

Antibacterial Activity of Garlic Ethanolic Extract Against Multi Drug Resistant Bacteria Isolated from Patients with Urinary Tract Infection in Khartoum City, Sudan.

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ABSTRACT: Background Urinary tract infection (UTI) is a major cause of morbidity and may sometimes lead to mortality, and represents a major health threat due to antibiotic resistance and high recurrence rate. The study aimed to testing the antibacterial activity of *Allium sativum* ethanolic extract against multidrug resistant urinary tract bacterial isolates. **Methods** This was laboratory-based study included a total of one hundred bacterial isolates were collected from different Hospitals in Khartoum city, Sudan. **Results** The result showed that amoxicillin had high resistant rates (97.9%) followed by Cephalexin (95.6%) then Cefotaxime (85.5%) , Co-trimoxazole (77.3%) and Ciprofloxacin (72.9%) and finally nitrofurantoin (40.9%). **Conclusions** Garlic ethanolic extract have a high antibacterial activities against pathogenic bacteria (*E.coli*, *K.pneumoniae* , *P.aeruginosa* and *E.faecalis*), and standard isolates (*E coli* ATCC 8729 and *S aureus* ATCC 6538).

Keywords: Antibacterial Activity, Garlic, MDR, UTI, Sudan.

Introduction

Urinary tract infections (UTIs) are the second most common infection present in community practice. Worldwide, about 150 million people are diagnosed with UTI each year [1-4]. UTIs are often treated with broad-spectrum antibiotics that affect both Gram-positive and Gram-negative bacteria [5-11].

Garlic (*Allium sativum* L.) is one of the oldest vegetables, and has been used as a medicinal plant [12]. Garlic has been used to treat bacterial related diseases such as pile, cough and rheumatism; and to alleviate tumor, cardiovascular diseases and ageing [13-17].

The study aimed to detect the antibacterial activity of Garlic ethanolic extract against multi drug resistant clinical isolates from urinary tract infections in Khartoum city, Sudan.

Materials and methods

Study design

The study design was laboratory-based study, conducted in Khartoum city hospitals, Sudan, included 100 different multi drug resistant clinical pathogens isolated from patients with urinary tract infection.

Inclusion criteria

Most common pathogenic multi drug resistant urinary tract bacterial isolates.

Exclusion criteria

Nonpathogenic urinary tract bacterial isolates and others pathogenic non multi drug resistant urinary tract bacterial isolates.

Laboratory procedure

Bacterial smear was made and allowed for air drying then Gram conventional method was applied [18]. Then biochemical tests were performed included Litmus milk reduction test [19]. Kligler's iron agar test (KIA) and Indole test were applied as conventional method [20]. And antibacterial susceptibility testing such as Modified Kirby-Bauer Method [21].

All bacterial isolates were tested against 6 different antimicrobial agents (amoxicillin, ciprofloxacin, cephalixin, cefotaxime, cotrimoxazole, nitrofurantoin)

Preparation of *Allium sativum* ethanolic extract

Extraction was carried out according to known conventional method [22]. Then the extract allowed to air till complete the dryness. Yield percentage was calculated as followed:

Weight of extract/weight of Sample (Garlic bulbs)

Sample	Weight of sample	Weight of extract	Yield%
1	450 grams	23.34 grams	5.18%

Also, we performed Cup-plate agar diffusion method as known protocol in literature [23].

Statistical analysis

Data was computed and analyzed by using Statistical Package for Social Sciences (SPSS) computer software version 20 to check frequency and mean.

Results and discussion

Clinical isolates frequency

In this study 100 clinical microorganisms were isolated from urine samples and tested by different biochemical tests and the result showed that; 56(56%) *Escherichia coli*, 26(26%) *Klebsiella pneumoniae*, 15(15%) *Enterococcus faecalis*, 3(3%) *Pseudomonas aeruginosa*. (Figure 1)

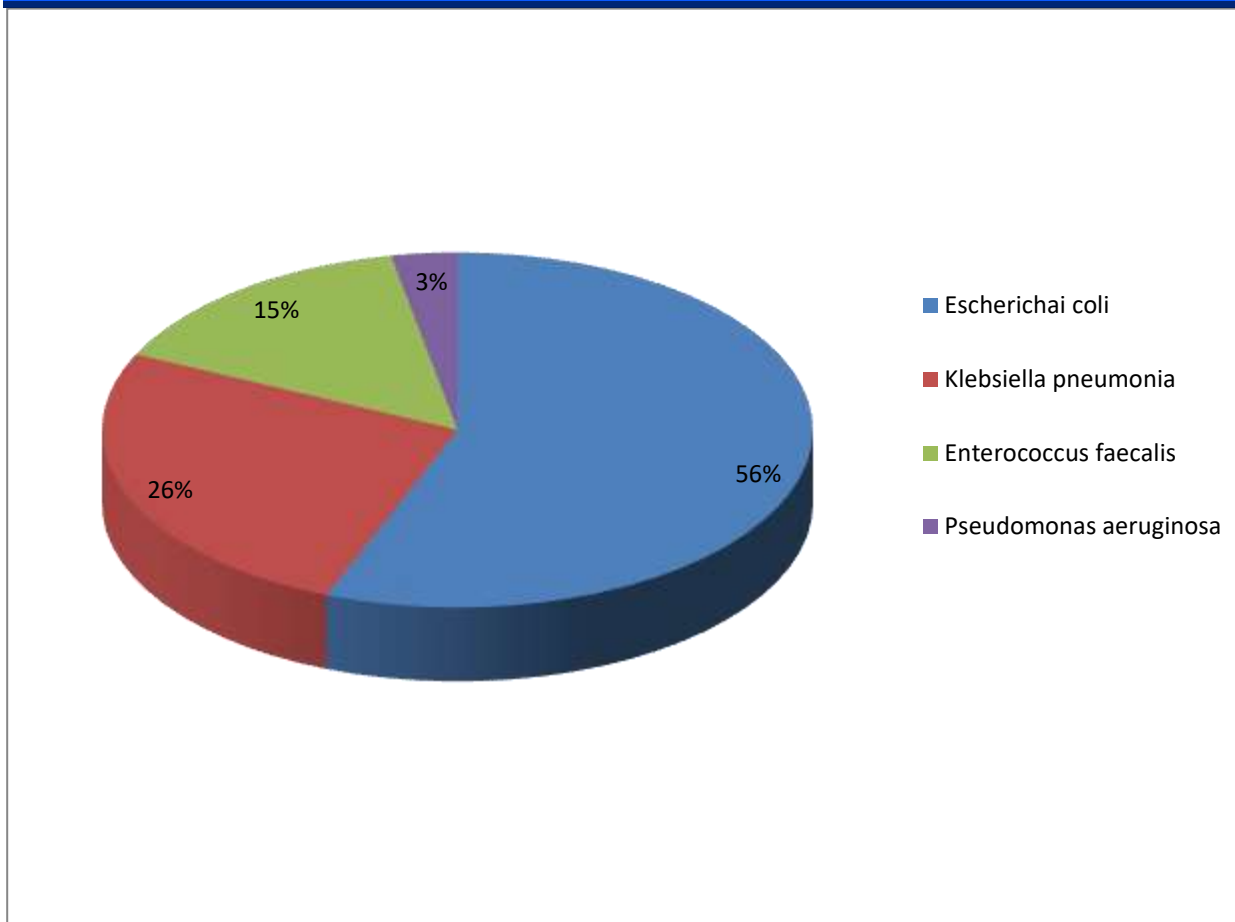


Figure 1. frequency of clinical isolates.

Susceptibility of Clinical isolate to selected antibiotics

Table1. Antimicrobial susceptibility testing results of UTIs isolates

Antibiotics M.O	CIP		AMX		COT		CTX		CN30		NIT	
	S No / %	R No / %	S No / %	R No / %	S No / %	R No / %	S No / %	R No / %	S No / %	R No / %	S No / %	R No / %
<i>E. coli</i> NO=56	10 17.9%	46 82.1%	1 1.8%	55 98.8%	8 14.3%	48 85.7%	2 3.6%	54 96.4%	4 7.2%	52 92.8%	40 71.4%	16 28.6%
<i>K. pneumoniae</i> NO=26	8	18 69.3%	0 0%	26 100%	6	20	2 7.7%	24	1	25	10	16 61.5%

	30.7%				23.1%	76.9%		92.3%	3.9%	96.1%	38.5%	
<i>E. feacalis</i>	4	11	1	14	3	12	2	13	1	14	14	1
NO=15	26.6%	73.4%	6.6%	93.4%	20%	80%	13.3%	86.7%	6.6%	93.4%	93.4%	6.3%
<i>P. aeruginosa</i>	1	2	0	3	1	2	1	2	0	3	1	2
NO=3	33.3%	66.7%	0%	100%	33.3%	66.7%	33.3%	66.7%	0%	100%	33.3%	66.7%
Total and %	13	77	2	98	18	78	7	93	6	94	65	35
	27.1%	72.9%	2.1%	97.9%	22.7%	77.3%	14.5%	85.5%	4.4%	95.6%	59.1%	40.9%

Key words: CIP: Ciprofloxacin, AMX: Amoxicillin, COT: Co-trimoxazole, CTX: Cefotaxime, CN30: Cephalexin, NIT: Nitrofurantoin, R: Resistant, S: Sensitive, M.O: microorganisms.

Susceptibility of standard isolates to *Allium sativum* ethanolic

extract

Antimicrobial activity of *Allium sativum* ethanolic extract was firstly screened against reference strains (*S.aureus* ATCC 6538 and *E.coli* ATCC 8729).

Table2. Antibacterial activity of *A.sativum* ethanolic extract against standard strains

Standard strains	Mean of inhibitory zone in mm			
	Conc100 mg/ml	Conc50 mg/ml	Conc25mg/ml	Conc12.5mg/ml
<i>S.aureus</i> ATCC 6538	27	27	23	20
	VA	VA	VA	VA
<i>E.coli</i> ATCC 8729	22	22	21	17.5
	VA	VA	VA	A

Key words:

VA: Very active

A: Active

PA: Partial active

NA: Non active

Interpretation of results

After 24 hours incubation, antibacterial activity result was expressed in diameters of inhibition zones were measured in millimeter <9mm zones was considered as inactive, 9-12mm as partially active while 13-18mm as active and > 18mm as very active.

Susceptibility of clinical isolates to *Allium sativum* extract

Table3. Antibacterial activity of *A.sativum* ethanolic extract against bacterial isolates.

Clinical isolates	Mean of inhibitory zone in mm			
	Conc100 mg/ml	Conc50 mg/ml	Conc25mg/ml	Conc12.5mg/ml
<i>Escherichia coli</i>	15.9	13.7	10.5	6.6
	A	A	PA	NA
<i>Klebsiella pneumoniae</i>	14	12.1	8.4	6.3
	A	PA	NA	NA
<i>Enterococcus faecalis</i>	17.8	16.3	12	8.1
	A	A	PA	NA
<i>Pseudomonas aeruginosa</i>	17.3	15.2	9	6
	A	A	PA	NA

Activity of *Allium sativum* extract against Gram positive standard and Clinical isolates.

Table4. Shows comparison between inhibitory zone (mm) of *Allium sativum* extract on *S.aureus* ATCC and *Enterococcus faecalis*.

Bacterial isolates	<i>S.aureus</i> ATCC6538	<i>Enterococcus faecalis</i>
Concentrations		
Concentration of 100 mg/ml	27(VA)	17.8(A)
Concentration of 50 mg/ml	27(VA)	16.3(A)
Concentration of 25 mg/ml	23(VA)	12(PA)
Concentration of 12.5 mg/ml	20(VA)	8.1(NA)

Key words:

VA: Very active

A: Active

PA: Partial active

NA: Non active

Activity of *Allium sativum* extract against Gram negative standard and Clinical isolates

Table5. Shows comparison between *Allium sativum* extract (mm) on *Escherichia coli* ATCC and other gram-negative clinical isolates.

Bacterial isolates	<i>Escherichia coli</i> ATCC 8729	<i>Escherichia coli</i>	<i>Klebsiella pneumonia</i>	<i>Pseudomonas aeruginosa</i>
Concentrations				

Concentration of 100 mg/ml	22(VA)	15.9(A)	14(A)	17.3(A)
Concentration of 50 mg/ml	22(VA)	13.7(A)	12.1(PA)	15.2(A)
Concentration of 25 mg/ml	21(VA)	10.5(PA)	8.4(NA)	9(PA)
Concentration of 12.5 mg/ml	17.5(A)	6.6(NA)	6.3(NA)	6(NA)

Key words:

VA: Very active

A: Active

PA: Partial active

NA: Non active

The antimicrobial activity of garlic ethanolic extract against MDR uropathogenic bacteria illustrated in image represented Figure (2).

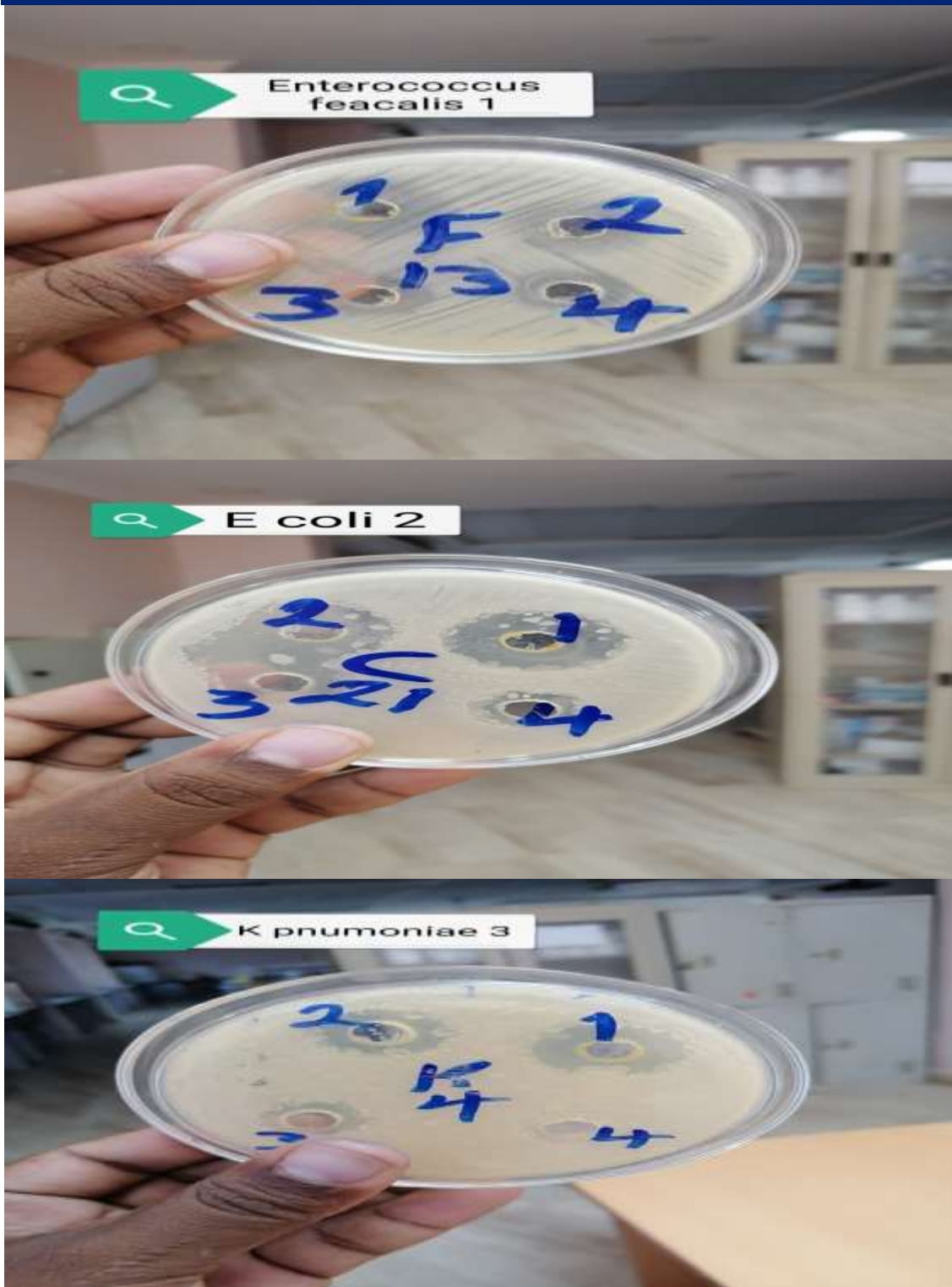


Figure 2: Pictures showing antimicrobial activity of garlic ethanolic extract against MDR uropathogenic bacteria.

Key words: 1=conc100%, 2=conc50%

3=conc25%, 4=conc12.5%

The present study was conducted to evaluate the antibacterial activity of Garlic ethanolic extract against multi drug resistant bacteria isolated from patients with urinary tract infections ,100 clinical isolates were tested as follows; 56(56%) *Escherichia coli*, 26(26%) *Klebsiella pneumoniae*, 15(15%) *Enterococcus faecalis*, 3(3%) *Pseudomonas aeruginosa*.

The result of antimicrobial sensitivity testing showed that amoxicillin had high resistant rates (97.9%) followed by Cephalexin (95.6%) then Cefotaxime (85.5%), Cotimoxazole (77.3%) and Ciprofloxacin (72.9%) and finally nitrofurantoin (40.9%) according previous report that showed increasing rate of resistance of these antibiotics that due to overuse and misuse of these medication [24-28].

Antibacterial activity of Garlic ethanolic extract was clearly detected on Gram positive (*E. faecalis*) and Gram negative (*E. coli*, *K. pneumoniae* and *P. aeruginosa*) clinical isolates and also on standard strains (*S. aureus* ATCC 6538 and *E. coli* ATCC 8729), this results agreed with previous study which demonstrate activity of garlic extract against uropathogenic bacteria isolated from female[29-32].

Conclusions

The study concluded that Garlic ethanolic extract have a high antibacterial activities against pathogenic bacteria (*E. coli*, *K. pneumoniae*, *P. aeruginosa* and *E. faecalis*), and standard isolates (*E. coli* ATCC 8729 and *S. aureus* ATCC 6538).

The study recommends that evaluate the antimicrobial activities of garlic using large sample size.

List of Abbreviations

A. sativum: Allium sativum

SPSS: Statistical Packages for Social Sciences

UTIs: urinary tract infections

Declarations

Ethics approval and consent to participate

Ethical approval was obtained from the from Ministry of Health Ethical Research Committee in accordance with the Declaration of Helsinki Principles, and the agreement was taken from Dentistry hospital administration before sample and data collection. The patient's information was highly secured and not used for other purposes than scientific inquiry. The aims and objectives of the study along with its procedure, methods, risks and benefits of this study were explained to each participant in easily understandable local language, and written informed consent was taken from each patient.

Ethical clearance code number: MH-RES/17-022-08

Date: 20/11/2022

Ethics approval and consent to participate (Plant guidelines)

The collection of the plant parts used in this study Allium sativum leaves complied with local and national guidelines. The plant material was cultivated or wild, local and national guidelines were followed, the permissions or licenses were obtained from the from National Centre for research, Khartoum-Sudan.

License code number: NRC-RES/17-022-11

Date: 22/11/2022

Consent for publication

Not applicable.

Availability of data and materials

The data sets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

Authors declare that they have no competing interests.

Funding

Not applicable.

Authors' contributions

HAB and SMS conceived the design and carried out the experiments. AAI obtained, analyzed and interpreted the data. HAB and SMS wrote and revised the manuscript. AAI provides financial support for all experiments. All authors have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript .

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