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# Garlic: An Effective Functional Food to Combat the Growing Antimicrobial Resistance

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## ABSTRACT

Emerging multidrug resistant bacterial infections are burning public health concerns worldwide. There is an urgent need to explore alternative antimicrobial agents for effective management of bacterial infections. Garlic (*Allium sativum*) has been traditionally used for the treatment of different diseases since ancient times. The present study aims to evaluate the antimicrobial activity of aqueous extract *of Allium sativum* against multidrug resistant clinical isolates of pathogenic bacteria found in human urine in cases of urinary tract infection (UTI). A total of 166 uropathogens were evaluated for antibiotic susceptibility, 56% clinical isolates were found to have high degree of resistance with multiple antibiotic resistance (MAR) index >0.5. About 82% bacterial isolates with MAR index >0.5 were found to be susceptible to crude aqueous garlic extract. Thus, aqueous garlic extract (AGE) was found to possess effective anti-bacterial activity against multidrug resistant clinical pathogens and may be tested further as a natural dietary component to manage drug resistance bacteraemia. Therefore, alternate medicine practices with natural plant extract including garlic may be of great importance in combating public health challenges like UTI.

*Keywords:* Garlic, Multidrug resistant, antibacterial activity, *Allium sativum*, aqueous garlic extract, urinary tract infections.

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## INTRODUCTION

Urinary Tract Infection (UTI) is one of the most common extra intestinal bacterial infections and the second most common infectious disease encountered in community practice. UTI alone poses a serious health problem affecting millions of people each year with total cost for treatment being in

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billions of dollars. Worldwide, about 150 million people are diagnosed with UTI each year (Gupta & Stamm, 2001). Antibiotics have revolutionized medicine in many respects; their discovery was a turning point in medicinal history. Regrettably, the use of these wonder drugs has been accompanied by the rapid appearance of resistant strains (Liu & Pop, 2009). As has been reported earlier on, major UTI causing isolates were identified as *Escherichia coli, Enterobacter* sp., *Klebsiella* sp., *Pseudomonas aeruginosa,* and *Staphylococcus aureus* (Kapur *et al*., 2013). In recent years, several reports from the scientific community have raised concerns that antibacterial drug development at its current pace will not adequately address the problems posed by antibiotic resistance among important bacterial pathogens leading to diseases (Manjunath *et al*., 2011). Emerging antimicrobial resistance compels once again to look back into traditional medicines or herbal products, which may provide appropriate/acceptable alternative solutions. Plants derived products have made large contributions to human health and wellbeing (Karuppiah & Rajaram, 2012). Traditional medicine, if used appropriately shows higher therapeutic efficacy with fewer side effects and cost effective. Hence, there is an imperative need to make judicious use of natural bioactive substances with established safety index to tackle increasing AMR.

Garlic is one of the edible plants which has generated a lot of interest throughout human history as a medicinal cure/remedy for many diseases. Ancient medical texts from Egypt, Greece, Rome, China and India each describe medical applications of garlic. Garlic is one of the earliest documented examples of a plant employed for treatment of disease and maintenance of health (Rivlin, 2001). A wide range of microorganisms including bacteria, fungi, protozoa and viruses have been shown to be sensitive to garlic preparations (Koch & Lawson, 1996). Allicin and other sulphur compounds are thought to be the major antimicrobial factors in garlic. Garlic is effective against a number of gram-negative, gram-positive and acid-fast bacteria including *Staphylococcus, Salmonella, Vibrio, Mycobacteria, Proteus* species and *Helicobacter pylori* along with antifungal, antiparasitic and antiviral activity (Cellini *et al*., 1996; Ankri & Mirelman, 1999). A recent study from the University of East London has shown that aqueous extracts of allicin when formulated into a simple cream are able to kill vast swathes of the so-called "superbug" MRSA (methicillin resistant *Staphylococcus aureus*) (Nummer *et al*., 2011). The present study aims to evaluate the antimicrobial activity of aqueous extract of *Allium sativum* against multi drug resistant clinical isolates of pathogenic bacteria found in human urine with the hope to develop novel treatment regimen for drug resistant UTI.

## MATERIALS AND METHODS

### *Source of Bacterial Test Isolates and Antibiotic Sensitivity Testing*

The test organisms were bacterial isolates from the urine samples submitted by patients

having suspected urinary tract infections. Urine samples from 166 patients who had UTI confirmed by positive urine culture reports were used for this study. The cultures were maintained in the laboratory. The antibiotic susceptibility patterns of the test organisms were performed as per standard Kirby–Bauer disc diffusion assay (Bauer *et al*., 1966). All antibiotic discs (Ampicillin 10µg; Gentamicin 30µg; Cefuroxime 30µg; Kanamycin 30µg; Amikacin 30µg; Ciprofloxacin 5µg and Penicillin 2 units) and media used were obtained from Himedia Labs, India. Results were interpreted based on the diameter of the observed zone of inhibition. Following the Clinical and Laboratory Standards Institute Guidelines, the obtained results were categorized into three groups: Sensitive (S); Intermediate (I); Resistant (R) (CLSI, 2006).

*Multiple Antibiotic Resistances (MAR) Index of Test Isolates*

The antibiotic susceptibility patterns obtained from the standard Kirby–Bauer disc diffusion procedure was used for calculating the MAR index. The MAR index is the ratio of number of antibiotics ineffective against the organisms to the total number of antibiotics exposed (Krumperman, 1983). A MAR value near to 1 indicates that all tested antibiotics are ineffective. The clinical isolates which had MAR>0.5 were used to test the antimicrobial effects of fresh aqueous garlic extract in the liquid form.

***Preparations of the Fresh Aqueous Garlic Extract (AGE)***

Fresh bulbs of *Allium sativum* were purchased from local vegetable shop in Hyderabad. Individual cloves were separated. Fifty grams of the garlic cloves (weighed after peeling the outer covering) were surface sterilized with 70% ethanol and rinsed thoroughly with sterile water, for 4-5 times. The cloves were sliced, crushed using mortar and pestle and suspended in 50ml of sterile distilled water. The extract was further homogenized using a Waring blender at 2000 rpm for 5mins at room temperature. The froth was allowed to settle down for 30 min at room temperature before filtering through a single layer of muslin cloth and later through pre sterilized Whatman filter paper No. 1. The filtrate was filter sterilized using 0.45µm filter (Millipore, India). The filtrate was collected in sterile tubes. This 1g/ml fresh AGE was stored at $4^0$C till further use.

*Determination of Minimum Inhibitory Concentration (MIC) of the Fresh Aqueous Garlic Extracts (AGE) Using Macro Broth Dilution Method*

The minimum inhibitory concentration (MIC) of the fresh aqueous garlic extract was determined by the broth dilution method. Different concentrations of fresh aqueous garlic extract were prepared ranging from 70mg/ml to 10mg/ml from stock of 1gm/ml in LB broth. The final volume in each tube was made to 5ml by adding LB broth.

Then, 0.1 ml of fresh microbial culture ($10^7$ cells) was inoculated in all the tubes. For positive control, no fresh aqueous garlic extract (AGE) was added and for negative control only LB broth was taken without garlic extract and bacterial cells.

The tubes were kept for overnight shaking at 120rpm at $37^0$C. The Optical Density at 600nm ($OD_{600}$) was recorded for all the tubes. Bacterial growth kinetics profile was produced with respect to varying concentrations of fresh aqueous garlic extract. The tube which contained the least concentration of fresh AGE and whose $OD_{600}$ value was equivalent to the blank i.e. only LB broth was considered as the Minimum Inhibitory concentration (MIC) of the fresh garlic extract in mg/ml against bacteria after seven days of preparation of fresh aqueous garlic extract.

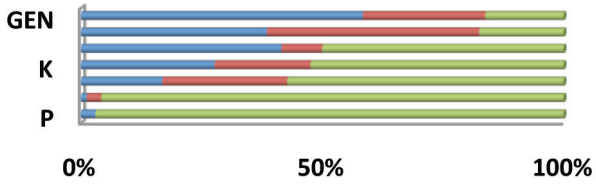
*Testing the Bio Efficacy of the Aqueous Garlic Extract (AGE)*

Bacterial strains (N=93) which showed resistance to 4 or more antibiotics out of 7 (MAR>0.5) were tested against AGE for growth inhibition. The bacterial strains were inoculated in 1ml LB broth and grown overnight at $37^0$C with continuous shaking. The next day before performing the antimicrobial assay, 50µl of overnight grown culture was aseptically transferred in fresh 5ml LB broth (pH 7.2) and placed in shaking incubator at $37^0$C for 3-4 hours. Thick LB agar plates were prepared by pouring 30ml of LB agar into sterile petri-dishes. Then, 0.1ml of the fresh bacterial broth culture was spread over the solidified LB agar plates using L-shaped glass spreader inside the laminar air flow chamber. The plates were incubated for 5 min at room temperature for the attachment of bacterial strains over the LB agar surface.

Agar well diffusion technique was used to test anti microbial activity of AGE. Wells (5 mm diameter) were made in the inoculated LB agar plates by using a sterile cork borer. Fresh AGE was (0.7mg /well) dispensed into the wells. The plates were initially kept at room temperature for 1 hour for the diffusion of the extracts in agar and later kept in bacterial growth incubator at $37^0$C in an up-side down position. The zones of inhibition were measured after 12-16 hours.

## RESULTS

The MAR index value calculated for the test organisms showed that 56% (93 of 166 isolates) of uropathogens had MAR index higher than 0.5, i.e. resistant to four or more antibiotics out of seven antimicrobial agents tested and 24% isolates had MAR $\geq$ 0.7, that is, they were resistant $\geq$ 5 antibiotics. The results also showed a very high incidence of resistance among uropathogens against penicillin and ampicillin, with 97% and 96%, respectively (see Fig.1). About 50% of bacterial isolates were resistant to even third generation antibiotics such as ciprofloxacin. Gentamicin was found to be the most effective drug against all common uropathogens in the studied cohort with only 16% (27/166) of clinical isolates being resistant to it. The next effective antibiotic was amikacin (17% resistance).




| | P | A | CXM | K | CIP | AK | GEN |
|---|---|---|---|---|---|---|---|
| S | 3.01 | 1.2 | 16.86 | 27.7 | 41.56 | 38.5 | 58.4 |
| I | 0 | 3.01 | 25.9 | 19.87 | 8.43 | 43.97 | 25.3 |
| R | 96.98 | 95.78 | 57.23 | 52.41 | 50 | 17.47 | 16.26 |

Fig.1: Resistance and susceptibility profile of uropathogens to antimicrobial agents (N=166; values expressed in percentage) [P=Penicillin, A=Ampicillin, CXM=Cefuroxime, K=Kanamicin CIP=Ciprofloxacin, AK= Amikacin, GEN=Gentamicin]

In the present investigation, antibacterial activity of AGE was tested for 93 isolates having MAR index > 0.5 and it was observed that 82% (76 out of 93) of the multi drug resistant isolates showed a zone of inhibition larger than 10mm, proving the high degree of growth inhibitory activity of AGE against MDR uropathogens (Table 1). Among the 76 clinical pathogens *S. aureus* and *E. coli* were most susceptible followed by *Enterobacter* sp. and *Klebsiella* sp. AGE showed the highest diameter of zone of inhibition of 28 mm against *E. coli* followed by *S. aureus* (26 mm).

TABLE 1
Range of zone of inhibition with fresh AGE for various groups of uropathogens

| Clinical isolate | No. of isolates | ZOI |
|---|---|---|
| *E. coli* | 35 | 17-28 mm |
| *Enterobacter* sp | 19 | 13-25 mm |
| *Klebsiella* sp | 11 | 14-24 mm |
| *S. aureus* | 6 | 16-26mm |
| *P. aeruginosa* | 5 | 13-22 mm |

Minimum Inhibitory concentration (MIC) of fresh aqueous garlic extract was found to be 35mg/ml where fresh AGE resulted in effective inhibition of bacterial growth, as shown in Table 2 and Fig.2.

TABLE 2
Absorbance at 600nm of varying concentrations of fresh AGE for MIC determination

| Tube No | AGE Concentartion mg/ml) | $OD_{600}$ |
|---|---|---|
| 1 | 70 | 0.02 |
| 2 | 50 | 0.031 |
| 3 | 35 | 0.033 |
| 4 | 25 | 0.781 |
| 5 | 15 | 1.084 |
| 6 | 10 | 1.102 |
| 7 | Positive control | 1.105 |
| 8 | Negative control | 0.017 |

One of the representative clinical isolate of *Staphylococcus aureus*, isolated from the infected urine sample and found to be resistant to ampicillin, penicillin, kanamycin and cefuroxime, was tested for

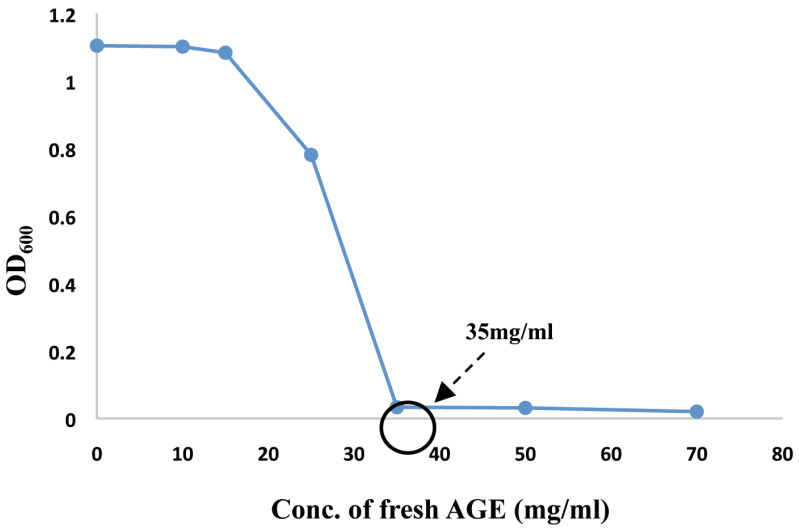



Fig.2: Absorbance at 600nm and concentration of fresh aqueous garlic extract, AGE (mg/ml) after 12 hour of incubation

the antimicrobial effect of AGE using water as the negative control and gentamicin as the positive control. AGE was found to be as effective as gentamicin (see Fig.3).

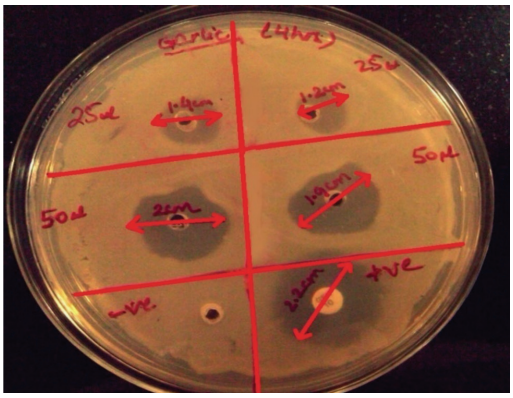



Fig.3: Zone of inhibition obtained with aqueous garlic extract against *Staphylococcus aureus* [+ve control is gentamicin and –ve control is water]

This provides a strong evidence for the antibacterial potential of fresh AGE in case of multi drug resistant infections, where most of the available antibiotics are not effective. AGE depicts the antimicrobial efficacy equivalent to one or the other antibiotics (generally prescribed in UTI cases) in 76 multidrug resistant uropathogens having MAR index > 0.5 (Table 3).

TABLE 3
Comparing the efficacy of AGE with other antibiotics used for uropathogens

| Range of ZOI (mm) | No. of isolates | Equivalent effective antibiotic |
|---|---|---|
| 11-15 | 25 | Gentamicin, Tobramicin, Levofloxacin |
| 16-20 | 44 | Amikacin, Naladixic acid, Trimethoprim, Ofloxacin |
| >20 | 07 | Cotrimoxazole, Cefturoxime, Piperacillin |

## DISCUSSION

Garlic has been studied for its antibacterial activity against a wide range of bacteria

but limited data is available for its efficacy in case of infectious diseases like UTI. The results of this work provide evidence that the fresh aqueous garlic extract possesses significant antibacterial activity. Similar results were obtained by Sharma *et al* (2009) in India (Sharma & Patel, 2009), where they showed a strong antimicrobial activity of *Allium sativum* against *Vibrio cholera*. The results of the present study support the use of natural products as affordable effective substitutes and/or additives for reducing the disease burden and overall cost of disease management. Garlic has recognized traditional medicinal applications (Ross *et al*., 2001); similar to other organosulphur and phenolic compounds have been reported to be involved in the antimicrobial activity (Raja *et al*., 2011; Johnson *et al*., 2011; Nweze *et al*., 2012; Aboaba & Efuwape, 2001). Several studies have reported *in vitro* evidence of the antimicrobial activity of fresh and freeze dried garlic extracts against many bacteria (Rees *et al*., 1993), fungi and viruses (Weber *et al*., 1992). However, ours is the first study to report the antibacterial activity of AGE against MDR bacterial isolates from the infected urine samples leading to UTI.

It is interesting to note that even crude extracts of this plant showed good activity against multidrug resistant strains where antibiotic therapy had limited or no effect. This provides hope for developing alternative drugs which may be of help in fighting the menace of growing antibacterial resistance. To conclude, there is evidence that garlic has potential in the treatment of UTI and may be other microbial infections. Considering the morbidity rate and economic burden of infectious diseases like UTI, use of garlic in an appropriate form might contribute substantially to public health. However, it is necessary to determine the bio availability, side effects and pharmaco-kinetic properties in more details.

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