is activated and then Alliin is converted to allicin by this enzyme. In fresh crushed or chopped garlic, pure allicin is stable only for a short time. However, after crushing or cutting garlic, allowing garlic to sit for 10 minutes may help to boost level[7].

At present, we study the antimicrobial property of garlic against *Acinetobacter* species by using standard methods.

*Author for correspondence

Article Received on: 11.11.2022 Revised on: 12.01.2023 Accepted on: 07.03.2023

2. Materials and Methods

A total of 100 isolates were collected from clinical specimens of patients of MGM Hospital in Kamothe, Navi Mumbai, during one year between February 2021 to February 2022. The isolates were identified as *Acinetobacter* species by using Standard Microbiological Procedures (Gram stain, motility, biochemical tests)

2.1 Determination of Susceptibility to Antibiotics

The Antibiotic Susceptibility Test was performed by Kirby Bauer Disk Diffusion Method[8] by using CLSI guidelines 2021.

2.2 Preparation of Standard Dilutions of Garlic Extract

Each time, 100 per cent garlic powder was inoculated on nutritional agar media and incubated at 37°C overnight to ensure sterility. By diluting the concentrated (100%) powder with adequate sterile distilled water, the garlic extract was diluted to 10–25%, 50%, 75% and so on[9].

2.3 Well Diffusion Method

Mueller Hinton Agar (MHA) was put into 90mm plates with a 3–4 mm agar depth. The test culture was smeared equally over the plate in three directions with a sterile cotton swab to obtain an even inoculum. For 3–5 minutes, the plates were left to dry. With the help of a sterile cork borer, wells of 5 mm diameter were cut on the surface of the agar. 5%, 10%, 25%, 50%and 100% solutions (v/v) of garlic extract(powder) were added to different wells and in one well, normal saline was added. The plates were incubated at 37°C for 22 h. The zone of inhibition was measured by a scale to the nearest mm including disc diameter[10].

2.4 Preparation of Antibiotic Garlic Disk

The filter paper discs were prepared by punching 6mm diameter holes in the Whatman filter paper, which was then sterilized in an autoclave.

The antibiotic solutions are put on each disc using a mechanical pipette during disc impregnation.

The discs were dried in an incubator and kept at - 20°C in tiny ampoules with a desiccant[9].

3. Result

The prevalence of *Acinetobacter* species was highest in the IPD ICU patients in comparison with IPD ward patients which was 47.00% and 40.00% respectively. Comparatively the isolates of *Acinetobacter* species were less in OPD patients (13%) (Figure 1).

Out of all *Acinetobacter* species isolated, *Acinetobacter baumannii* was (68%) and *Acinetobacter lwoffii* was (32%) (Figure 2). Seventy-nine per cent of *Acinetobacter* showed resistance to at least 14 antibiotics or more.

3.1 Susceptibility Pattern of Acinetobacter Species with Garlic

As we increase the concentration of garlic the susceptibility of *Acinetobacter* species goes on increasing for garlic. Seventy-five per cent of *Acinetobacter* species showed Susceptibility to garlic (Figure 3).

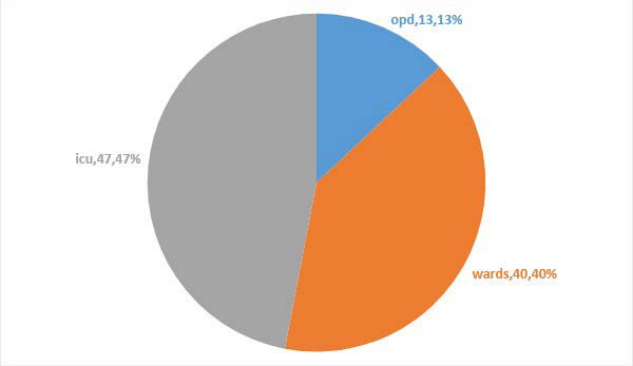

Figure 1. Prevalence of *Acinetobacter* species.

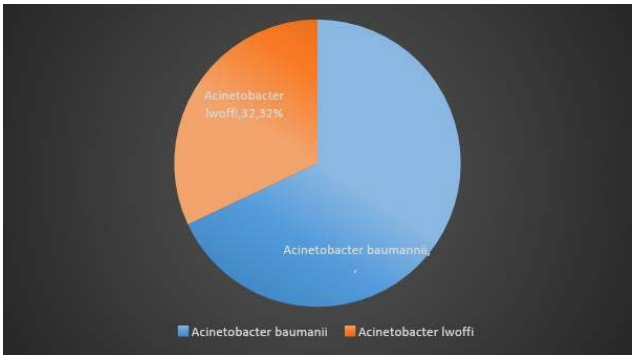

Figure 2. Prevalence of *Acinetobacter baumannii* and *Acinetobacter lwoffii.*

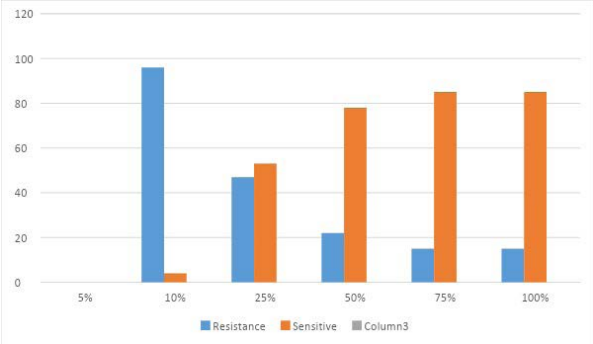

Figure 3. Susceptibility pattern of Acinetobacter species with garlic.

3.2 Susceptibility of both *Acinetobacter baumannii* and *Acinetobacter lwoffii* for Garlic in Well Diffusion and Disk Diffusion Method

Susceptibility of both *Acinetobacter baumannii* and *Acinetobacter lwoffii* for garlic is the same in well diffusion as well as Disk diffusion method (Figures 4, 5 and 6).

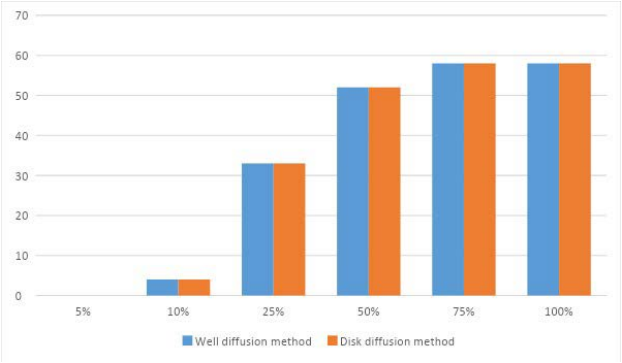

Figure 4. Susceptibility of *Acinetobacter baumannii*.

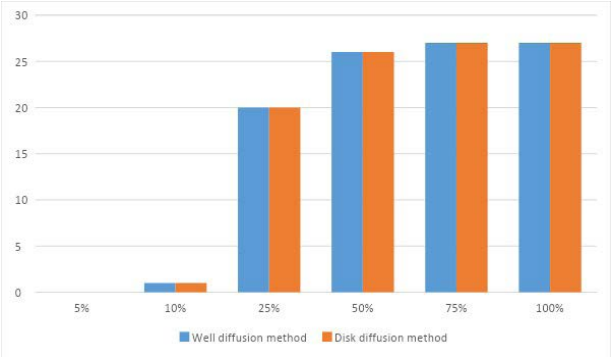

Figure 5. Susceptibility of *Acinetobacter lwoffii.*

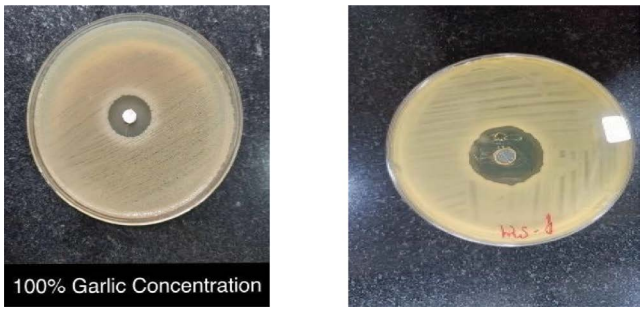

Figure 6. Susceptibility of both Acinetobacter in well diffusion as well as disk diffusion method.

3.3 Comparison of Susceptibility of *Acinetobacter* Species with the Conventional Antibiotics with Commercially Available Garlic

For treatment of infections caused by *Acinetobacter baumannii* levofloxacin can be given. But if resistance is detected for Levofloxacin, then along with higher antibiotic Polymyxin B, garlic extract can be given as an adjuvant for treatment.

Susceptibility of *Acinetobacter lwoffii* is more for garlic (84.3%) as compared to Levofloxacin (75%), for treatment levofloxacin along with garlic extract can be given. If resistance is detected for levofloxacin, a higher antibiotic Polymyxin B can be given for treatment (Table 1).

Table 1. Comparison of susceptibility of *Acinetobacter* species with antibiotics and garlic

***Acinetobacter* species**	**Antibiotic**	**Antibiotic**	**Garlic**	**Garlic**
	First line antibiotic	Second line anti-biotl c	Well diffusion method	Disk diffusion method
Acinetobacter baumannii	Levofl-oxacin (64.7 %).	Polymyxin B (75%).	85%	85%
Acinetobacter lwoffii	Levofl-oxacin (75%).	Polymyxin B(100%)	84.3%	84.3%

4. Discussion

According to the current study, 87% of *Acinetobacter* species were isolated from Inpatient (IPD) patients, while the remaining 13% were identified from Outpatient patients (OPD). Amongst these 87 inpatients, the highest number of *Acinetobacter* species were isolated from MICU (25.28%) followed by SICU (19.5%), Medicine (13.8%), EMS ICU (6.8%), COVID ICU (6.8%), Orthopedics (6.8%), FSW (6.8%), Surgery (4.5%), Respiratory Medicine (4.5%), and MSW (4.5%). In a study done by S. Das, *et al*., ICUs had the greatest percentage of *Acinetobacter baumannii* strains (23.3%), followed by the Orthopedics ward (21.7%), and the General ward (21.7%), from the Surgery Ward (20%) respectively[11].

In this study among all *Acinetobacter* species, *Acinetobacter baumannii* was found to be the maximum (68%) and the remaining isolate was *Acinetobacter lwoffii* (32%). In a study done by Victor Moses Musyoki[12] *Acinetobacter baumannii* was found in 95 per cent of the samples tested. Other species found in low numbers included *Acinetobacter lwoffii* (3%), *Acinetobacter haemolyticus* (1%), *Acinetobacter calcoaceticus* (n = 1) and *Acinetobacter junii* (n = 1).

As we increase the concentration of garlic the susceptibility of *Acinetobacter* species goes on increasing. It shows the highly preventing activity towards the growth of *Acinetobacter* species.

In a study done by Ivana Cirkovic, *et al*.,[13] the agar diffusion method was used to assess the antibacterial properties of Ethanol garlic extract, which revealed that it has high antimicrobial activity against Gram-positive bacteria but not Gram-negative bacteria. *S. aureus* was shown to be the most sensitive bacteria whereas *P. aeruginosa* was found to be the most resistant. In another study done by Nima Hosseini Jazani, *et al*.,[14] Seventy-five per cent of the *Acinetobacter* strains were Multi-Drug Resistant (MDR) strains, meaning they were resistant to at least 12 antibiotics and more. This study found that chloroformic garlic extract has a bactericidal effect against multi-antibiotic-resistant *Acinetobacter* strains. It means that multidrug-resistant *Acinetobacter* bacteria with high antibiotic resistance may be sensitive to garlic extracts in small doses.

The susceptibility pattern of *Acinetobacter baumannii* and *Acinetobacter lwoffii* for garlic is the same in the well diffusion method and disk diffusion method. In one study done by Qurat Ul Ain *et al*.,[15] 10 different plant materials were taken to see their antimicrobial activity against 15 clinical isolates of MDR *A. baumannii* by using the agar well diffusion method. They used the plants included: betel leaves, lemon peel, lemon seeds, bael leaf, watermelon peel, garlic, bitter gourd pulp, bitter gourd seeds and vinca rosea leaves. They observed betel leaf ethanol extract showed a promising effect on *A. baumannii* isolates and garlic, bitter gourd, bitter gourd seeds and vinca rosea leaf showed weak activity against only a few strains.

5. Conclusion

Out of all *Acinetobacter* species isolated, the maximum strains were *Acinetobacter baumannii* (68%) and *Acinetobacter lwoffii* (32%).

As the concentration of garlic extract goes on increasing like 25%, 50%, 75% and so on the susceptibility of both *Acinetobacter baumannii* and *Acinetobacter lwoffi* also goes on increasing.

Susceptibility of both *Acinetobacter baumannii* and *Acinetobacter lwoffii* for garlic is the same in good diffusion as well as disk diffusion method.

Garlic consumption may be used as an economic way to the treatment bacterial infectious disease caused by *Acinetobacter* species to reduce the problem of multi–drug resistance.

6. References

1. Ananthanarayan R. Ananthanarayan and Paniker's textbook of microbiology. Orient Blackswan; 10th ed; 2017. p. 404-5.
2. Sastry AS, Bhat S. Essentials of medical microbiology. JP Medical Ltd; 3rd ed; 2021. p. 645-646.
3. Jung J, Park W. Acinetobacter species as model microorganisms in environmental microbiology: current state and perspectives. Applied Microbiology and Biotechnology. 2015; 99(6):2533-48. https://doi.org/10.1007/s00253-015-6439-y PMid:25693672
4. Yadav S, Trivedi NA, Bhatt JD. Antimicrobial activity of fresh garlic juice: An *in vitro* study. Ayu. 2015; 36(2):203. https://doi.org/10.4103/0974-8520.175548 PMid:27011724 PMCid:PMC4784133
5. Strika I, Bašić A, Halilović N. Antimicrobial effects of garlic (*Allium sativum* L.). Bulletin of the Chemists and Technologists of Bosnia and Herzegovina. 2017; 47(7): 17-20.

6. Bayan L, Koulivand PH, Gorji A. Garlic: A review of potential therapeutic effects. Avicenna J Phytomed. 2014; 4(1):1-14.
7. Calsamiglia S, Busquet M, Cardozo PW, Castillejos L, Ferret A. Invited review: Essential oils as modifiers of rumen microbial fermentation. Journal of Dairy Science. 2007; 90(6):2580-95. https://doi.org/10.3168/jds.2006-644 PMid:17517698
8. Sharma R, Otieno S, Otieno S. Kirby Bauer disc diffusion method for antibiotic susceptibility testing. Microbe Notes; 2022.
9. Vineetha N, Vignesh RA, Sridhar D. Preparation, standardization of antibiotic discs and study of resistance pattern for first-line antibiotics in isolates from clinical samples. International Journal of Applied Research. 2015; 1(11): 624-31.
10. Yadav M, Bohra R, Gupta N. *In vitro* determination of antibacterial effect of garlic (*Allium sativum*) on *Staphylococcus aureus* and *E. coli*. Int J Curr Microbiol Appl Sci. 2019; 8(09):498506. https://doi.org/10.20546/ijcmas.2019.809.060
11. Das S, Basak S. *Acinetobacter baumanii* complex and its beta-lactamase production: Are we moving towards pre antibiotic era? Int J Health Sci Res. 2018; 8(3):60-69.
12. Musyoki V, Masika M, Mutai W, Gitau W, Kuria A, Muthini F. Antimicrobial susceptibility pattern of acinetobacter isolates from patients in Kenyatta National Hospital, Nairobi, Kenya. Pan African Medical Journal. 2019; 33. https://doi.org/10.11604/pamj.2019.33.146.17220 PMid:31558943 PMCid:PMC6754852
13. Cirkovic I, Jovalekic M, Jegorovic B. *In vitro* antibacterial activity of garlic and synergism between garlic and antibacterial drugs. Archives of Biological Sciences. 2012; 64(4):1369-75. https://doi.org/10.2298/ABS1204369C
14. Jazani NH, Shahabi S, Ali AA, Zarrin S, Daie NA. *In vitro* antibacterial activity of garlic against isolates of *Acinetobacter sp*. Journal of Biological Sciences. 2007; 7(5):819-22. https://doi.org/10.3923/jbs.2007.819.822
15. Ain Q, Naeem S, Naim A. Antibacterial activity of ethanolic plant extracts on multidrug-resistant *Acinetobacter baumannii* clinical isolates. Pakistan Journal of Botany. 2022; 54(5). https://doi.org/10.30848/PJB2022-5(35)