IOSR Journal Of Pharmacy
(e)-ISSN: 2250-3013, (p)-ISSN: 2319-4219
www.iosrphr.org Volume 5, Issue 4 (April 2015), PP. 36-40

Isolation and Identification of Coliform Bacteria from Drinking Water Sources of Hazara Division, Pakistan

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ABSTRACT: *In Pakistan there is not a good awareness about water borne diseases. It is just due to lack of knowledge and infrastructure and it is not a hidden thing that in Pakistan water borne diseases are not different from world. In this study determination of coliforms specially E.coli, P.aeruginosa, Salmonella and H. pylori were isolated and identified by using 100 ml of drinking water sample from common sources. WHO recommendation tells us that any 100 ml water sample used for deinking must not contain any coliforms in it. In this study a total of 90 samples were collected from 3 different cities of Hazara Division (Mansehra, Abbottabad and Haripur). To find out pathogenic bacteria culturing technique was used followed by staining for identification of bacterial specie. In Mansehra 15 samples (16.66%) were found pathogenic, 18 samples (20%) from Abbottabad and 16 samples (17.77%) from Haripur respectively. Four Different bacterial species were found i-e E.coli, P. aeruginosa, Salmonella and H. pylori. Ecoli was mostly isolated specie that was identified in 24 samples (26.66%) followed by P. aeruginosa 11 samples (12.22%), H. pylori 8 samples (8.88%) and Salmonella 6 samples (6.66%). This study concludes that disinfection of water should be implemented to reduce water borne diseases, water supplying departments have to follow WHO standards for better public health and to control disease outbreak by coliforms.*

KEYWORDS: *E.coli, P. aeruginosa, Salmonella, H. pylori, Contamination,*

I. INTRODUCTION

Earth consist of approximately 70% surface area covered with water and remaining is land which have only 2% water which is drinkable [1]. Water is an important chemical molecule containing feature of life it can be dissolved into organic compounds, salts, inorganic compounds and gases that are involved in metabolic processes because it is universal solvent and due to that it provides stability to membrane system, macro molecules, hemostatis, transportation and thermal regulation of body [2,3,4,5]. All cells of body contain water as an important component. Water content of a single cell is 45% to 95% and microorganism contains 80% of body weight as water and human contains water i-e 70% of their body weight. It is thermal regulator of human body and normal human body contains 42 liters of water in them [6]. Whenever 2.7 liters of water loss from body it can leads to headache, dehydration and weakness. Water is equally important and critical for both humans and environment and it is a key issue in form of drinking water [7]. Dams, canals and wells show importance of water and the impact of human beings on water cycle. Environmental effects like migration of peoples and animals, land losses, change of environmental factors, depletion of biological resources shows that these activities are noticeable [8]. Pathogenic contamination of water is also important threat for living organisms. In Asian regions peoples those are living near to rivers are at high risk of their lives because of sewage pollution which is directly disposed off from chemical factories and septic tanks that are the main reservoir of pathogens involves in water borne diseases [9]. Developing regions lack in provision of safe drinking water to their peoples and in Africa and Asia almost 800 billion individuals using unsafe drinking water which results in suffering of individual from water borne diseases [10].

II. OBJECTIVES OF STUDY

1. To find out the presence of coliforms in drinking water of Hazara Division (Mansehra, Abbottabad, and Haripur cities).
2. To find out the storage effect in households on the presence of coliforms.
3. To find out the prevalence of bacterial pathogens in drinking water of Hazara Division.
4. To find out the quality of water used for the purpose of drinking of Hazara Division.

III. METHODOLOGY

SAMPLING SITES

Current study was carried out to examine the quality of drinking water of Hazara Division, Pakistan. In Hazara division Municipal Corporation store water and supplied it to local population through pipe lines. Knowing the public health risk from unsafe drinking water three cities i.e Mansehra, Abbottabad and Haripur were chosen to study the quality of daily used drinking water.

SAMPLE COLLECTION

A total of 90 samples were collected from different demographic location of Mansehra, Abbottabad and Haripur including Rural and Urban areas (30 from each city). 100 ml water sample was collected and transferred it into disposable sterilized test tubes. Also pH of water was tested by using combi 3 dipsticks.
After collection of sample test tubes were tightly closed to avoid any contamination and protection to make it protected from environmental pathogen contamination.

STERILIZATION

At first Petri plates, test tubes and other instruments like flasks etc were sterilized using spirit and allowed to cool down after that they were autoclaved at 121^0C. After that Petri plates were dried in laminar flow hood in presence of UV light.

PREPRATION OF CULTURING MEDIA

Ingredients of media were taken in conical flasks and mixed using international criteria for Media preparation [11]. The samples were autoclaved to remove suspension and bacteria at 110°C by plugging them with cotton and covering them with aluminum foils. After sterilization media was transferred to Petri plates and incubate at 30°C for 24 hours,

CULTURING

With the help of streaking water samples were streaked on prepared culture and incubate it for 24 hours at 30°C. Placed petri plated upside down to prevent any environmental contamination.

GRAM STAINING

Bacterial growth appeared were obtained and fixed on glass slide and stained using crystal violet for 30 seconds and then washed using distilled water. After that Gram Iodine was applied for 10 sec and used 95% Acetone alcohol as decolorizing agent and finally safranin were applied and wash slide with water dried and observed using microscope.

OXIDASE TEST

Oxidase regent was prepared using manufacturer instructions and then drop 2-3 drops of it on filter paper placed in petri plate. Then by moving some bacteria to regent showed those bacteria which changes color to deep purple on treated filter paper within 10 seconds were report as oxidase +ive.

IV. RESULTS & DISCUSSION

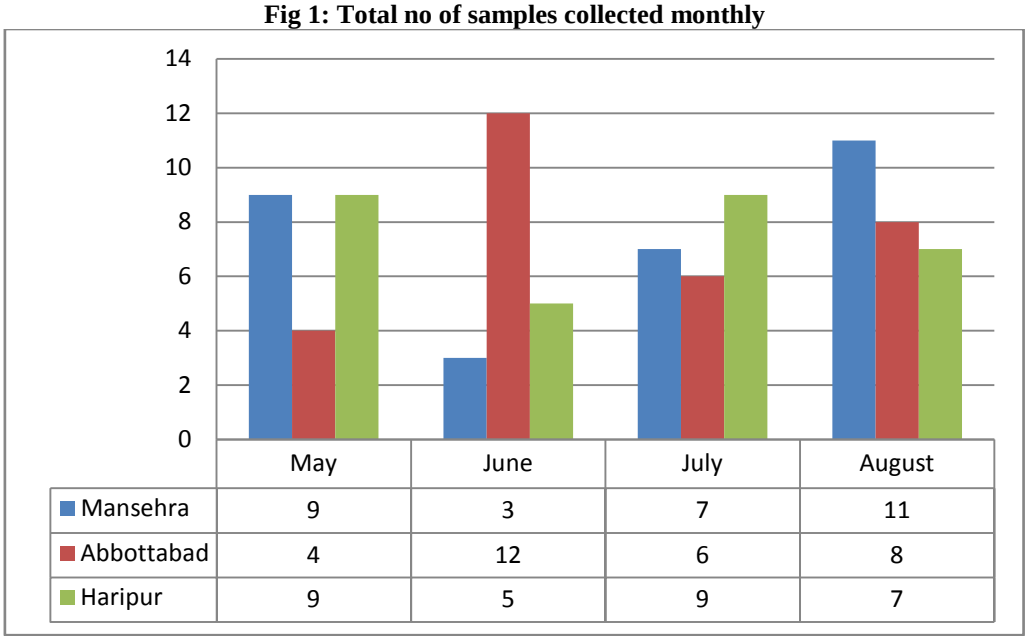
A total of 90 samples were collected from different demographic locations of Hazara division from 1st May 2014 to 30th August 2014. Rural and urban areas of three major cities of Hazara division were selected i-e Mansehra, Abbottabad, Haripur and samples were collected. 30 samples were collected from each city to achieve a good comparison as shown in Fig 1. After a careful experimental work Abbottabad stand top for having most number of coliforms found in drinking water samples with 18 samples (20%) followed by Haripur city were total no of coliform identified were in 16 samples (17.77%) and in Mansehra bacterial species were found in 15 samples (16.66%) as shown in Fig 2. Four Bacterial species were found i-e E.coli, P. aeruginosa, Salmonella and H. pylori. Ecoli was mostly present specie and it was identified in 24 samples (26.66%) followed by P. aeruginosa in 11 samples (12.22%), H. pylori in 8 samples (8.88%) and Salmonella in 6 samples (6.66%) as shown in Fig 3. In Mansehra most prevailing pathogen found was E.coli in 8 samples (8.88%) > P. aeruginosa in 3 samples (3.33%) > Salmonella in 2 samples (2.22%) > H. pylori in 1 samples (1.11%). In Abbottabad E.coli were found in 6 samples (6.66%) > H. pylori in 4 samples (4.44%) > P. aeruginosa in 2 samples (2.22%) > Salmonella in 0 samples (0%). In Haripur city E.coli were found in 10 samples (11.11%) > P. aeruginosa in 6 samples (6.66%) > Salmonella in 4 samples (4.44%) > H. pylori in 3 samples (3.33%) as shown in Fig 4. pH of samples recorded for every sample and it shows the mean ph in month of June 7.9 > July 7.8 > August 7.6 > May 7.4 respectively as shown in Table 1. Temperature of Hazara division recorded per month and mean temps shows that month of July 33 ^{0}C > June 32 ^{0}C > August 26 ^{0}C > 22.05 ^{0}C respectively as shown in Table 2.It has beeen the goal to achive diagnostics for target coliforms in clinical labs and from the last decade successful efforts have been made [12]. According to Stevens *et al., E.coli* is main indicator for fecal contamination [13]. Jay stated that *E.coli* presence is indication of enteric patrhogens [14]. According to Baudart *et al.,* Water quality is directly proportional to presence of coliforms in water [15]. Bej *et al.,* and Petit *et al.,* studies showed that *E. coli* is mostly concerned with fecal pollution [16,17]. According to Baudizsova *E. coli* should be used as a prime bacteria as indicator for pathogenic contamination of water [18]. Kudryavtseva and Edberg *et al.,* reported that *E. coli* survival depends upon environmental factors and type of water they mostly survived 4 to 12 weeks at moderate temperature [19,20]. In a study by Havelaar *et al., P. aeruginosa* was most prevalent possibly because of its mesophilic nature [21]. According to Jarvis and Martine nosocomial pneumonia respiratory tract infections are because of *Pseudomonas* [22]. In developed countries there are very rare cases to isolate *Salmonellae* because of management of system [23,24].

Okonko *et al*., stated that pH value of samples were within range which is mostly confirmed by other authors [25]. Sautour *et al.*, study presented that Bacterial survival greatly depend upon incubation temperature [26]. According to W.H.O bacterial growth increases when temperature increases and it will lowers down when temperature drops [27]. Kirchman and Rich stated that bacterial species respond quickly to higher temperature when there is avalibilty of dissolved organic matter [28].

V. CONCLUSIONS

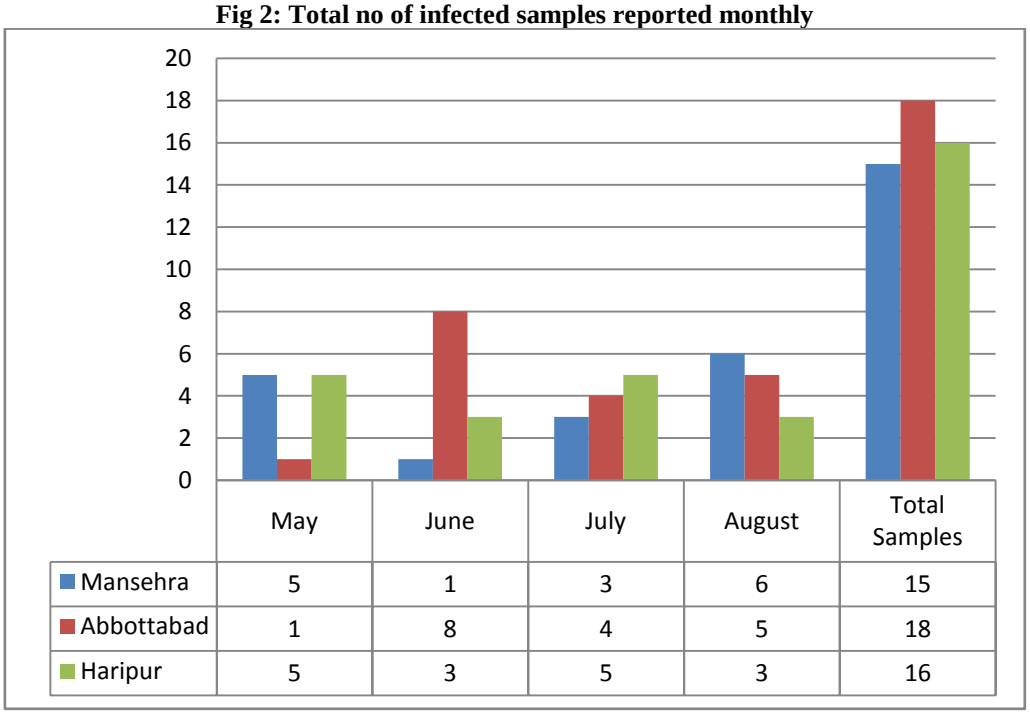
Water used for drinking is highly contaminated in Hazara Division (Mansehra, Abbottabad and Haripur cities). As summer progressed no of pathogenic bacteria increased isolation of four different bacterial species indicate high no of water borne diseases in Hazara division. So water authorities should have to take steps to control coliforms in drinking water in order to prevent population from water borne diseases.

Fig 1: Total no of samples collected monthly



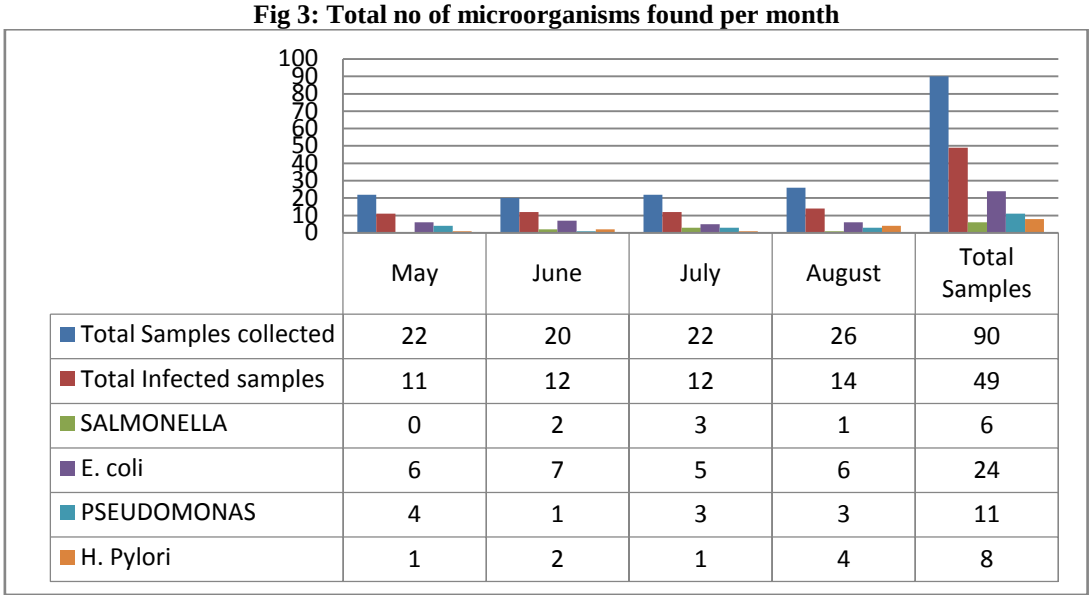
	May	June	July	August
Mansehra	9	3	7	11
Abbottabad	4	12	6	8
Haripur	9	5	9	7

Fig 2: Total no of infected samples reported monthly



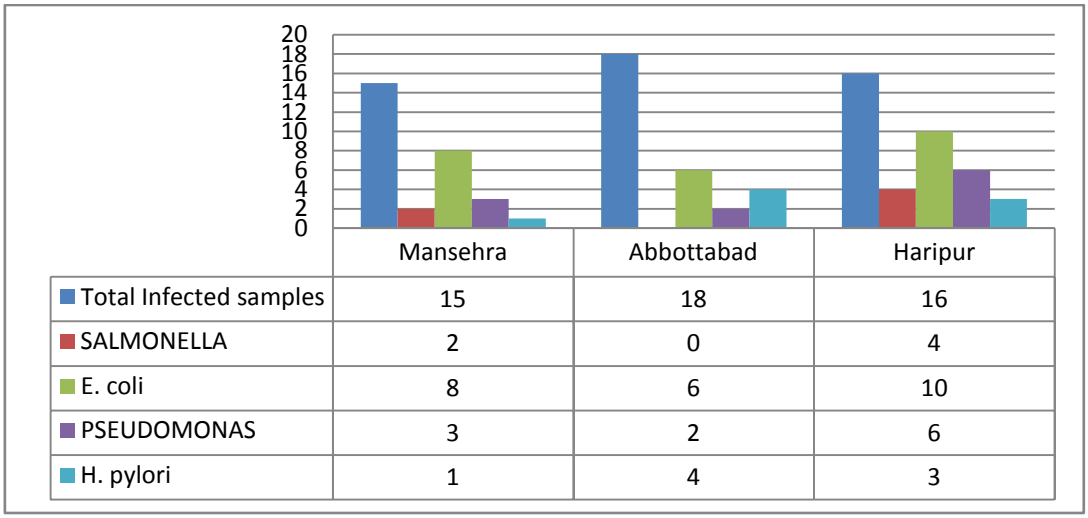
	May	June	July	August	Total Samples
Mansehra	5	1	3	6	15
Abbottabad	1	8	4	5	18
Haripur	5	3	5	3	16

Fig 3: Total no of microorganisms found per month



	May	June	July	August	Total Samples
Total Samples collected	22	20	22	26	90
Total Infected samples	11	12	12	14	49
SALMONELLA	0	2	3	1	6
E. coli	6	7	5	6	24
PSEUDOMONAS	4	1	3	3	11
H. Pylori	1	2	1	4	8

Fig 4: Total no of microorganisms found according to demography



	Mansehra	Abbottabad	Haripur
Total Infected samples	15	18	16
SALMONELLA	2	0	4
E. coli	8	6	10
PSEUDOMONAS	3	2	6
H. pylori	1	4	3

Table 1: pH of Water Samples,

Month	Minimum	Maximum	Mean
May	7	7.8	7.4
June	7.2	8.6	7.9
July	7.5	8.1	7.8
August	7.2	8	7.6

Table 2: Temp of Hazara Division

MONTH	MINIMUM ^{O}C	MAXIMUM ^{O}C	MEAN ^{O}C
May	17.6	26.5	22.05
June	26	38	32
July	30	36	33
August	22	30	26

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