IN VITRO ANTIBACTERIAL ACTIVITY OF ONION (*ALLIUM CEPA*) AGAINST CLINICAL ISOLATES OF *VIBRIO CHOLERAE*

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Background: Cholera is a major public health problem in developing countries of the world. Bacterial resistance, lack of surveillance data and proper microbiological facilities are major problems regarding diagnosis of cholera. The spread of microbial drug resistance is a global public health challenge that results in increased illness and death rate. Newer antimicrobials or agents are urgently required to overcome this problem. This work was therefore done to investigate the antimicrobial potential of onion against thirty-three clinical isolates of *Vibrio cholerae*. **Methods:** The extract was prepared by reflux extraction method. Antibacterial screening of clinical isolates of *V. cholerae* was done by agar well diffusion method. Agar dilution method was used to assess the Minimum Inhibitory Concentration (MIC). **Results:** All tested strains of *V. cholerae* were sensitive to onion (*Allium cepa*) extracts of two types (purple and yellow). Purple type of extract had MIC range of 19.2–21.6 mg/ml. The extract of yellow type onion had an MIC range of 66–68.4 mg/ml. **Conclusion:** The results indicated that onion (*Allium cepa*) has an inhibitory effect on *V. cholerae*. Keeping in view the anti-bacterial activity of this compound can be exploited as a therapeutic agent in an animal model. This finding is a positive point for further investigation of this herb of traditional medicine.

Keywords: *Vibrio cholerae*, resistance, *Allium cepa,* antibacterial activity, extract

INTRODUCTION

Vibrio cholerae, the causative organism of cholera is a gram negative bacterium responsible for severe morbidity and mortality in developing countries of the world including Pakistan. It has caused seven pandemics of the world.[1] Actual number of cholera cases and cholera induced deaths are under reported due to under estimation of true prevalence of the disease, lack of public education, failure to report cholera to WHO, limitations of data collection system, lack of detection in remote areas and political expediency of the nations avoiding the potential economic impacts of releasing such information.[2]

Cholera can be treated with oral rehydration solution and antimicrobial agents like tetracycline, ampicillin, nalidixic acid, erythromycin and furazolidone. Antimicrobial resistance has developed against these antibiotics.[3] Resistance to more than one antibiotic is now common among the clinical isolates. There are reports of multi drug resistant *V. cholerae* appearing with increasing frequency.[4] World Health Organization has suggested an urgent need to find new antimicrobial or new approach to combat this serious issue. According to WHO more than 80% of the world population relies on traditional medicine for their primary health care needs.[5]

Allium is the largest and important representative genus of the *Alliacae* family comprises 450 species. Onion *(Allium cepa)* is a bulbous plant widely cultivated in almost every country of the world with leading production in China, India and United States. It is rich in proteins, carbohydrates, sodium, potassium and phosphorus. Traditionally onion has been used to treat intestinal infections. It has been reported to be an antibacterial, antiviral, antiparasitic, antifungal and has antihypertensive, hypoglycemic, antithrombotic, antihyperlipidemic, anti inflammatory and antioxidant activity.[6]

In the light of above mentioned properties, the present study was conducted to investigate the antibacterial activity of *Allium cepa* against clinical isolates of *V. cholerae*.

MATERIAL AND METHODS

A total of 33 bacterial isolates of *V. cholerae* were used. Out of these eight clinical isolates were obtained from an epidemic in Muirpur Khas, Sindh. Rest of the clinical isolates were collected from Combined Military Hospital, Lahore (n=4), Institute of Public Health, Lahore (n=2) and National Institute of Health, Islamabad (n=19). The isolates were identified on the basis of their morphology, cultural characteristics and API 20NE (Biomeurix France). Final confirmation was done on the basis of serology. Susceptibility pattern of all the clinical isolates was also done by using Kirby Bauer, Disc Diffusion method according to CLSI guidelines 2009.[7]

Two types of *Allium cepa* (purple and yellow) were purchased from local market of Lahore. They were washed with water; air dried and then soaked in absolute ethanol (97%) for 5 days at room temperature with intermittent shaking. They

were filtered under ultraviolet light using filter paper. Prepared extracts were collected and stored in dark, amber colored bottles in refrigerator till use.

Screening for antibacterial activity of both types of ethanolic extracts of onion was done by agar well diffusion assay. One strain was picked up at random for screening purpose. Broth cultures equal to 0.5 McFarland standards were prepared according to method described in CLSI (2009). Four wells of equal size on each MH agar plate were made by 9 mm cork borer. A total of 120 l of extract from each dilution (doubling dilution; 50%, 25%, 12.5%, 6.25% and were poured into each well with micropipette. Phenol 6% and normal saline were used as positive and negative control respectively. The diameter of the clear zone around each well was measured in mm by digital calipers (sylvac fowler ultra- cal II).

Minimum Inhibitory Concentration (MIC) of each extract was determined by agar dilution assay. The extracts were diluted with 50% propylene glycol and different dilution concentrations were prepared. The flasks were autoclaved after which they were put in water bath at 50 ºC to avoid any solidification. Mulleur Hinton plates were prepared having 12, 14.4, 16.8, 19.2, 21.6, 24, 26.4, 28.8, 31.2, 33.6, 36, 38.4 mg/ml concentration of purple type of extract, and 58.8, 60, 61.2, 62.4, 63.6, 64.8., 66, 67.2 mg/ml with yellow type of extract.[8,9] They were allowed to solidify at room temperature and kept in refrigerator till use.

Three control plates were also set, one with MH agar inoculated with all strains to confirm the viability of the cultures, second control plate contained medium only and the third plate had extract incorporated medium to check the sterility of medium and the extract. MIC was recorded as the lowest concentration of the extract at which visible bacterial growth was completely inhibited. The experiment was performed in triplicate.

RESULTS

Sensitivity pattern of the clinical isolates is shown in Figure-1. Screening of antibacterial activity of both the types of extracts is given in Figure-2.

Table-1 shows zones of inhibition of ethanolic extracts of onion against *V. cholerae*. MIC results revealed that with EEP, 50% of the clinical isolates were inhibited at 19.2 mg/ml, 90% were inhibited at 21.6 mg/ml where as with EEY, 50% isolates were inhibited at 67.2 mg/ml and 90% strains were inhibited at 68.4 mg/ml. (Table-2). Significant difference is observed in the mean MIC of purple and yellow types of onion (p<0.001), (Table-3).

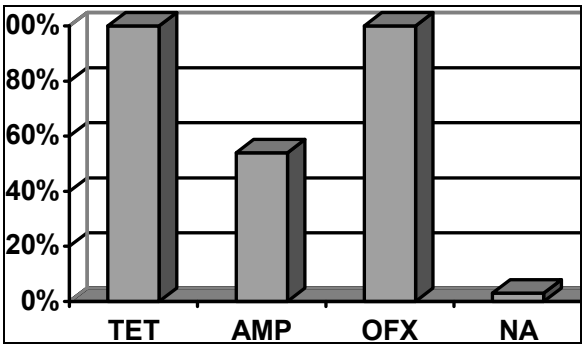

Figure-1: Susceptibility pattern of *V. cholerae* to antibiotics that are sensitive to these drugs

TET: Tetracycline 30 g, AMP: Ampicillin 10 g, OFX: Ofloxacin 5 g, NA: Nalidixic Acid 30 g.

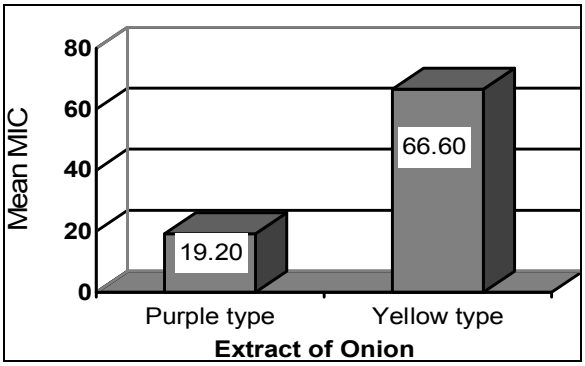

Figure-2: Comparison of MICs of ethanolic extracts of two types of *A. cepa*

Mean MIC= in mg/ml. Extract= type of extract.

Table-1: Zone of inhibition of ethanolic extracts of onion *(Allium cepa)* against *Vibrio cholerae* (UHS 8) by agar well diffusion method

Concentrations of extracts (%)	Zone of inhibition (mm)	
	EEP Mean±SD	EEY Mean±SD
100	25.83±2.18	23.16±0.28
50	23.57±0.84	18±0.5
25	16.57±1.93	15±1.0
12.5	14.63±2.43	13±0.5
6.25	12.89±2.43	0±0

EEP: Ethanolic extract of purple type of onion, EEY: Ethanolic extract of yellow type of onion.

Table-2: MIC range of ethanolic extract of purple and yellow type of onion against *V. cholerae*

Extract Type	Concentration Range (mg/ml)	MIC_{50}	MIC_{90}	MIC_{100}
EEP	19.2–21.6	19.2	21.6	21.6
EEY	66–68.4	67.2	68.4	68.4

EEP: Ethanolic extract of purple type of onion, EEY: Ethanolic extract of yellow type of onion.

Table-3: Mean MIC of purple and yellow types of onion (n=33)

V. cholerae)	EEP Mean±SD	EEY Mean±SD	*p*
MIC (mg/ml)	19.20±2.40	66.60±1.54	<0.001

EEP= Ethanolic extract of purple type of onion, EEY= Ethanolic extract of yellow type of onion

DISCUSSION

Allium cepa extract has been extensively studied for its antimicrobial activity against a wide range of bacterial, fungal and parasitic organisms. However, a limited data is available so far regarding its efficacy against *V. cholerae*.

Both the extracts exhibited antibacterial activity against *V. cholerae*. The antibacterial activity of purple type of *Allium cepa* extract was found to be better as compared to yellow type of *Allium cepa* extract**.**

Agar well diffusion method showed that both the tested extracts had antibacterial activity against *V. cholerae*. The results showed that EEP has better antibacterial activity against *V. cholerae* than EEY. Antibacterial activity of both extracts has been increased by increasing their concentration. EEP showed activity even at 6.25% whereas EEY demonstrated at 12.5% and showed no activity at 6.25%.

Our results are comparable with a study conducted by Nelson *at al* revealed that ethanolic extract of onion gave 11 mm zone of inhibition with MIC 0.8 mg/ml against *Pseudomonas aeruginosa* and 9 mm zone of inhibition with MIC 0.8 mg/ml.[8,9]

According to a study in most of the plants bulbous extracts are more effective as antibacterial agents as they have *Alliicine*. Allium plants have the higher concentration of *Allicine* in their bulbs than other organs.[10]

In a study conducted by N. Benkeblia, in Algeria, red/purple onion exhibited better antibacterial activity as compared to yellow onion against *S. aereus* and *Salmonella enteritidis*. The zone of inhibition of extracts increased with increasing concentration of extracts.[11] In a study by Rehan Irkin, in Turkey, onion extract with ethyl alcohol has inhibited *Aspergillus niger*.[12] In a study conducted by Mahesh in India, antimicrobial activity of certain plants was evaluated using the disc diffusion method against certain bacteria.[13]

The onion bulbs contain numerous organic sulphur compounds including Trans-S-(1-propenyl) cysteine sulfoxide, S-methyl-cysteine sulfoxide, S-propylcycteine sulfoxides and cycloallicin, flavinoids, phenolic acids, sterols including cholesterol, stigma sterol, b-sitosterol, saponins, sugars and a trace of volatile oil compounds mainly of sulphur compounds. The presence of these compounds may explain the anti-microbial activity of this plant.[14]

Recently in a study by Sharma *et al* in India in 2009, antibacterial activity of certain plants was evaluated against *V. cholerae*. In this study MIC was determined using disc diffusion method and revealed that *Allium sativum* was best vibriocidal among the plants used and had MIC of its aqueous extract is found to be 5–15 mg/dl and with acetone extract it was found to be 2.5–5 mg/dl.[15]

The same results were obtained in another study conducted by Sharma *et al* in India in 2009. The MIC was determined by disc diffusion method.[16]

The antibiotic sensitivity pattern of certain antibiotics has also been determined according to CLSI standards. Difference in susceptibility of *V. cholerae* was also observed in this study. According to Scrascia *at el* in 2003, 22% strains are resistant to tetracycline as compared to our study in which 100% strains are sensitive to tetracycline. Forty-seven percent strains are resistant to ampicillin while in our study 54% strains are sensitive to ampicillin. Difference in antibiotic sensitivity pattern of the organism to certain antibiotics is also observed in literature.[4]

It is evident from our study that this is a significant difference in the MICs of the two types of extracts of onion. This might be due to the difference in their constituents or might be due difference in the method of extract making or due to difference in the temperature while making agar dilution plates.

As no data is available regarding antibacterial effect of onion against *V. cholerae,* antibacterial activity of garlic is determined in studies.

It is hoped that this study would lead to the establishment of some new and more potent antimicrobial drug of natural origin and native plants. It is very important to develop guidelines for all procedures adopted in evaluating antibacterial activity of onion and analyse extracts of onion of different regions for the actual ingredient which is responsible for their antibacterial activity.

CONCLUSION

It is concluded from this study that *Allium cepa* extract has antimicrobial activity against *V. cholerae*. It is expected that using natural products as therapeutic agents will probably not elicit resistance in micro-organisms. It is essential that research should continue to isolate and purify the active components of this natural herb and use in experimental animals.

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