Comparative assessment of garlic extract versus amikacin against some common pathogens: A prospective experimental study

Iqra Kanwal[1], Sheikh Abdul Khaliq[2], Bilqees Fatima[3]

ABSTRACT

Objective: To compare antibacterial effect of garlic, (a natural product) against four pathogens (Escherichia. coli, Klebseilla. pneumoniae, Staphylococcus. aureus and Pseudomonas.aeruginosa) in comparison with a standard antibiotic Amikacin.
Study Design: Prospective experimental study.
Place and Duration: Pharmaceutical Microbiology Lab, Department of Pharmaceutics, Faculty of Pharmacy, Hamdard University, Karachi from 02nd January 2018 to 25th June 2018.
Methodology: A number of 150 selected micro-organisms that are Escherichia. coli, Klebseilla. pneumoniae, Staphylococcus. aureus and Pseudomonas. aeruginosa, are collected from clinical laboratories of Karachi. Minimum sample size of study is 143 isolates. Disks of Amikacin (30 µg) were used for this purpose and garlic was taken as 100% crude extract. Disk diffusion method and well diffusion method were employed for Amikacin and garlic respectively. Student t-test was applied to detect the significance.
Results: Crude garlic extract is more effective than Amikacin against three out of four pathogens used in this study; E.coli, S. aureus and P.aeruginosa (p=0.0001), while in case of K. pneumoniae Amikacin has similar efficacy in contrast to crude garlic extract. Overall efficacy of crude garlic extract was better against standard antibiotic Amikacin (p=0.0001).
Conclusion: Garlic has a potent effect against a variety of micro-organisms especially those who have developed resistance to the standard antibiotics. It is also found to be effective against organisms that have developed multidrug resistance.
Keywords: Amikacin, Antibacterial, Disc diffusion, Garlic, Pathogen, Resistance

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INTRODUCTION

The discovery of powerful and safe antibiotics is evidently the most prominent medicinal advancement ever, which has greatly decreased the rate of mortality as well as morbidity related to the infectious diseases[1]. But with an increased use of these agents the antibiotics are developing the resistance against them day by day[2]. Fleming and Walter were first to notify about the ability of micro-organisms to adapt to the changing environment while accepting the noble prize in 1945[3]. The antibiotic resistance has extended throughout the world[4]. With the passage of time, the resistance is increasing widely and frequently in community as well as hospitals[5]. The antibiotic resistance is a leading danger to mankind,[6] as its outcomes includes the failure of treatment resulting in dangerous concerns specifically in patients with critical illness[7]. The scientists have recognized that extended usage of antimicrobials may brought about resistance and is the fundamental factor for development of resistance with high rate[8]. Based upon these findings, scientists have directed their concentration towards plant derived products[9]. Garlic is known for treatment of various diseases including infectious diseases for years[10]. Pasteur was the one who pointed out the antimicrobial properties of garlic first time in the late 50's[11]. In addition to antibacterial activity, garlic can also act against cancer, low blood sugar level, cardiac issues and inflammatory processes[10,12]. Garlic has a broad spectrum of activity and act against both Gram-negative as well as Gram-positive pathogens. It is observed that garlic also work against those organisms that have developed resistance to standard

1. M.Phil. Scholar of Pharmaceutics
2. Assistant Professor of Pharmacy
3. Assistant Professor of Pharmaceutics

Faculty of Pharmacy, Hamdard University, Karachi, Pakistan

Correspondence:
Iqra Kanwal
M.Phil. Scholar of Pharmaceutics, Faculty of Pharmacy, Hamdard University, Karachi, Pakistan
Email: dr.iqrakanwal@yahoo.com

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antibiotics[9]. So we conducted this study with an objective to compare antibacterial effect of garlic, (a natural product) against four pathogens (Escherichia. coli, Klebseilla. pneumoniae, Staphylococcus. aureus and Pseudomonas.aeruginosa) in comparison with a standard antibiotic Amikacin.

METHODOLOGY

This Prospective experimental study was conducted in the Pharmaceutical Microbiology Biology Laboratory of Department of Pharmaceutics, Faculty of Pharmacy, Hamdard University, Karachi. Duration of study is 2nd January 2018 to 28th June 2018. Minimum sample size of study was determined by precision analysis technique i.e. 143 isolates[13].

A total of 150 clinical isolates were collected from a pathological laboratory, which consisted of E.coli (n=66), K. pneumoniae (n=43), S. aureus (n=24) and P.aeruginosa (n=17), these isolates are from individuals with one infection only. Those individuals found to have more than one infection were excluded from study.

Garlic was purchased from a local market at Karachi. Garlic cloves were peeled and washed with ethanol for sterilization. When ethanol completely evaporated, the cloves were crushed using sterile mortar and pestle. The paste was then filtered by a muslin cloth, and the extract obtained was said to be a 100% crude garlic extract[14]. Disk diffusion method was used for testing Amikacin with a disk content of 30 µg as described in Kirby-Bauer method[15], while in case of crude garlic extract, agar well diffusion method was used.[9] The isolates were first inoculated in trypton soya broth and then kept at 37℃ for growth of micro-organisms. The 0.5 McFarland standard was used to compare the growth of micro-organisms in trypton soya broth. The isolates were then spread at Mueller Hinton agar using a sterile cotton swab. The disks of Amikacin were placed on the external surface of agar using sterile forceps[9]. A hole of 10 mm diameter was created in agar using sterile cork borer,[16] and 50 µl crude garlic extract was poured in the hole using a micropipette.[2] The medium was then placed in an autoclave for 24 hours at 37 ℃ for incubation. After 24 hours the plates were taken out and zone of inhibition (ZOI) were measured using a ruler[17].

Data Analysis: The data analysis was carried out using SPSS 20 software. Student t-test was used to compare the results of Amikacin and crude garlic extract for finding the differences and significance in zone of inhibitions at a 95% confidence interval, the $p<0.05$[18]

RESULTS

Among 150 clinical isolates, Escherichia coli was found to be most frequent specie with 44%(n=66) followed by K. pneumoniae 28.67%(n=43), S. aureus 16%(n=24) and P.aeruginosa 11.33%(n=17) (Fig-1).

P.aeruginosa was found to be most susceptible towards Amikacin with ZOI 91.66% while S. aureus was most resistant with ZOI 52.94%. (Table-I).

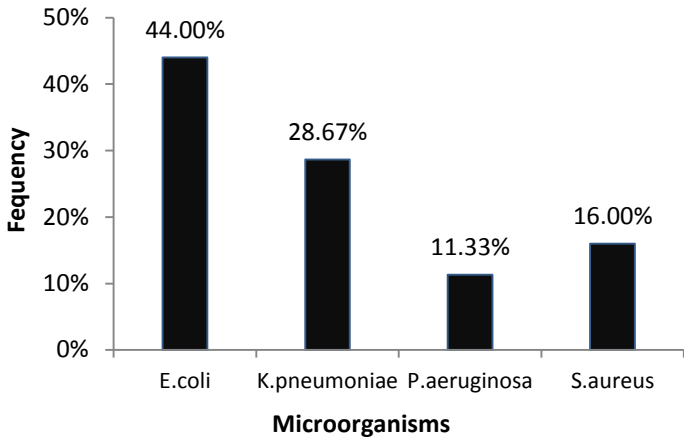

Figure- 1: Frequency distribution of microorganisms (N=150)

The zone of inhibitions (ZOI) of Amikacin was compared with the zone of inhibition of crude garlic extract. The maximum zone of inhibition of garlic extract was against S. aureus and minimum zone was formed against K. pneumoniae. In case of P.aeruginosa, S. aureus and E.coli the zone of inhibition formed by crude garlic extract was greater than that of Amikacin while in case of K. pneumoniae Amikacin was found to have similar effect like crude garlic extract. (Table-II)

DISCUSSION

The present study was conducted to evaluate the antibacterial effect of garlic against different pathogens. It is reported in various studies that garlic has an antibacterial activity against a wide variety of micro-organisms including those that have become resistant to the standard antibiotics[11,19]. Current study focuses the antibacterial effects of garlic compared with Amikacin. The study reveals that S. aureus have developed highest resistance against Amikacin among the four micro-organisms; E.coli, K. pneumoniae, S. aureus and P.aeruginosa while P.aeruginosa is most susceptible organism.

In contrast to Amikacin, garlic has been proved to have better efficacy against three of the four micro-organisms that are E.coli, S. aureus and P.aeruginosa ($p=0.0001$) which is also documented in some other studies[9,20]. While in case of K. pneumonia, Amikacin and garlic extract have similar antibacterial efficacy ($p>0.05$). Momoh et al. reported the activity of garlic against S. aureus and P.aeruginosa[21]. In another study it was found that garlic can inhibit growth of E.coli, K. pneumoniae, S. typhi, S. mutans andB. cereus[22]. Iwalukon et al. in their study suggested that garlic is effective against susceptible as well as resistant strains of common pathogens including S. pneumoniae, S. pyogenes, H. influenza, P. aeruginosa, C. albicans, E.coli, Shigella sp. as well as S. typhi[20]. Palaksha et al. also reported high sensitivity of E.coli and S. aureus towards garlic[14].

Gulfraz et al. also reported the antibacterial activity of different extracts of garlic against S.aureus, P. aeruginosa, E. coli, K. pneumoniae and S. epidermidis[10].

Table-I: Zone of Inhibitions in millimeter (mm) by Amikacin (Standard Antibiotic) (N=150)

MICRO-ORGANISMS	AMIKACIN [R= ≤ 14mm, I= 15-16 mm, S= ≥ 17 mm]					
	S*		I**		R***	
	ZOI**** (mm)	%S	ZOI**** (mm)	%I	ZOI**** (mm)	%R
E.coli	18.9	81.81%	15.83	4.54%	13.44	13.63%
K. pneumoniae	18.55	62.79%	15.37	9.30%	13.2	27.90%
P.aeruginosa	17.93	91.66%	~	~	12.55	8.33%
S. aureus	17.64	41.17%	15	5.88%	13.61	52.94%

***Sensitive, **Intermediate, ***Resistance, ****Zone of inhibitions**

Table-II: Comparative mean zone of inhibitions of Amikacin and Garlic extract (N=150)

Micro-organisms	Amikacin	Garlic Extract	t-test	p-value
E.coli	17.45 mm*	20.15 mm*	t=3.646 at 95%CI	0.0001
S. aureus	17.48 mm*	27.62 mm*		
P.aeruginosa	15.35 mm*	25.12 mm*		
K. pneumoniae	16.77 mm*	16.02 mm*	t=0.669 at 95%CI	>0.05

***millimeter**

Fratianni et al. also found out a high sensitivity of E.coli, P. aeruginosa and S. aureus to garlic extract[23].

Study can give comprehension that garlic has a potent effect against most of the microbes that have developed resistance against other antimicrobial agents. Garlic is also found to be effective against nosocomial infections, which does not respond to the standard antibiotics[2]. Many studies suggested that crude garlic is more effective against pathogens in contrast to the standard antibiotic[24].

Pathogens are capable of adaptation with their environments rapidly due to which the antimicrobial resistance is increasing day by day[6,8]. This uncontrolled and growing resistance has become a real danger to mankind. Consequences of such resistance may rise in ratio of morbidity and mortality versus treatment failures and high cost of health care[4,25]. In this era, the need of agents other than antibiotics is greatly increased than ever[9]. Researchers are focusing towards the natural products especially plant derived compounds[9].

CONCLUSION

Garlic has a potent effect against a variety of micro-organisms especially those who have developed resistance to the standard antibiotics. It is also found to be effective in case of multidrug resistance in selected micro-organisms. So in future it can work to eradicate infections, formulations development is required containing garlic as anti-infective.

CONTRIBUTION OF AUTHORS

Kanwal I: Conceived idea, Manuscript writing
Khaliq SA: Study supervisor, Designed the study, Final proofreading.
Fatima B: Literature search, Statistical analysis

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