TO STUDY THE INHIBITORY EFFECT OF GARLIC EXTRACT ON UROPATHOGENIC E.COLI

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ABSTRACT

OBJECTIVE: The present study aims to study the inhibitory effect of garlic extract on uropathogenic E.coli and to detect biofilm production among them. Garlic was selected as it has wide range of antimicrobial activity.

METHOD: Organism isolated from urine sample of UTI patients were tested against different concentration of Aqueous Garlic Extract for its antimicrobial activity by Agar Well Diffusion Method.

RESULT: Prevalence of E.coli was found to be 44.41%. All four different concentrations of Aqueous Garlic Extract i.e. 20%, 40%, 60% and 80% showed zone of inhibition in uropathogenic *E.coli.*

CONCLUSION: The Study showed that Aqueous Garlic Extract was effective against uropathogenic *E.coli* which were isolated from urine samples of UTI.

Key words: Urinary Tract Infections, Aqueous Garlic Extract, Uropathogenic *E.coli.*

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INTRODUCTION

Antibiotics are among the most successful drugs ever developed. Soon after their discovery, however, it became evident that bacteria could become resistant to them. [1]

Urinary tract infection (UTI) is defined as the microbial invasion of tissues of the urinary tract extending from the renal cortex to the urethral orifice. UTI is classified by the site of infection (the bladder [cystitis], kidney [pyelonephritis], or urine [bacteriuria]), UTI can be asymptomatic or symptomatic, characterized by a wide spectrum of symptoms ranging from mild irritative voiding to bacteremia. The most common uropathogens identified in patients with UTI are enteric gram-negative bacteria, with *E. coli* being the most common followed by Klebsiella, Pseudomonas aeruginosa, and Proteus mirabilis. In India UTI accounts for

nearly 7 million office visits and 1 million emergency department visits, resulting in about 1,00,000 hospitalizations. [2]

Despite the fact, that both the genders are susceptible to infection, women are frequently vulnerable to UTI due to both anatomy and physiology of urogenital structures. [3]

UPEC possesses a number of virulence characteristics that enable it to penetrate and damage host tissues, hurt mucosal surfaces, get past host defense mechanisms, and trigger an inflammatory response in the host. Another pathogenic factor that helps the strains stay in the genitourinary tract for a long time and obstruct the removal of germs is the production of biofilms. Biofilms are organized populations of bacteria that are attached to any abiotic or biological surface by a self-produced exopolysaccharide matrix. [4] The incrementing scope of pathogenic resistance to antibiotics has encouraged the search for antivirulence natural extracts. Plants have been able to give new compounds of great benefit to humanity. Many approaches have been aimed to find natural biological principles in plant. [5]

Garlic (Allium sativum), an essential food ingredient worldwide, has long been known to have antibacterial, antifungal and antiviral effects. The main antimicrobial constituent of garlic, allicin, is generated by the enzyme alliinase when garlic is crushed. [6] One of the active ingredients in freshly crushed garlic homogenates, allicin, possesses a range of antibacterial properties. Pure allicin has been shown to have antibacterial properties, it can combat a variety of Gram-positive and Gram-negative bacteria, including enterotoxicogenic strains of Escherichia coli that are resistant to drugs. [7]

MATERIALS AND METHODS

The present study was carried out in MGM Medical College and Hospital, Kamothe, Navi Mumbai.

Garlic Sample Processing

Aqueous Garlic Extract was prepared by collecting fresh garlic from market. Garlic was peeled, sterilized with ethanol, then evaporating of ethanol was accomplished in sterilized in laminar air flow. By using sterile mortar and pestle 50 g of garlic was homogenized and 100ml of distilled water was added, then it was allowed to stand for a time of 72 hours. Sterilization of mixture was performed by filtration through a Millipore filter paper 0.45. Then it was stored in sterile bottle and kept at 4^0C. [8]

Sample collection and Bacterial strain

Urine was collected in a wide mouth screw capped sterile container by various methods. *Escherichia coli* was identified using Standard Microbiological methods. [9]

Antimicrobial Susceptibility testing

Antimicrobial Susceptibility testing was performed as per the guidelines of Clinical and Laboratory Standard Institute (CLSI). Commercially available antibiotic disc from Hi-Media were used for the study. Following are the names of the antibiotics: Cephalothin CEP (30mcg), Ofloxacin OF (2mcg), Ceftizoxime CZX (30mcg), Levofloxacin LE (5mcg), Cefuroxime CXM (30mcg), Nitrofurantoin NIT (300 mcg), Cefotaxime CTX (30 mcg), Trimethoprim TR(5mcg), Amoxiclav (amoxicillin/clavulanic acid) AMC (30mcg), Amikacin AK (30mcg), Norfloxacin NX (10mcg), Tobramycin TOB (10mcg).

Bacterial suspension which was used for testing was compared to 0.5 McFarland standard solution. [10]

Antibacterial activity of Garlic

Agar well diffusion method was used for testing the antibacterial activity of garlic against Uropathogenic E. coli. Aqueous Garlic Extract was diluted with sterile distilled water to obtain varying concentrations of 20%, 40%, 60%, 80%. Sterile Muller Hinton agar plates were uniformly seeded with bacterial suspension of *Escherichia coli.*

Then with the help of sterile cork borer well of 8mm were punched into the agar medium and varying concentration of honey was added to the wells and the plates were incubated at 37^0C for 24 hours. After the incubation period, the zone diameter of inhibition was measured. One plate was seeded with Distilled water, instead of honey which was considered as Negative Control. [10]

Biofilm detection in UPEC

Simple qualitative method was used to detect the biofilm formation by Congo Red Agar (CRA) method.

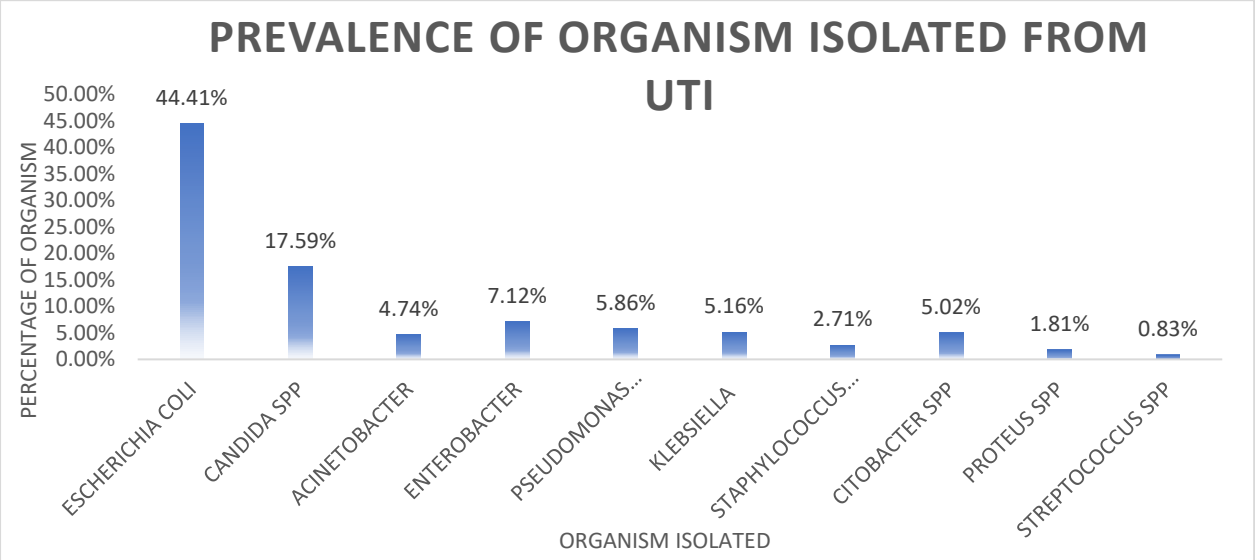
This method involves use of special media that is Brain Heart Infusion (BHI) agar with Sucrose and Congo red in the following composition: BHI agar-52 g/L; sucrose-36 g/L; agar-10 g/L; Congo red-0.8 g/L. Congo red was prepared as concentrated solution and autoclaved. It is added to the medium when agar is cooled to 55°C and poured into petri-plates. Plates were inoculated and incubated for 24–48 h at 37°C. Black colonies with dry crystalline morphology was considered positive for biofilm producing organisms while weak or nonbiofilm producing organisms appeared to be pink in colour. [11]

RESULTS

A total of 716 urine samples of UTI patients were received among which 318 (44.41%) yielded growth of *Escherichia coli*. Out of 716 Uropathogens isolated, 318 were isolated from males and 398 were isolated from females.

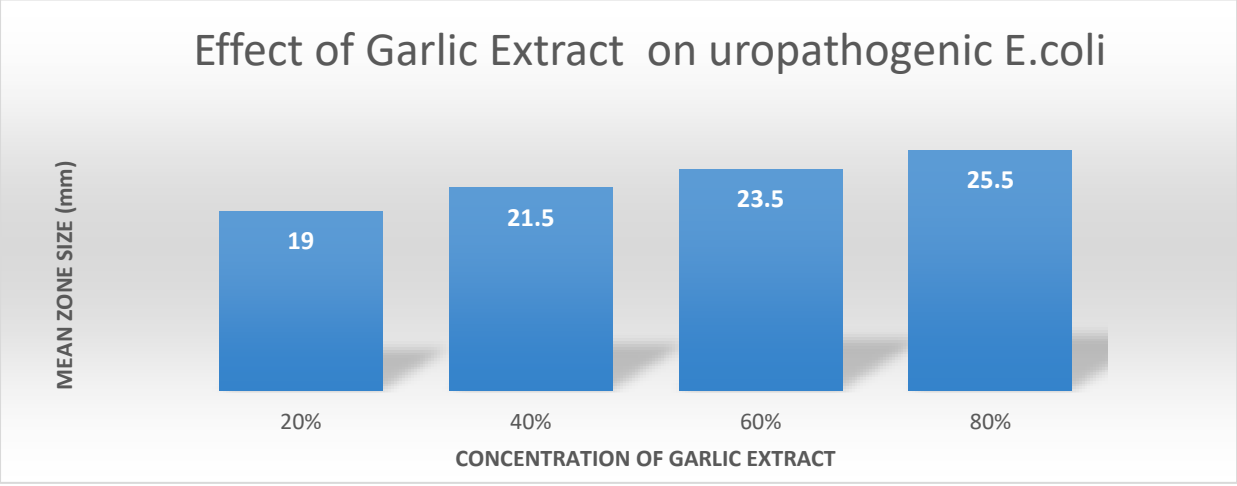
Table no. 1: Prevalence of organisms isolated from UTI (n= 716)

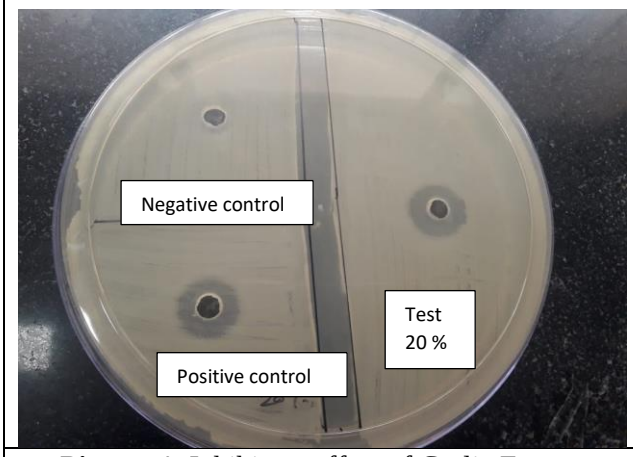
ORGANISM	NO. OF ISOLATE	PERCENTAGE
ESCHERICHIA COLI	318	44.41%
CANDIDA SPP	126	17.59%
ACINETOBACTER SPP	34	4.74%
ENTEROBACTER SPP	51	7.12%
PSEUDOMONAS AERUGINOSA	42	5.86%
KLEBSIELLA SPP	37	5.16%
STAPHYLOCOCCUS AUREUS	20	2.71%
CITOBACTER SPP	36	5.02%
PROTEUS SPP	13	1.81%
STREPTOCOCCUS SPP	06	0.83%



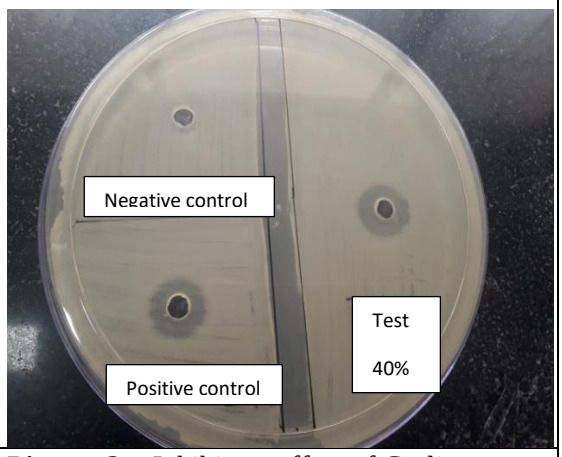
Prevalence of uropathogenic *E. coli* was found to be 44.41%. The major predisposing factor associated with UTI was found to be Catheterization (19.83%) followed by past history of UTI (4.88%). Uropathogenic *E. coli* isolated were tested for antimicrobial activity of aqueous garlic extract of four different concentrations i.e 20%, 40%, 60%, 80%. All 318 *E. coli* isolates showed good zone of sensitivity, the mean zone size being 19.16 mm for 20% Garlic Extract, 21.43 mm for 40% Garlic extract, 23.50mm for 60% Garlic Extract and 25.77 mm for 80% Garlic Extract.

Graph no. 2: Effect of Garlic Extract on Uropathogenic *E. coli*

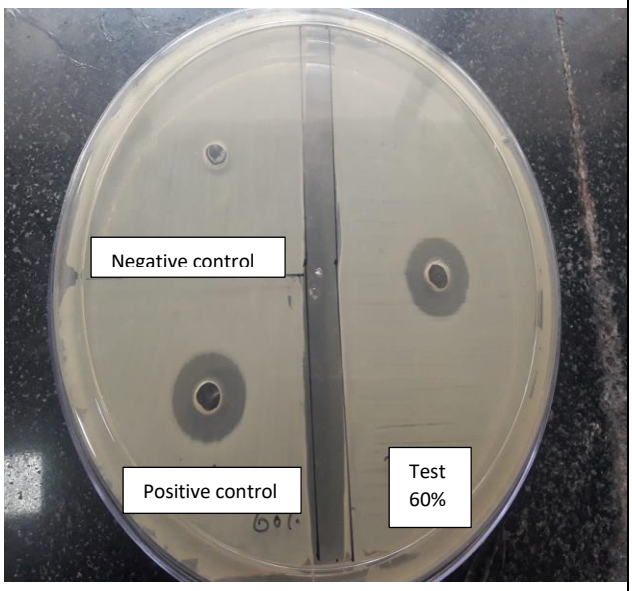




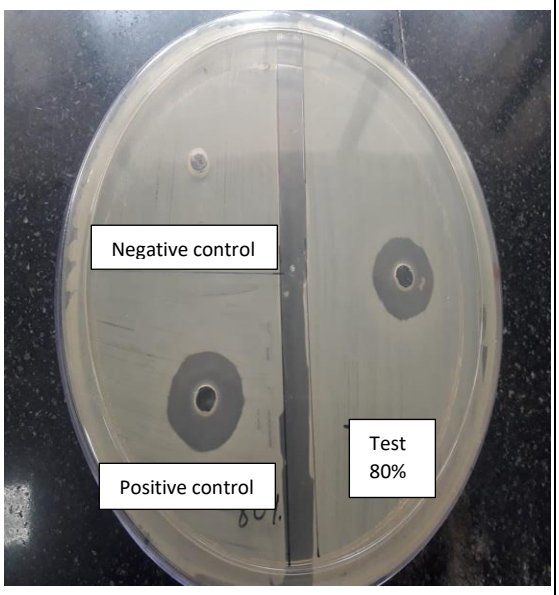
Picture 1: Inhibitory effect of Garlic Extract (20%) On Uropathogenic *E. coli*



Picture 2: Inhibitory effect of Garlic Extract (40%) On Uropathogenic *E. coli*



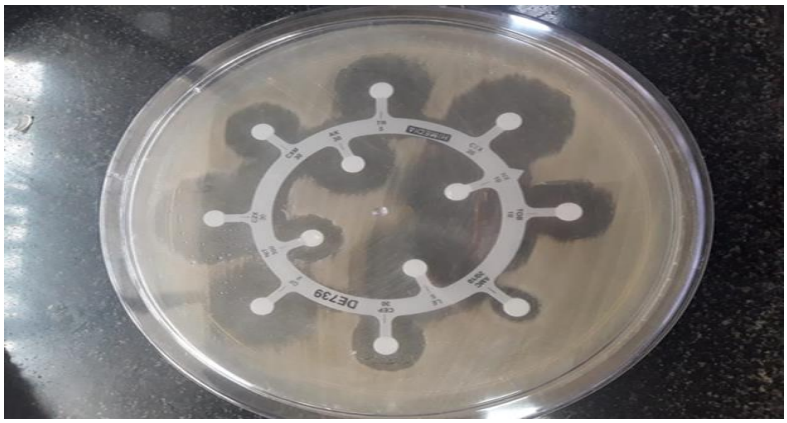
Picture 3: Inhibitory effect of Garlic Extract (60%) On Uropathogenic *E. coli*



Picture 4: Inhibitory effect of Garlic Extract (80%) On Uropathogenic *E. coli*

Table no. 2: Antibiotic discs

NAME OF ANTIBIOTIC	ABBR	DISC CONCENTRATION	% OF SENSITIVITY	% OF RESISTANCE
Cephalothin	CEP	30mcg	30 %	70 %
Ofloxacin	OF	2mcg	69.41 %	30.59 %
Ceftizoxime	CZX	30mcg	59.41 %	40.59 %
Levofloxacin	LE	5mcg	73.53 %	26.47 %
Cefuroxime	CXM	30mcg	37.05 %	62.95 %
Nitrofurantoin	NIT	300 mcg	80 %	20 %
Cefotaxime	CTX	30 mcg	50.58 %	49.42 %
Trimethoprim	TR	5mcg	52.94 %	47.06 %
Amoxiclav (amoxicillin/ clavulanic acid)	AMC	30mcg	36.47 %	63.53 %
Amikacin	AK	30mcg	89.41 %	10.59 %
Norfloxacin	NX	10mcg	54.11 %	45.89 %
Tobramycin	TOB	10mcg	78.82 %	21.18 %



Picture 5: Antimicrobial susceptibility testing

Antibiotic Susceptibility testing showed following results, highest sensitivity was shown by Amikacin (89.41%) followed by Nitrofurantoin (80%).

DISCUSSION

This study was carried out in the department of Microbiology, at Mahatma Gandhi Mission Medical College and Hospital, Kamothe, Navi Mumbai. Rate of *E. coli* isolate from urine samples was found to be **(44.41%)** followed by Candida spp **(17.49%).** This was found to be more as compared to study by Manjula Mehta, Sonia Bhardwaj and Jyoti Sharma where prevalence rate of *E. coli* was **41%.**

Gender wise female **(55.58%)** were more susceptible to UTI than male **(44.41%)**. Age wise, elderly i.e., 61 & above year **(37.15%)** were more susceptible. Major predisposing factor in the study was found to be catheterization (**19.83%)**. Effect of Garlic Extract on Uropathogenic *Escherichia coli*, which was performed by well diffusion method in which effect of four different concentrations of aqueous garlic extract was tested against on Uropathogenic *Escherichia coli*. Mean zone size of 20% aqueous garlic extract was 19.16mm. Mean zone size of 40% aqueous garlic extract was 21.42 mm, for 60% it was 23.50 mm and for 80% it was 25.7 mm respectively. The results show that all four concentrations of aqueous garlic extract were effective against Uropathogenic *Escherichia coli* in urinary tract infections. Comparing my results with other study done by Alyasari, H.F., Al-khafaji, J.K. where they found that the diameter of the inhibition zone ranged from 27.0 to 28.0 mm for garlic extract, is quite similar to diameter of the inhibition zone in my study. Another study by Banfitebiyi G. *et al* states that the sizes of inhibition zones are inversely proportional to the increase in dilution of AGE. They found that the diameter zone of inhibition of AGE varies respectively from 29±2 - 32±4.5 mm and 24±1 - 25±1 mm at 1000 and 500mg/ml.

Maximum number of uropathogenic *E. coli* were sensitive to amikacin with 89.41% sensitivity followed by nitrofurantoin (80%). Minimum sensitivity by *E. coli* was shown to Cephalothin (30%). The results of my study are almost similar with the study by Mohammed A. Almalki et al in which 182 (86.25%) *E. coli* were sensitive to Amikacin. Biofilm detection in Uropathogenic *Escherichia coli* which was performed by Congo red agar method. Biofilm production was seen in 7% Uropathogenic *Escherichia coli* which is less as compared to study done by Seema Mittal et al in which 13.5% *Escherichia coli* were positive for biofilm production by microtiter plate (MTP) method.

CONCLUSION

The study was performed at Mahatma Gandhi Mission Medical College and Hospital, Kamothe, Navi Mumbai.

Prevalence of uropathogenic *E. coli* among patients with Urinary Tract Infection was found to be **44.41%**. Predisposing factors associated with urinary tract were studied and it can be concluded that catheterization **(19.83%)** is an important predisposing factor to UTI. Also, this infection was more common in those who had past history of UTI.

Antibiotic susceptibility test showed that most *Escherichia coli* were sensitive to Amikacin (AK) with **89.41%** sensitivity followed by Nitrofurantoin (NIT) with **80%** sensitivity. Apart from this antibiotic susceptibility pattern showed good sensitivity to many other antibiotics, including Cephalothin, Ofloxacin, Ceftizoxime, Levofloxacin, Cefuroxime, Cefotaxime, Trimethoprim, Amoxiclav (amoxicillin clavulanic acid), Norfloxacin, and Tobramycin.

Biofilm detection among Uropathogenic *Escherichia coli* was done by Congo red agar method and it showed that **22 (7%)** of uropathogenic *Escherichia coli* produced biofilm.

Inhibitory Effect of Garlic Extract on Uropathogenic *Escherichia coli:* All four concentrations of aqueous Garlic Extract i.e., 20%, 40 %, 60%, 80% showed good zone of inhibition. Thus, for urinary tract infections caused by *Escherichia coli*, along with antibiotic therapy, Garlic is effective and can be used as an adjuvant in urinary tract infections.

To conclude *Escherichia coli* remains the most common pathogen causing UTI. Catheterization is one of the most common predisposing factors for development of UTI, hence strategies should be formulated to minimize use of catheters. Also, the antibiotic sensitivity pattern is constantly changing hence it is necessary to collect data and implement antibiotic stewardship. Biofilm production can increase resistance to commonly used antibiotic. Aqueous garlic extract was seen to have good potential against uropathogenic *Escherichia coli* and hence can be used as adjunct treatment along with commonly used antibiotics however that will require further testing about its effect on virulence factors of Escherichia coli and various organisms and also perform further studies in vivo**.**

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