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Original Research Article

Isolation of *Vibrio cholerae* from sewerage and awareness level regarding Cholera among the residents of Lahore

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ABSTRACT

Background: Cholera is a major public health concern which is caused by *Vibrio cholerae*, found in freshwater, sewage and clinical reservoirs. The Water and Sanitation Agency (WASA) maintains water supply and sewerage services in Lahore, Pakistan. This study aimed to isolate *V. cholera* from sewage managed by WASA and to evaluate the awareness and sociodemographic factors linked to *V. cholerae* isolation and cholera awareness in Lahore residents.
Methods: A cross-sectional study was conducted on 100 sewerage sample from 8 towns of Lahore to isolate V. cholera. 300 individuals each representing 01 household were interviewed to assess the level of knowledge, attitude and practice regarding cholera. Sewerage samples were analyzed using the TCBS agar and Oxidase test. The serotype of positive samples was found using antisera.
Results: V. cholera (serotype O1) was isolated from 2 out of 100 sewerage samples from 2 out of 8 towns of Lahore. In areas where *V. cholera* was detected the average score of knowledge, attitude and practice among the residents was less as compared to the areas where it was not detected. The history of acute watery diarrhea among household was statistically significant in areas where V. cholera was detected (p value 0.044).
Conclusion: The isolation and the detection of V. cholera serotype O1 signifies the fact that *V. cholera* is present in Lahore. The residents of the area where *V. cholera* was detected had low level of knowledge, attitude and practice as compared to the areas where it was not detected.

Keywords: Awareness, Sewerage, Surveillance, *V. cholerae*, Wastewater

INTRODUCTION

Vibrio cholerae is a gram-negative, anaerobic, comma shaped, facultative and non-spore forming bacteria.[1] It can exist either as a biofilm or can be motile. The bacteria can survive in aquatic environment as well as the gastrointestinal tract depending on various factors such as nutrients, temperature, oxidative stress and protozoa in the aquatic environment and low pH, increased osmolality, bile, decreased level of iron and various nutrients in the gastrointestinal environment of human

host. The bacteria can survive under stressful conditions by adapting to various stress responses.[2]

V. cholera has more than 200 serotypes based on the presence of O antigen on lipoprotein layer of cell membrane. Serotype O1 and O139 are associated with causing diarrhea in humans. The serotype O1 is further divided into two biotypes, one is classical and the other is E1 Tor and two major serotypes Inaba and Ogawa. After entering human small intestine, the virulence genes are induced by the bacteria and under suitable host conditions are expressed leading to production of cholera toxin causing acute watery diarrhea, dehydration and can lead towards metabolic acidosis and hypovolemic shock. The main cause of death due to cholera is the negligence in treating the dehydration. 3 The main symptom of cholera is acute watery diarrhea that can progress to hypovolemic shock and death within 12 hours if left untreated. The cholera toxin activates adenylate cyclase thus increasing cAMP level increases the secretion of chloride and bicarbonate ion and decreases the absorption of sodium ion, causing acute water loss.[4]

John Snow discovered the link between consuming contaminated water and cholera transmission back in 1854 during the Soho Cholera outbreak in London. He identified the possible source of contaminated water and upon intervention, the Cholera outbreak was controlled. This discovery provided evidence that the presence of cholera can serve as an indicator for access to social utilities.[5] In 1854, Fillipo Pacini identified *V. cholera* under the microscope for the first time. After 29 years, Koch and his team found bacteria in the gut of individuals who had died of cholera and mentioned the link of cholera with bacteria. Later he isolated the bacteria on pure culture, marking a significant advancement in public health.[6]

Approximately, one billion people are at risk of developing cholera in the countries where cholera is endemic. At present, there are 51 countries endemic for cholera in Africa, South Asia and Southeast Asia.[7] According to the World Health Organization (WHO), 1.3 to 4.0 million cases of cholera and 21,000 to 143,000 deaths due to cholera have been reported globally. And the cases of cholera being reported have been rising continuously for the past few years. The data from 2020 has shown that 323,369 cases and 857 deaths have been reported from 24 countries.[8]

In Africa, 3,221,050 suspected cases were reported to WHO from 1970-2011 making 46% of global suspected cases. In 2014, Africa reported 105,287 cases of Cholera and amongst these 83% were from The Democratic Republic of Congo, Ghana and Nigeria. The case fatality ratio was higher than 5% in African region.[9] In Bangladesh, it is estimated that nearly 100,000 cases and 45,000 deaths occur due to Cholera. In 1990, atypical variant of *V. cholera* serotype O1, biotype E1 Tor was found in Bangladesh.[10] Cholera is endemic in Pakistan and has a potential to become an epidemic with a single laboratory confirmed case. In 2022, the province of Sindh reported 234 confirmed cases of Cholera whereas Balochistan province reported 31 and Punjab province reported 25 confirmed cases.[11] Cholera is a significant public health problem particularly in many developing countries where the provision of safe drinking water and sanitation remain compromised. The route of transmission is fecal-oral and the risk factors include consuming contaminated diet and unsanitary conditions.[12] Cholera is treated as gastroenteritis and fluid replacement remains the first line treatment option. Various antibiotics like chloramphenicol, streptomycin, tetracycline, azithromycin and ciprofloxacin have been used by clinicians in the past for the treatment of cholera, but antibiotic drug resistance has posed a new threat.[13]

Food and Drug Administration (FDA) has approved the use of oral Vaxchora for the prophylaxis of Cholera and can provide protection against cholera serotype O1 for two years. Vaxchora is a single dose live attenuated vaccine, given orally.[14] Oral cholera vaccines (OCV) recommended by WHO include Dukoral, Shanchol, and Euvichol-Plus.[8] Cleaning of water by chlorination or boiling, using hygienic measures, proper sanitation of infrastructure, using clean and separate toilets can reduce the incidence of cholera. Handwashing before and after meal could also decrease the risk of getting affected by cholera. Cholera was identified in freshwater environment of North India suggesting that water could be a reservoir for highly divergent strains of *V. cholera*. A study showed that the cause of contamination in drinking water is due to mixing of sewage in drinking water pipes.[15] *V. cholera* can also spread through contaminated wastewater, however limited data is available to find out the fate of *V. cholera* in wastewater.[16] *V. cholera* can be isolated from wastewater bodies supplementing available water sources reservoirs, the key importance of isolating from wastewater is its role in spreading microbial pollutants, causing health hazards in communities. The wastewater treatment plants receive water from these sources for the purpose of treatment. However, the use of water samples for surveillance is superior to wastewater isolation.[10] *V. cholera* can be isolated from stool cultures using the conventional methods of culture. The rapid diagnostic tests are useful during the period of epidemics to analyze and screen suspected stool specimens, water/food samples.[17] The patient of cholera has potentially shed 107 to 109 virulent strains/ml in acute watery diarrhea.[18] *V. cholera* can be isolated from the fecal matter using a culture media. It can either be inoculated directly onto the TCBS or after enrichment into Alkaline peptide water for 6-8 hours at 35-37 °C. After the overnight growth of *V. cholera* 2-3 mm size of yellow, shiny colonies are formed. After the confirmation the antisera which are *V. cholera* O1 polyvalent is used to find out the serotype which could further be tested to find out the subtype using the Ogawa and Inaba anti sera.[19] Hounmanou et al reported that *V. cholera* was isolated from wastewater,

vegetables and fish using the PCR method for sample analysis during the time of no outbreak. The prevalence of *V. cholera* was 36.7% in wastewater, 21.7% in vegetables and 23.3 % in fish. 20 In 2021, Zohra et al conducted a study in Pakistan where wastewater samples from 12 districts were tested using RT-PCR technique and 22 out of 160 samples came positive for Vibrio cholera. The factors influencing frequency and duration of cholera include poor sanitation, temperature and rainfall. In 2005, WHO introduced a system of Disease Early Warning System (DEWS) throughout Pakistan for the early diagnosis and prompt treatment and reduction of outbreaks of various diseases. However, cholera is rising in Pakistan but no proper environment surveillance data is available. [21]

During an outbreak of cholera in Rawalpindi in 2017, Akram K reported that the consumption of contaminated water was the most obvious cause for the outbreak during flood. The analysis of water samples showed the presence of coliform organisms. The serotype Inaba was also isolated from the stool samples of the cases.[22] The importance of using the wastewater for the surveillance of bacterial and viral infections lie in the fact that the untreated wastewater increases the incidence of microbial diseases. In pandemics occurring due to viruses, wastewater analysis could be used for the surveillance.[23] Reidl J et al, reported that the outbreak of cholera in endemic areas is linked with poor sanitation, overcrowding, consuming contaminated water and food such as oyster, crabs and shellfish. However, contaminated water is the main source of epidemic.[4] A meta-analysis by Richterman A et al, suggested that cholera is associated with water, sanitation and hygiene, socioeconomic status and intrinsic patient characteristics.[24] Study conducted in Bangladesh shows that the spread of diarrhea among the household is linked with a contact with a patient of cholera.[25] Cholera epidemic of Yemen in 2016 signifies that when environmental conditions become favorable, they could cause the spread of Cholera. The first wave occurred during dry season and the second wave occurred during the rainy season and the incidence was more during rainy season.[26] Cholera outbreak was declared in Karachi after 129 laboratory confirmed cases were reported in 2022 and at that time Khan HA et al, reported that the main factors related to the outbreak were poor hygiene, over-crowding, poverty and climate change.[27]

METHODS

Study design

A descriptive cross-sectional study was conducted in eight administrative zones (towns) of Lahore under the control of WASA. The study included sewerage (primary, secondary and tertiary open drains) from eight towns of Lahore and the residents of the eight WASA-administrative zones of Lahore. The study was conducted for a month from 11th October 2022 till 10th November 2022. Sampling frame of eight administrative regions of Lahore was developed with the help of WASA. Drains for sampling were selected randomly using random number generator. Non-probability convenience sampling was used to interview the residents of the area.

Sample size

Sewerage sample

Sample size was calculated using WHO sample size calculator. The proportion of V. cholera in sewage was taken as 13%. 21 Taking level of significance of 5% and error margin as 7%. The calculated sample size was 89. 100 samples of sewerage water were taken.

Survey regarding cholera from residents

Sample size was calculated using WHO sample size calculator. The proportion of knowledge regarding cholera was taken as 53%. 24 Taking level of significance of 5% and margin of error as 10%. The calculated sample size was 96. 300 individuals were interviewed.

Inclusion criteria

Wastewater samples from the primary, secondary drains and Tertiary drains, individuals from all socioeconomic group, male, female and children>5years age, having symptoms of acute watery diarrhea (AWD), having no symptoms of AWD, known or not known contact with AWD patients

Exclusion criteria

Wastewater drains not under the control of WASA, and children >5years age.

Data collection

The data was collected from 8 towns of Lahore. We collected 15 ml of waste water sample including 10 samples from Gulberg town, 20 samples from Nishter town, 15 samples from Wagha town, 10 samples from Iqbal town, 10 samples from G.B town, 5 samples from Jubilee town, 23 samples from Ravi town and 7 samples from Shalimar Town for bacteriological waste water sample collection in sterilized and sealed glass containers. The glass bottles had a rope tied at their mouth and the bottle was immersed into the drain taking the sample from surface to mid-stream level near to the wall of the drain. Each container was labelled by giving a unique identification name before the sample collection using a permanent ink marker. After that samples were stored in cold box. GIS coordinates and the air temperature from where the sample was collected were noted. The waste water samples along with a form mentioning the details about the sample were delivered to the laboratory of IPH and stored in refrigerator. At the

time of collection of waste water sample, three houses in the vicinity were interviewed using a well-structured and pre-formed questionnaire.

Assessment of knowledge, attitude, and practice (KAP) scores

Knowledge assessment

To assess the level of knowledge, a total of four questions were administered, with a cumulative score of 12. Responses of "Yes" were assigned a score of 1. Responses of "No" and "Don't know" were assigned a score of 0. For the question regarding the spread of cholera, each correct option was awarded a score of 1, while the incorrect option of touching the ill received a score of 0. For the question on the best method for hand washing, the response "water and soap for 20 seconds" was scored 2, and "water only" was scored 1.

Attitude assessment

The assessment of attitudes comprised seven questions, with a total score of 7. Responses of "Yes" were assigned a score of 1. Responses of "No" and "Don't know" were assigned a score of 0.

Practice assessment

The practice assessment included a total of 18 questions, both asked and observed, with a cumulative score of 57. Responses of "Yes" were assigned a score of 1. Responses of "No" and "Don't know" were assigned a score of 0.

Sewerage sample analysis

The wastewater samples were enriched in Alkaline Peptone water for 9 hours. After enrichment, it was allowed to grow on the TCBS agar for overnight. The culture media having yellow, shiny, 2-3 mm colonies were screened further using the Oxidase test. The specimens having oxidase positive result were labelled as *V. cholera* positive. The antisera were used to determine the serotype of *Vibrio cholera*.

Data analysis

Statistical Package for Social Sciences (SPSS) Version 24.0 was used for statistical analysis. Frequency tables were generated for all possible variables. Means and other parameters of central tendency were calculated for continuous data. Chi Square was applied to find out association between categorical variables. Means were compared using student's t test where applicable. Scatterplot was used for continuous data. The GIS coordinates were noted in spreadsheet and map was formed using Google maps.

RESULTS

A total of 100 waste water samples were collected from 8 towns of Lahore. 2 out of 100 (2%) came positive for V. cholera on Oxidase test having serotype O1 using antisera from Iqbal Town (Primary drain, Kharak Nala) and Nishter Town (Secondary drain, Kot Lakhpat). One out of ten (10%) samples from Allama Iqbal Town and one out 20 (5%) samples from Nishter Town showed positive results on Oxidase test whereas 98 out of 100 (98%) waste water samples showed negative Oxidase test, even though the TCBS agar showed yellow colonies growth. (Table 4).

There was no rainfall during the time of our sample collection. 211 out of 300 (70.3%) participants were males and 89 out of 300 (29.7%) were female participants with ages ranging from 14 years to 87 years. The mean age for the male participants was 42.77±13.32 and for female participants was 39.33±12.06. 12.7% participants had the history of acute watery diarrhea in the past one month and 87.3% had no history of acute watery diarrhea in the past one month. 6.7% were hospitalized for the diarrhea and 93.3% were not hospitalized. 6% showed the history of diarrhea among other members of family and 94% did not show any history of diarrhea among other members of family. Although just 01 out of 300 (0.3%) showed the frequency of number of individuals in home affected with cholera. 7% showed the contact with a cholera positive patient and 93% showed no known contact with cholera positive patient (Table 6).

The areas where *V. cholera* was detected the average score of knowledge among the residents was 4.333±0.8165 as compared to the areas where V. cholera was not detected was 5.571±1.4710 which is statistically significant (p=0.041) (Table 5). The areas where V. cholera was detected the average number of toilets in a house were 3.00±0.632 as compared to the areas where *V. cholera* was not detected were 2.34±1.242. This difference is statistically not significant (p=0.50) (Table 6).

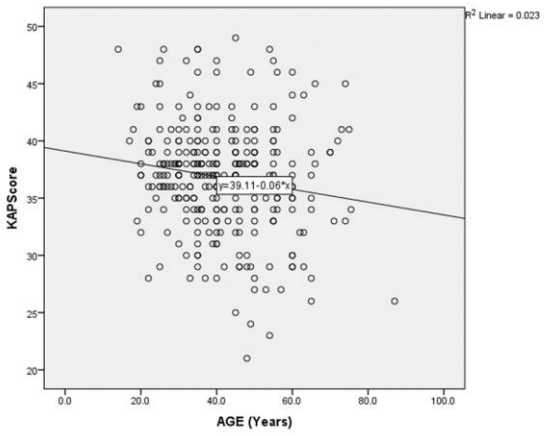

Figure 1: Correlation between age with KAP regarding cholera.

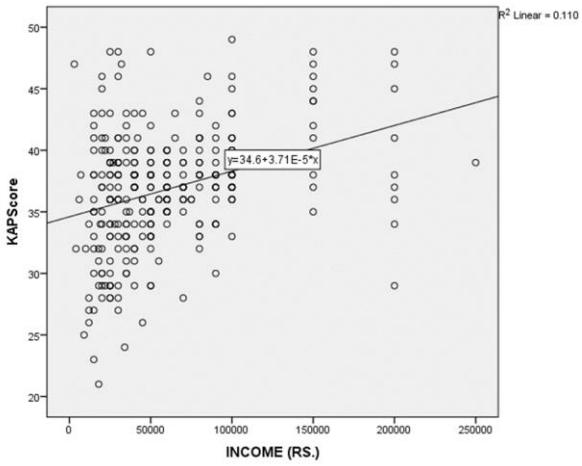

Figure 2: Correlation between income and KAP regarding cholera.

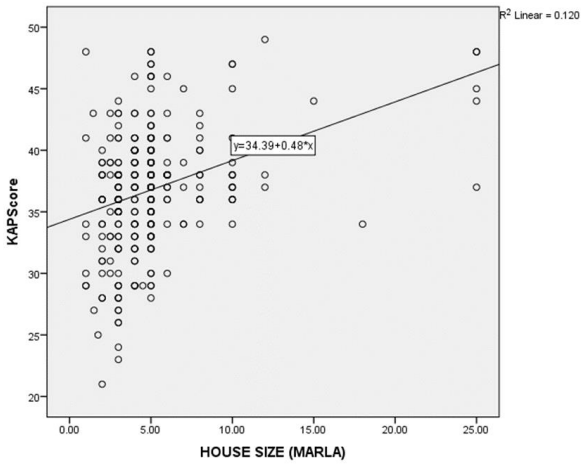

Figure 3: Correlation between house size and KAP regarding cholera.

Table 1: Questionnaire for assessment of knowledge of participants regarding cholera.

Knowledge		
S. no.	**Questions**	**Score**
1.	Do you know about cholera?	
	Yes	1
	No	0
	Don't know	0
2.	Do you know about spread of cholera?	
	Dirty hands	1
	Dirty water	1
	Dirty food	1
	Touching ill	0
	Flies	1
	Dirty toilet	1
3.	Do you know about the best way of hand washing?	
	Water and soap	2
	Only water	1
	20 seconds	2
	Don't know	0
4.	Do you know that cholera can spread through dirty toilet?	
	Yes	1
	No	0
	Don't know	0
	Total	12

Table 2: Questionnaire for assessment of attitude of participants regarding cholera.

Attitude		
S.no.	**Questions**	**Score**
1.	Do you think dirty water can spread cholera?	
	Yes	1
	No	0
	Don't know	0
2.	Do you think tap water is fit for drinking?	
	Yes	1
	No	0
	Don't know	0

Continued.

Attitude		
3.	Do you think we should wash vegetables/fruit before eating?	
	Yes	1
	No	0
	Don't know	0
4.	Do you think we should cover edibles?	
	Yes	1
	No	0
	Don't know	0
5.	Do you think we should dispose garbage at proper place?	
	Yes	1
	No	0
	Don't know	0
6.	Do you think hand washing can prevent diseases?	
	Yes	1
	No	0
	Don't know	0
7.	Do you think we should clean hands with soap?	
	Yes	1
	No	0
	Don't know	0
	Total	**7**

Table 3: Questionnaire for assessment of practices of participants regarding cholera.

Practice		
S. No	**Questions**	**Score**
1.	Use any method to clean water	
	Yes	2
	No	0
2.	Name of method to clean water	
	Boil	2
	Filter	2
	Cloth/strainer	1
	Other	1
3.	Do you wash vegetables/fruits before eating?	
	Yes	2
	No	0
	Sometimes	1
4.	Do you cover edibles?	
	Yes	2
	No	0
	Sometimes	1
5.	How often do you eat from outside in a week?	
	Don't eat	3
	Once	2
	Twice	1
	More than twice	0
6.	Is the soap always available in washing area/toilet?	
	Yes	1
	No	0
	Don't know	0
7.	Do you wash hands with soap?	
	Always	2
	Sometimes	1
	Don't	0

Continued.

Practice		
8.	When do you wash hands with soap?	
	After using toilet	2
	Before eating/cooking	2
	After eating/cooking	1
	After cleaning house	2
	After changing diaper of a child	2
9.	How long do you wash your hand?	
	30 seconds	2
	20 seconds	2
	10 seconds	1
	Don't notice	0
10.	Where do you trash garbage?	
	Garbage cart	2
	Street	0
	Garbage dump	0
	Other	0
11.	How often do you clean the toilet in a week?	
	Daily	4
	More than thrice	3
	Twice	2
	Once	1
	Don't know	0
Observations		
12.	Food items properly covered	
	Yes	1
	No	0
13.	Flies absent	
	Yes	1
	No	0
14.	Garbage properly covered	
	Yes	1
	No	0
15.	Toilet cleanliness	
	Yes	1
	No	0
16.	Soap available	
	Yes	1
	No	0
17.	Nails cleanliness	
	Yes	1
	No	0
18.	Sewage system	
	Closed	1
	Open	0
	Total	57

Table 4: Sewerage sample analysis.

S. no.	Town (n=8)	No. of samples taken (n=100)	*V. cholera* detected	No. of samples positive for cholera (n=100)	%	Average Temperature (°C)	No. of residents interviewed (n=300)
1.	Gulberg	10	Nil	0	0	30	30
2.	G.B	10	Nil	0	0	29	30
3.	Iqbal	10	Detected	1	10	31	30

Continued.

S. no.	Town (n=8)	No. of samples taken (n=100)	*V. cholera* detected	No. of samples positive for cholera (n=100)	%	Average Temperature (°C)	No. of residents interviewed (n=300)
4.	Jubilee	5	Nil	0	0	28	15
5.	Wagha	25	Nil	0	0	31	45
6.	Ravi	23	Nil	0	0	30	69
7.	Nishter	20	Detected	1	5	32	60
8.	Shalimar	7	Nil	0	0	30	21

Table 5: Mean comparison of *V. cholera* and KAP score

Variables	*V. cholera* detected	*V. cholera* not detected	t test	P value	Remarks
	Mean±SD	Mean±SD			
Knowledge score	4.333±0.8165	5.571±1.4710	0.191	0.041	Significant
Attitude score	5.833±0.4082	6.058±0.6072	0.764	0.368	Not significant
Practice Score	24.0±2.284	25.207±4.0652	0.155	0.469	Not significant

Table 6: Mean comparison of *V. cholera* and sociodemographic variables.

Variable	*V. cholera* detected	*V. cholera* not detected	t test	P value	Remarks
	Mean±SD	Mean±SD			
Income (Rs.)	66583±34103.397	58755±42905.021	0.879	0.658	Not significant
House size (Marla)	6.3333±2.875	4.9803±3.479	0.640	0.345	Not significant
No. of rooms in house	3.83±0.753	3.11±1.334	0.200	0.188	Not significant
No. of washrooms in house	3.00±0.632	2.34±1.242	0.046	0.50	Not significant
AWD in past one month	1.83±0.408	1.87±0.332	0.574	0.767	Not significant

Table 7: Comparison of *V. cholera* and sociodemographic (categorical) variables.

Variable		*V. cholera* detected		*V. cholera* not detected		P value	Remarks
		n	%	n	%		
Education	Illiterate	1	16.7	59	20.1	1.000	Not significant
	Literate	5	83.3	235	79.9		
Family type	Nuclear	2	1.3	153	98.7	0.435	Not significant
	Extended	4	2.8	141	97.2		
Hospitalized	Yes	1	5.0	19	95.0	0.341	Not significant
	No	5	1.8	275	98.2		
AWD among household	Yes	2	11.1	16	88.9	0.044	Significant

The residents living in the area where V. cholera was detected, 16.7% were illiterate and 83.3% were literate as compared to the residents where V. cholera was not detected 20.1% were illiterate and 79.9% were literate (p=1.000) (Table 7).

The residents living in the area where V. cholera was detected, 11.1% had a history of AWD among their household members as compared to the residents where V. cholera was not detected 1.4% had no history of AWD among their household members (p=0.044) (Table 6).

The level of KAP when compared with various sociodemographic variables showed the following results. The average score for knowledge for males was 5.408±1.4491 as compared to the females was 5.876±1.4757, which is statistically significant (p=0.011). (Table 7). The average score for knowledge among illiterate was 4.833±1.5422 as compared to the literate

was 5.725±1.3991, it is statistically significant (p=0.000). The average score for attitude among illiterate was 5.933±0.8410 as compared to the literate was 6.083±0.5268, which is statistically insignificant (p = 0.192). The average score for practice among illiterate was 22.117±4.5737 as compared to the literate was 25.950±3.5058, a statistically significant result (p= 0.000) (Table 4). The correlation between age and KAP score was r=-0.125 This correlation is weakly negative. (Figure 1) The correlation between income and KAP score was r=0.332. This correlation is weakly positive. (Figure 2) The correlation between house size and KAP score was r=0.347. This correlation is weakly positive (Figure 3).

Table 8: Mean comparison of KAP score and sociodemographic variables.

KAP score	Variable		t test	P value	Remarks
	Mean±SD	Mean±SD			
Knowledge score	**Male**	**Female**			
	5.408±1.4491	5.876±1.4757	0.710	0.011	Significant
Attitude score	6.043±0.6422	6.079±0.5051	0.314	0.638	Not significant
Practice score	24.611±3.7366	26.539±4.4109	0.021	0.000	Significant
Knowledge score	**Illiterate**	**Literate**	0.479	0.000	Significant
	4.833±1.5422	5.725±1.3991			
Attitude score	5.933±0.8410	6.083±0.5268	0.001	0.192	Not significant
Practice score	22.117±4.5737	25.950±3.5058	0.000	0.000	Significant
Knowledge score	**Nuclear family**	**Extended family**	0.642	0.680	Not significant
	5.581±1.4499	5.510±1.4959			
Attitude score	6.045±0.5387	6.062±0.6690	0.172	0.809	Not significant
Practice score	24.865±3.6575	25.524±4.3971	0.027	0.160	Not significant

DISCUSSION

Cholera is waterborne infectious disease with history dating back to the 19th century.[5,6] It has almost become unknown in developed countries but it can become an epidemic in developing countries.[12,28] According to WHO and other studies conducted in Pakistan show that Pakistan is endemic for cholera.[11,21,22,29] The isolation of *V. cholera* from sewerage water suggests that cholera is present in Lahore District as evident in our study which is an alarming situation for developing countries like Pakistan.

Considerable number of studies conducted in Africa, Yemen, Bangladesh and Pakistan show that cholera tend to increase during hot and rainy season.[16,26,29,30] In our study cholera was isolated during the months of October and November with average temperature of 30°C with no rainfall suggests that due to climate change the summer season has prolonged as compared to the winters and the bacteria are adapting according to the changing environment, thus increasing number of cholera cases.

Studies conducted internationally and nationally have shown that various methods are used to isolate V. cholera from water or fecal samples, particularly during the time of an epidemic.[17,31] In studies conducted by Houmanou YM et al in Tanzania, Islam MS et al in Bangladesh, and Zohra T et al in Pakistan show that the water sample could be tested for the detection of microbial contamination and could be further tested using Rapid Diagnostic Test (RDT), culture or PCR.[20,21,32] For fecal matter, RDT, culture on TCBS agar with further confirmation using the oxidase test. The serotyping could be done using the antisera. The strains of *V. cholera* could be compared using the electrophoresis.[19] However, in this study the sewerage samples were used for the isolation of *V. cholera* and the method that we opted for the isolation of *V. cholera* was TCBS agar and oxidase test. The antisera were used to detect the Serotype O1. More samples of *V. cholera* could have tested positive for cholera if PCR or other method of isolation were used.

Even though more than 200 serotypes of V. cholera have been discovered so far, but serotype O1 is notorious for causing cholera epidemics.[3,10] The serotype detected in this study was serotype O1 which is a serious concern.

Studies conducted in The United Kingdom and India have shown that the isolation of *V. cholera* from the aquatic environment, fish and the aquatic birds. However, limited data is available to isolate it from wastewater.[16,28] Our study shows that wastewater is one of the sources for spreading V. cholera in Pakistan apart from other sources such as contaminated food and drinking water & poor sanitary conditions. The importance of using the wastewater for isolation of *V. cholera* lies in the fact that our study could be a fruitful addition to the already available but limited data. Various studies conducted in developing countries such as Africa, Yemen, Bangladesh show that the level of KAP about the risk factors of cholera is associated with age, gender, income, education, family type and house size and various other sociodemographic factors.[24,26,33–35] Studies done in Pakistan also show that cholera is related with low socioeconomic condition, overcrowding, unhygienic

practices and unclean drinking water.[21–23,27,29,36,37] In our study, the areas where *V. cholera* was detected had low level of KAP as compared to the areas where *V. cholera* was not detected, hence suggesting that the incidence of cholera is related with sociodemographic factors and the hygienic practices opted by the community.

Our study suggests that the areas where *V. cholera* was detected, level of education, income, family type, house size, average number of rooms and toilets in a house were not linked with the causative factor for cholera even though these factors influence the outcomes of *V. cholera*. [26,37,38] The results might have been different if the sample size was large enough.

A study conducted by Weil et al, in Bangladesh reported that one of the factors that contact of an individual with a cholera patient could influence the development of AWD.[25] In our study, it was noted that the individuals who were tested positive for *V. cholera* showed the history of *V. cholera* among household. The visible justification to this is that people living in a household share toilet, food items, common source of drinking water and other inanimate objects thus causing disease in healthy people.

Even though studies conducted in Africa, Bangladesh and Pakistan show that the higher level of education could help individuals to adopt better knowledge, attitude and practices towards cholera prevention and treatment.[27,34,37,38] However, our study shows that there is no significant impact of education regarding awareness of cholera. The possible reason could be that formal education alone does not affect the level of awareness regarding cholera. This highlights the need for including health education in school curriculum.

The level of KAP about the risk factors of cholera is related with gender and age of an individual as suggested by various studies conducted in Africa and Bangladesh.[24,25,34,35] Our study shows that the level of KAP was more in men as compared to the females, and younger individuals as compared to the elderly and children. This signifies that male population and younger individuals have more exposure to the knowledge about the preventive measures of cholera as elderly and children are dependent on others for information.

It is apparent from various studies conducted in developing countries such as Africa, Yemen and Bangladesh that better the per capita income and a bigger house better will be the level of KAP regarding the awareness of cholera.[26,35,38] This relation was evident in our study as the income and the house size of the sample population increased the level of KAP regarding awareness of cholera. This could be due to the fact that the people with higher income are able to attain the better access to health-related knowledge. The limitations of this study were small sample size, financial constraints, Laboratory constrains regarding complete investigation of sewage water, Unavailability of laboratory confirmed cases of cholera.

CONCLUSION

The isolation of *V. cholera* from sewerage and detection of serotype O1 signifies the fact that *V. cholera* is present in Lahore, Pakistan. The residents of an area where *V. cholera* was detected had low level of knowledge, attitude and practice as compared to the areas where *V. cholera* was not detected. Those who were young, had higher income and larger house size had better KAP score. The level of awareness regarding cholera was not related to literacy status.

Recommendations

Sewage testing should be made a part of routine surveillance protocol during an outbreak to ensure timely control of the disease. The waste water treatment system and disinfection of sewage water of Lahore needs to be upgraded to prevent the spread the water borne diseases. Preventive strategies such as handwashing need to be encouraged in order to improve the current health status of the community. Health education should be included in school curriculum, seminar and print media could be used to spread awareness regarding cholera among masses

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