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Synergistic antibacterial activity of garlic extract against uropathogenic gram-negative bacilli

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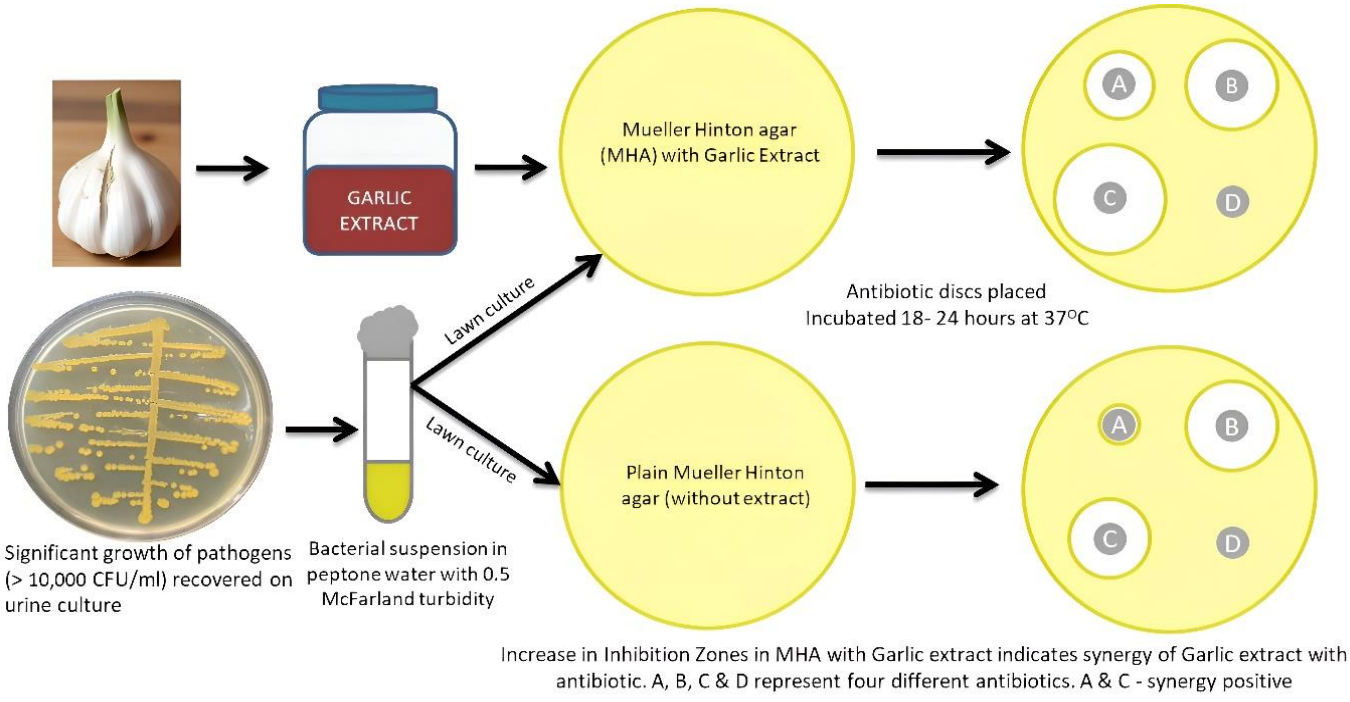
ABSTRACT

Garlic extract is known to have distinct antibacterial activity and synergistic effect with various antibacterial agents. This study was carried out to evaluate synergistic activity of garlic extract with antibiotics against uropathogenic gram-negative bacteria isolated from patients with symptomatic urinary tract infection. Gram negative bacterial isolates with significant bacteriuria (>10,000 CFU/ml) recovered on urine culture from patients with symptomatic UTI were included in the study. Biochemical identification antibiotic susceptibility tests were performed as per standard microbiology procedures. The synergy of garlic extract with antibiotics was determined by disc diffusion method. A total of 92 bacterial isolates, including 54 multi-drug resistant bacterial isolates, were obtained from 53 female and 39 male patients.



Escherichia coli was the most common (55.4%), followed by Klebsiella pneumoniae (25%), Non-fermenter gram negative bacilli (9.8%), Citrobacter spp (7.6%), and Proteus vulgaris (2.2%). Resistance to cefotaxime, nalidixic acid, norfloxacin cotrimoxazole and nitrofurantoin was 72.8%, 64.1%, 50%, 47.8% and 45.7% respectively. A large majority of these isolates showed synergy of garlic extract with amikacin (80.4%), gentamicin (83.7%), imipenem (83.7%), meropenem (77.2%), fosfomycin (82.6%), nitrofurantoin (80.4%), cefoperazone-sulbactam (77.2%). Furthermore, addition of garlic extract effectively improved susceptibility of these gram-negative uropathogenic bacteria to amikacin, gentamicin, imipenem and nitrofurantoin. Our study demonstrated synergistic effects of garlic extract with several antibiotics against uropathogenic bacteria, suggesting its potential use as an adjunctive therapy with antibiotics. However, further studies are required before it can be introduced for clinical use.

Keywords: Garlic extract, Allicin, Antibacterial synergy, Uropathogens, Multidrug-resistant bacteria.

INTRODUCTION

Urinary tract infection (UTI) is one of the most common bacterial infections in community as well as in hospitals. It affects 150 millions of individuals worldwide each year[1]. As per an estimate, about 12%-16% adult patients have an indwelling urinary catheter at some time during hospital stay and CA-UTIs account for 12-30% of infections in acute care hospitals[2]. These infections lead to significant health complications unless treated effectively. The clinical presentation of UTI can range from asymptomatic bacteriuria or mild infection with vague manifestations to severe complicated infections such as bacteremia pyelonephritis, purulent urethritis, prostatitis, urethral stricture, urolithiasis, urinary tract obstruction and subsequent hydronephrosis[3]. Due to the variable and wide spectrum of clinical presentations, there is ample scope of antibiotic misuse and overuse in UTI cases giving rise to development of resistance to commonly used antibiotics. As antibiotic resistance continues, the treatment options are increasing becoming ineffective. Furthermore, the slow pace of development of new antibiotics is posing an additional challenge. Hence, there is a growing interest on natural antibacterial agents which can be introduced as an adjunctive or alternative therapy to conventional antibiotics. Garlic (Allium sativum) has been known for centuries for its medicinal properties and have been used as a remedy for infections. Researchers have recognized garlic extract for its antimicrobial properties and synergistic action with antibiotics. The objective of this study was to evaluate synergistic activity of garlic extract with antibiotics against uropathogenic bacteria isolated from patients with symptomatic urinary tract infection.

MATERIAL AND METHODS

Study Design

A laboratory-based cross-sectional study was carried out in department of Microbiology at a tertiary care hospital in South India during July 2023 to September 2023. Gram negative bacterial isolates from patients with symptomatic UTI during the period of study were included. National Healthcare Safety Network (NHSN) criteria was employed to define symptomatic UTI[4]. Significant growth of uropathogens (> 10,000 CFU/ml) recovered on culture of urine samples from patients with any one of the following symptoms - fever >38.0°C, suprapubic tenderness, costovertebral angle pain or tenderness, urinary urgency, frequency, dysuria in absence of catheter were included. Bacterial isolates from infants, asymptomatic bacteriuria, and duplicate isolates from same patient were excluded. Because of difference in antibiotic panel, gram-positive bacterial isolates and *Pseudomonas aeruginosa* were also excluded from the analysis.

Urine Culture

Urine samples received in Microbiology laboratory are processed as per standard microbiology procedures. A growth of 10,000 CFU/ml or more is considered as significant for which biochemical identification of the pathogen and antibiotic susceptibility tests are performed.

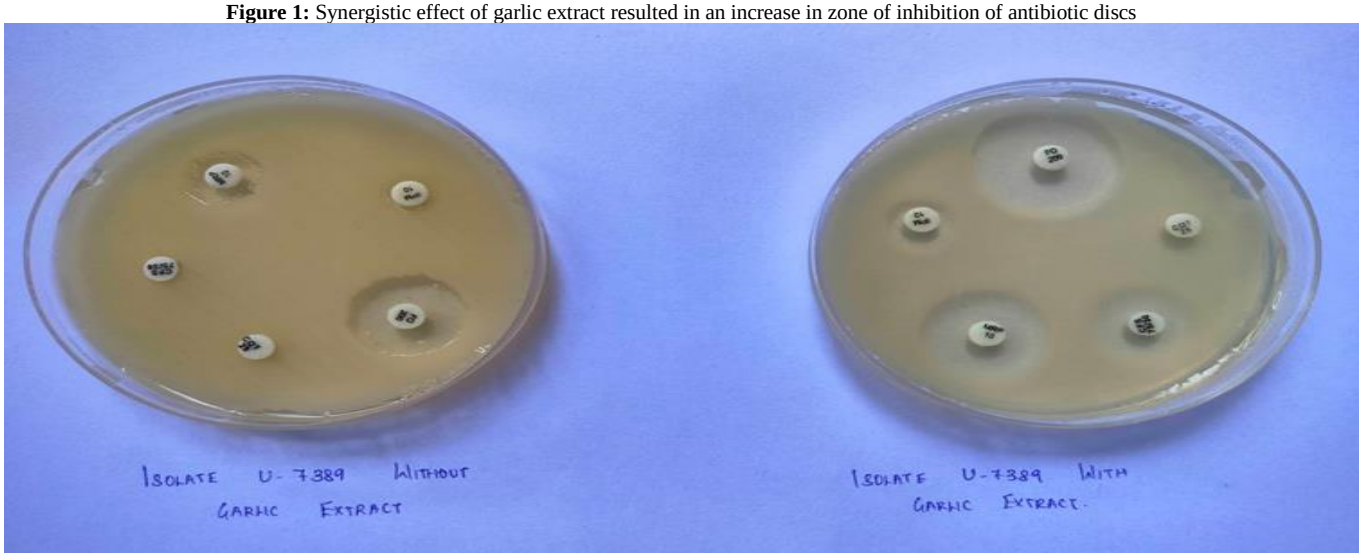
Antibiotic Susceptibility Testing

The bacterial isolates was done by Kirby Bauer's disk diffusion technique on Mueller Hinton agar (MHA) as per the latest Clinical Laboratory Standards Institute (CLSI) guidelines using a panel of antibiotics comprising of co-trimoxazole (1.25/23.75 µg), cefotaxime (30 µg), amikacin (30 µg), gentamicin (10 µg), imipenem (10 µg), meropenem (10 µg), nitrofurantoin (300 µg), nalidixic acid (30 µg), norfloxacin (10 µg), fosfomycin (200 µg) and cefoperazone-sulbactam (75/30 µg)[5].

Synergistic Antibacterial Activity of Garlic Extract

The antibacterial synergy was determined by disc diffusion method as described by Li *et al.*[6]. The antibiotic susceptibility was performed using two sets of MHA plates with same antibiotics for each isolate. While plain antibiotic discs were applied on the first set of MHA, 50 µL of 100% garlic extract (v/v) (procured from commercial sources) was added by micropipette to each antibiotic discs on the second set of MHA. A sterile Whatman No. 1 filter paper discs of 6 mm diameter imbibed with 50 µL of sterile distilled water was also incorporated on the second set of MHA as control. The plates were left for 30 mins for absorption of the solution by the disks and then incubated at 37^{O}C for 18-24 hours. The sizes of zone of inhibition (ZOI) were compared between the MHA with antibiotic discs with garlic extract and without the extract. Presence of synergy was considered if an antibiotic disc with garlic extract displayed larger ZOI in comparison its counterpart *i.e.* antibiotic disc without garlic extract (Figure 1).

Figure 1: Synergistic effect of garlic extract resulted in an increase in zone of inhibition of antibiotic discs



Statistical Analysis

All laboratory data were entered into Excel (MS Excel 2011). Data was analysed for descriptive statistics. P value was calculated with the help of GraphPad software.

RESULT

A total of 92 bacterial isolates were obtained from 53 female and 39 male patients. Most of these patients were in 21-50 years age group (n=48, 52.2%), followed by patients above 50 years of age (n=34, 37%) (Table 1). *Escherichia coli* was the most common uropathogen (n=51, 55.4%), followed by *Klebsiella pneumoniae* (n=23, 25%), Non-fermenter gram negative bacilli (n=9, 9.8%), *Citrobacter species* (n=7, 7.6%), and *Proteus vulgaris* (n=2 , 2.2%). The isolates were tested against a panel of antibiotics using Kirby-Bauer disc diffusion method. Of these 92 isolates, 54 were multi-drug resistant *i.e. E. coli* (n=32), *K. pneumoniae* (n=12), NFGNB (n=6), *Citrobacter species* (n=3), and *Proteus vulgaris* (n=1). Resistance was most common against cefotaxime (72.8%), followed by nalidixic acid (64.1%), norfloxacin (50%), cotrimoxazole (47.8%) and nitrofurantoin (45.7%) (Table 2).

Antibiotic synergy of garlic extract was estimated from the increase in ZOI in presence of garlic extract. Although a large fraction of these isolates ranging from 39.1% to 83.7% showed synergistic action of garlic extract with antibiotics, few isolates also had unchanged or reduced zone sizes (Table 3). While majority of the isolates displayed synergy of garlic extract with amikacin (80.4%), gentamicin (83.7%), imipenem (83.7%), meropenem (77.2%), fosfomycin (82.6%), nitrofurantoin (80.4%), cefoperazone-sulbactam (77.2%), a smaller number of these isolates had synergy of garlic extract with cotrimoxazole (60.9%), cefotaxime (46.7%), nalidixic acid (39.1%), and norfloxacin (39.1%) (Table 3).

Because of the change of ZOI after addition of garlic extract, the susceptibility improved from Resistant to Intermediate (R→I) or Resistant to Sensitive (R→S) or Intermediate to Sensitive (I→S) in few isolates for these antibiotics, especially for amikacin, gentamicin, imipenem and nitrofurantoin (Table 4). Table 5 illustrates the changes in susceptibility after addition of garlic extract among MDR and non-MDR bacterial isolates. Significant improvement susceptibility (p value < 0.05) to gentamicin and nalidixic acid as a result of synergistic effect of garlic extract was observed among MDR isolates in comparison to non-MDR isolates (table 5).

Table 1: Age and gender-wise distribution of patients

Age groups	Male	Female
0-10 years	3	2
11-20 years	1	4
21-30 years	3	27
31-40 years	6	3
41-50 years	5	4
51-65 years	10	8
Above 65 years	11	5
Total	**39**	**53**

Table 2: Antibiotic resistance pattern of bacterial uropathogens

Antibiotics	Resistant	Intermediate	Sensitive
Cotrimoxazole	44 (47.8%)	3 (3.3%)	45 (48.9%)
Cefotaxime	67 (72.8%)	16 (17.4%)	9 (9.8%)
Amikacin	19 (20.7%)	34 (37%)	39 (42.4%)
Gentamicin	26 (28.3%)	15 (16.3%)	51 (55.4%)
Imipenem	28 (30.4%)	30 (32.6%)	34 (37%)
Meropenem	15 (16.3%)	6 (6.5%)	71 (77.2%)
Nitrofurantoin	42 (45.7%)	14 (15.2%)	36 (39.1%)
Nalidixic acid	59 (64.1%)	12 (13%)	21 (22.8%)
Norfloxacin	46 (50%)	4 (4.3%)	42 (45.7%)
Fosfomycin	9 (9.8%)	7 (7.6%)	76 (82.6%)
Cefoperazone-sulbactam	19 (20.7%)	7 (7.6%)	66 (71.7%)

Table 3: Change in zone sizes of bacterial uropathogens in presence of garlic extract

	Increased ZOI				No change in ZOI	Reduced ZOI
Antibiotics	**>10 mm**	**6-10 mm**	**1-5 mm**	**Total**		
Cotrimoxazole	3	7	46	56 (60.9%)	31 (33.7%)	5 (5.4%)
Cefotaxime	0	5	38	43 (46.7%)	47 (51.1%)	2 (2.2%)
Amikacin	0	7	67	74 (80.4%)	16 (17.4%)	2 (2.2%)
Gentamicin	1	6	70	77 (83.7%)	15 (16.3%)	0 (0.0%)
Imipenem	5	12	60	77 (83.7%)	9 (9.8%)	6 (6.5%)
Meropenem	4	7	60	71 (77.2%)	15 (16.3%)	6 (6.5%)
Nitrofurantoin	1	14	59	74 (80.4%)	15 (16.3%)	3 (3.3%)
Nalidixic acid	0	2	34	36 (39.1%)	54 (58.7%)	2 (2.2%)
Norfloxacin	0	1	35	36 (39.1%)	49 (53.3%)	7 (7.6%)
Fosfomycin	2	10	64	76 (82.6%)	7 (7.6%)	9 (9.8%)
Cefoperazone-sulbactam	2	11	58	71 (77.2%)	12 (13%)	9 (9.8%)

Table 4: Change in susceptibility of bacterial uropathogens in presence of garlic extract

	No change in susceptibility				Improved susceptibility			
Antibiotics	S→S	I→I	R→R	**Total**	I→S	R→I	R→S	**Total**
Cotrimoxazole	45	1	34	80 (87%)	2	6	4	12 (13%)
Cefotaxime	9	7	65	81 (88%)	8	3	0	11(12%)
Amikacin	39	11	12	62 (67.4%)	23	5	2	30 (32.6%)
Gentamicin	51	6	15	72 (78.3%)	9	10	1	20 (21.7%)
Imipenem	34	9	18	61 (66.3%)	20	6	5	31 (33.7%)
Meropenem	71	5	13	89 (96.7%)	1	0	2	3 (3.3%)
Nitrofurantoin	36	4	22	62 (67.4%)	10	11	9	30 (32.6%)
Nalidixic acid	21	12	57	90 (97.8%)	0	2	0	2 (2.2%)
Norfloxacin	42	2	45	89 (96.7%)	2	1	0	3 (3.3%)
Fosfomycin	76	1	1	78 (84.8%)	6	7	1	14 (15.2%)
Cefoperazone-sulbactam	66	3	10	79 (85.9%)	4	9	0	13 (14.1%)

Table 5: Comparison of change in susceptibility of MDR and non-MDR bacterial isolates in presence of garlic extract

Antibiotics	**Type of pathogen**	**Improved susceptibility**	**No change in susceptibility**	**P-value**
Cotrimoxazole	MDR	7	47	>0.05
	Non-MDR	5	33	
Cefotaxime	MDR	1	53	>0.05
	Non-MDR	10	28	
Amikacin	MDR	18	36	>0.05
	Non-MDR	12	26	
Gentamicin	MDR	17	37	0.014526
	Non-MDR	3	35	
Imipenem	MDR	20	34	>0.05
	Non-MDR	11	27	
Meropenem	MDR	2	52	>0.05
	Non-MDR	1	37	
Nitrofurantoin	MDR	16	38	>0.05
	Non-MDR	14	24	
Nalidixic acid	MDR	2	52	0.002163
	Non-MDR	0	38	
Norfloxacin	MDR	1	53	>0.05
	Non-MDR	2	36	
Fosfomycin	MDR	12	42	>0.05
	Non-MDR	2	36	
Cefoperazone-sulbactam	MDR	41	13	>0.05
	Non-MDR	37	1	

DISCUSSION

Garlic (*Allium sativum*) has been shown to have broad-spectrum antimicrobial activity, making it effective against a wide range of bacteria, fungi, and even viruses. The primary compound responsible for garlic's antibacterial properties is allicin, a sulfur-containing compound. Allicin exerts its antibacterial effects by interfering with the thiol groups in bacterial enzymes. This interference disrupts essential metabolic functions within the bacteria, ultimately leading to cell death. Additionally, garlic has been shown to exert antibacterial action by inhibiting the synthesis of nucleic acids and proteins in bacteria, further compromising their ability to produce biofilm, survive and multiply. Minimum inhibitory concentration (MIC), agar-well and disc diffusion methods have been utilized to determine antibacterial activity of garlic in various studies. Karuppiah *et al.* evaluated antibacterial action of garlic cloves against MDR clinical pathogens by disc diffusion methods and found that *E. coli, P. aeruginosa*, and *Bacillus sp.* were the most susceptible as they produced ZOI of 19.45 mm, 18.5 mm and 16.5 mm and MIC of 65.50, 58.50 and 80.10 µg/mL respectively[7]. Garlic extract was also reported to have potent inhibitory effect against MDR *E.coli* and *Salmonella* [8]. In our study, we found 58.7% (54 out of 92) isolates recovered from patients with symptomatic UTI were multi-drug resistant. These

isolates displayed a higher resistance against cefotaxime (72.8%), followed by nalidixic acid (64.1%), norfloxacin (50%), cotrimoxazole (47.8%) and nitrofurantoin (45.7%). This is similar to other studies from India [9, 10]. In a multi-centric study, increasing incidence of resistance against first- and second-generation cephalosporins, fluoroquinolones and co-trimoxazole among uropathogens was detected at the community-settings of different regions in India [10]. Likewise, uropathogens like *E. coli* and *K. pneumoniae* displayed higher resistance against cotrimoxazole, ciprofloxacin and cefuroxime in another study from Rajasthan [9].

We analysed the change in ZOI of each antibiotic in presence of garlic extract for 92 bacterial isolates in order to evaluate the synergistic action of garlic extract with antibiotics. Although there were several strains with no change in zone sizes and occasional isolates with reduced zone sizes, on an average 40-80% isolates showed increase in zone sizes due to synergistic action of garlic extract with antibiotics. The synergistic antibacterial action of garlic extract was estimated by qualitatively grading the increase in zone sizes as shown in table 3. About one third of our isolates displayed improved susceptibility from Resistant to Intermediate or Sensitive and Intermediate too Sensitive as a result of increase in ZOI for amikacin, gentamicin, imipenem and nitrofurantoin. Moreover, the improvement in susceptibility to gentamicin and nalidixic acid among MDR isolates was statistically significant compared to non-MDR isolates. These findings are consistent with recent studies which have evaluated the synergism of garlic extract with antibiotics [6, 11-13]. Li *et al.* utilized disc diffusion method to evaluate synergistic interaction of fresh garlic extract (FGE) with antimicrobials and observed an increase in cefoxitin, oxacillin, piperacillin zone sizes in Methicillin resistant *Staphylococcus aureus* (MRSA) isolates, cefotaxime, levofloxacin, ceftriaxone, ampicillin and cefazolin zone sizes in *Pseudomonas aeruginosa* and itraconazole and fluconazole zone sizes in *Candida albicans* respectively in presence of FGE[6]. They also reported an obvious increase of 29 mm and 30.5 mm in the zone size of itraconazole and fluconazole with FGE in *Candida albicans* [6]. In another study, Magryś *et al.* evaluated the synergistic interaction between FGE and antibiotics by checkerboard method [11]. They noted synergistic potentiation of antibacterial action of gentamycin and ciprofloxacin against MDR pathogens by garlic [11]. Pillai *et al.* confirmed the synergism of FGE with ampicillin by observing a sharp reduction in ampicillin MIC against S. aureus from 24 µg/ml to 2 µg/ml on addition of FGE [12]. Similar synergism was also observed in another study[13]. Furthermore, synergistic combinations of novel agents with antibiotics have recently been introduced as an effective strategy to eradicate infections caused by biofilm-producing and MDR-pathogens. In a study conducted by Bhatwalkar *et al.*, garlic was found to show potent anti-biofilm action as it significantly decreased biofilm formation by 35 to 59% and reduced total carbohydrate content of biofilm by 40% [14]. This is especially relevant in the context of urinary tract infections as biofilm production plays a major role in its pathogenesis.

CONCLUSION

Allicin and garlic derivatives are natural bioactive agents with strong antibacterial property. This study highlights the potential of garlic extract to enhance the efficacy of conventional antibiotics against gram-negative bacterial pathogens causing UTI. The synergistic effect of garlic extract with antibiotics is particularly noteworthy in the context of UTI as Garlic derivatives can be employed as a promising adjunctive to systematic antibiotic therapy as well as a coating agent on urinary catheter to prevent colonization of multi-drug resistant, biofilm forming uropathogenic bacteria. However, additional studies are necessary before it can be introduced for clinical use.

Conflict of interest

The authors have no conflicts of interest regarding this investigation.

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