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Diarrhea Outbreak Caused by Contaminated Water used for Vegetables Sale

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ABSTRACT

Water has always played a prominent role in human civilization. The water sources used for supplying water were not always clean, and coliforms are the major source of diarrheal infections especially in developing countries and hence contaminated water is the main reason for the spread of these coliforms with in a community by different ways. The contamination of water used in vegetables by pathogens cause diarrheal disease is the most important aspect of vegetables quality. This problem arises as a consequence of contamination of water by faecal matter, particularly human faecal matter, containing pathogenic organisms. Vegetable sellers usually use dirty/contaminated water to shower on vegetables to keep them fresh. This study describes the outbreaks of diarrheal diseases along with the reasons for the outbreaks. In our study we have used Membrane filter technique to test the quality of water used by the vegetable sellers. Purpose of this study is to detect the presence of coliforms which is associated with diarrheal infections due to fecal contamination. We examined the water samples by Membrane filter method in which we use 0.45µm filter paper which was soaked in peptone water and incubated at 37 ºC for 24 hours, this peptone water was further streaked on MacConkey and EMB media. Additional identification of microorganisms was done by microscopy and biochemical tests. Observed results revealed that the water is fecally contaminated and the microorganisms isolated were as with described percentages *E.coli* **12%, Klebsiella 40%, Proteus 44%, Pseudomonas 20%, Shigella 8%, Salmonella 4% and Enterobacter 4% who are the members of coliforms. This study helps in the identification of coliform from different sources which lead to a cause of diarrheal infection**

Key Words: Membrane filter technique, Coliforms.

INTRODUCTION

Normally fruits and vegetables carry nonpathogenic, epiphytic micro-flora. Produce may be contaminated with pathogens during production on the farm and all stages of product handling from harvest to the point of sale (Beuchat, 1996). On the farm the possible microbial hazards include the use of raw manure and contaminated soil amendments, dirty irrigation water, wild animals and birds, dirty farming equipments, critical health conditions or unhygienic conditions of the employee at the harvest and packaging could contaminate the product. Rinse water, dust, insects, harvesting equipments, transport containers, ice and transport vehicles, processing equipments, feces, wild and domestic animals are the post- harvest contamination of fruits and vegetables (Buchanan and Gibbons, 1974). Wide varieties of microorganisms including pathogenic microorganism's harbors fresh produce as raw agricultural products. Bacteria such as *Clostridium botulinum, Bacillus cereus, L.monocytogens* all are normally inhabitants of man soil and are capable of

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causing illness in humans, whereas, *E.coli,* Shigella, Salmonella and Campylobacter reside in the intestinal tract of animals and humans, can contaminate fruits and vegetables through contact with feces, untreated irrigation water or surface water and sewage. The membrane filter (MF) technique is fully accepted and approved as a procedure for monitoring drinking water microbial quality in many countries. This method consists of filtering a water sample on a sterile filter with a 0.45-µm pore size which retains bacteria, incubating this filter on a selective medium and enumerating typical colonies on the filter. Bacteria were identified based on colonial characteristics on nutrient and MacConkey agar, Gram coloration, motility and biochemical reactions using previously established schemes (Bernetter and Beuchat, 2001; Cheesbrough, 2000; Cowan and Steel, 1985).

MATERIALS AND METHODS

Study Duration: 06 months (July 2012 – December 2012).

Sample Size: 25 water samples collected from vegetable sellers from different regions of Karachi vegetable markets.

Requirement: 0.45 µm filter paper, Wire loop, Needle, Match box, Burner.

Glassware & Plastic ware: 25 sterile test tubes, Test tube stand, 5ml syringe for peptone water, 10ml syringes for water sample, Beaker for disposal of water, 25 sterile sample bottles, Slides.

Equipment: Sterile filter assembly.

Media: Peptone water, Mac Conkey agar, EMB agar, Sugar Tube (Glucose, Lactose, Mannitol, Sucrose).

Stain: Gram Stain.

Method: Different water samples were collected from different vegetable carts in 25 sterile test tubes and mark them as 1,2,3-25 respectively. Water samples were filtered through filter assembly, containing filter paper size 0.45µm by the help of syringe. The filtered water was collected in a beaker and was disposed off and the filter paper was placed in bottle (mark respective to the test tubes) containing peptone water and incubates the bottles at 37^{o}C for 24 hours. Take a loop full from the respective marked peptone bottles and streak on Mac Conkey agar and EMB agar plate which are also marked respective to the bottles. Incubate the agar plates at 37^{o}C for 24 hours in the incubator Observe the colonial morphology of colonies appearing on agar plates. Perform gram staining and perform biochemical test: (Urease test, Citrate test and TSI). Incubate the urease tubes, citrate tubes and TSI tubes in incubator at 37^{o}C for 24 hours. Observe the results and make conclusion.

RESULTS

The purpose of our study is to detect and isolate microorganisms/ coliforms from water samples collected from vegetable carts which the vegetable sellers shower on vegetables to keep them fresh, can cause diarrheal outbreak in a community. In this study we have isolated 12% *E. coli*, 40% Klebsiella, 44% Proteus, 44% *Pseudomonas aeruginosa*, 8% Salmonella, 4% Shigella and 4% Enterobacter by the help of cultural characteristics, microscopy and biochemical tests which includes (Urease test, Citrate test and TSI test) (Fig. 1-6) and the conformational identification was done by the

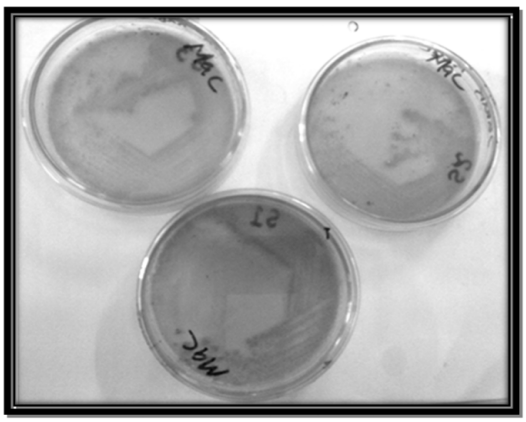

Figure 1: Gram-ve organism isolated on MacConkey agar

Table I. Primary Isolation Using Differential Media.

S.NO	MacConkey Agar	EMB Agar	Microscopy
1.	Non fermenting colonies and some are pink color, big, mucoid isolated Colonies appear.	Pinkish purple colored, spreading gummy colonies appear	Gram –ve scattered rods .
2.	Non fermenting and spreading colonies and 4-5 pink ,big, mucoid isolated colonies	Green metallic sheen appear and . gummy spreaded purple colonies	Gram –ve rods in chain and scattered also.
3.	Non fermenting and spreading colonies and some are pink, mucoid isolated colonies appear	Pink isolated and spreading gummy colonies and also purple very small Colonies appear	Gram –ve short scattered rods.
4.	Non fermenting spreading colonies	Isolated pink colored colonies.	Gram –ve short scattered rods.
5.	Non fermenting spreading colonies	Spreading pink colored colonies	Gram –ve short rods in chain.
6.	Non fermenting spreading colonies	Isolated gummy pink colored colonies	Gram –ve short rods in chain and scattered also.
7.	Non fermenting spreading colonies.	Isolated gummy pink colored colonies	Gram –ve short scattered rods.
8.	Non fermenting spreading colonies	Isolated gummy pink colored colonies	Gram –ve short scattered rods.
9.	Non fermenting spreading colonies.	Isolated gummy colonies and 2-3 are big gummy, black centered or nucleated colonies appear.	Gram –ve very small rods.
10.	Non fermenting spreading colonies	Gummy spreading colonies appear	Gram –ve big scattered rods.
11.	Non fermenting spreading colonies	Gummy spreading colonies appear	Gram –ve scattered rods.

12.	Non fermenting spreading colonies	Gummy spreading colonies appear	Gram –ve scattered rods.
13.	Non fermenting spreading colonies	Gummy spreading colonies appear	Gram –ve short scattered rods.
14.	Non fermenting spreading colonies	Non fermenting spreading colonies	Gram –ve short scattered rods.
15.	Non fermenting spreading colonies	Gummy spreading colonies appear	Gram –ve rods in chains.
16.	Non fermenting spreading colonies	Spreading pink and dark purple colonies	Gram –ve short scattered rods.
17.	Isolated mucoid, one pink colored spreaded colonies	Dark purple colonies and green metallic sheen appear.	Gram –ve short scattered rods.
18.	Spreading, mucoid and non fermenting Colonies appear	Dark purple mucoid and green metallic . sheen	Gram –ve scattered rods.
19.	Spreading, mucoid and non fermenting	Dark purple mucoid colonies appear	Gram –ve short scattered rods.
20.	Spreading, mucoid and non fermenting And also isolated colonies appear.	Pink and dark purple mucoid colonies	Gram –ve short scattered rods.
21.	Spreading and isolated mucoid colonies and 2-4 pink colored colonies appear	Dark purple mucoid colonies appear	Gram –ve short scattered rods.
22.	Spreading, mucoid, non fermenting and also isolated colonies appear	Pink spreaded, mucoid and dark purple colonies appear	Gram –ve short scattered rods.
23.	Spreading, mucoid, non fermenting colonies	Pink spreaded, mucoid and dark purple colonies appear	Gram –ve short scattered rods.
24.	Isolated mucoid and some are pink color mucoid colonies	Isolated mucoid and some are pink color mucoid colonies	Gram –ve short scattered rods
25.	Spreading, mucoid and non fermenting colonies appear	Dark purple mucoid or gummy colonies and some are black color colonies appear	Gram –ve short scattered rods.

Table II. Biochemical Properties.

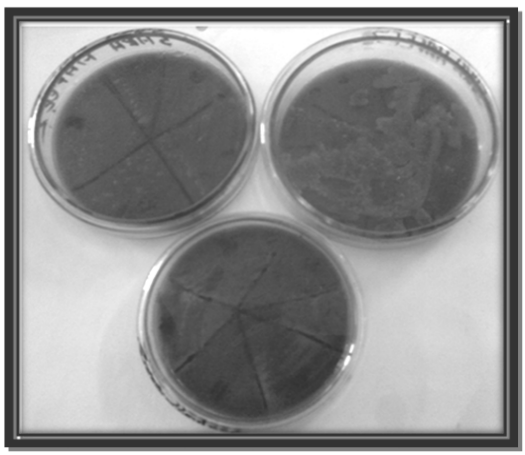

Figure 2: Gram-ve organism isolated on EMG agar

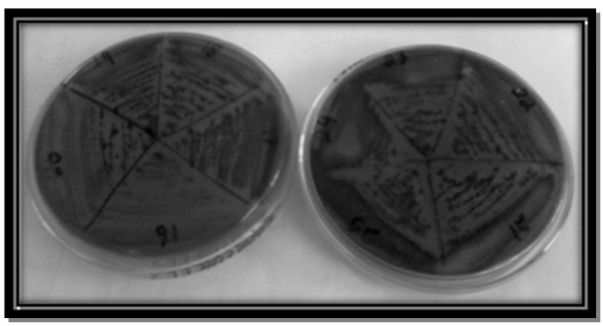

Figure 3: Gram-ve organism isolated on EMG agar

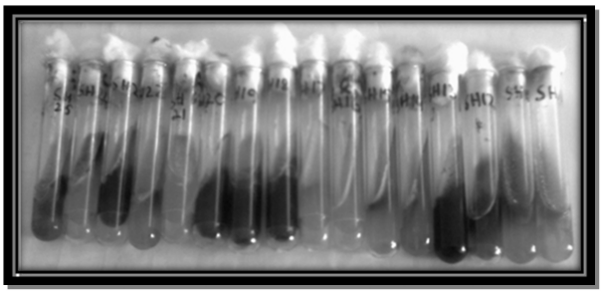

Figure 4: Results of TSI for identification of Gram-ve organism

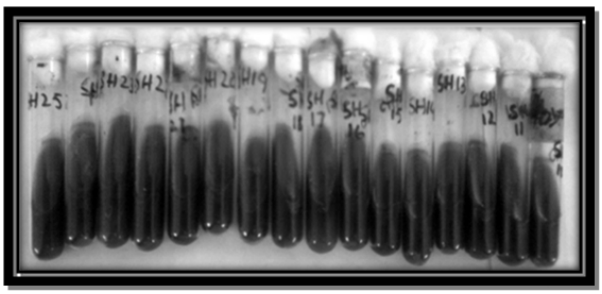

Figure 5: Results of Citrate test

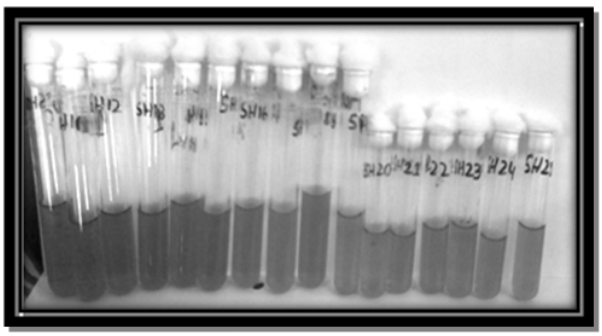

Figure 6: Results of Urease test for identification of Gram-ve organism

standard IMVIC formulas, as for E.coli (++--), Klebsiella (--++), Enterobacter (--++), Shigella (-+--), Salmonella (-+-+), Proteus (++--) and Pseudomonas aeruginosa (---+) (Table II.)

DISCUSSION

There are many different sources for the outbreak of diarrheal infections but the main source originates from fecal oral route of transmission so all those sources mainly involving contaminated water could be a major source of diarrheal infection. The purpose of our study is to identify different sources of diarrheal outbreak in a community and by the help of this study we concluded that the water used to shower on vegetables by the vegetable sellers was contaminated with fecal pathogenic microorganisms which are the leading cause of spread of diarrhea infection in a community and it become one of the many sources of diarrhea outbreak. The detection of water samples collected from vegetable carts was done by Membrane Filter Technique and it is the method most widely used for the enumeration of coliforms in drinking water. This technique, simple to perform and inexpensive, requires at least an overnight incubation period and a confirmation test (24 to 72 additional hours) after the initial typical colony investigation.

There are many other methodologies which are also designed for the rapid detection of coliforms from the contaminated water, which are less time consuming and give accurate results in a short period of time and are inexpensive such as Presence/Absence technique, Multiple- Tube Fermentation technique, MPN, SPC, etc. which we study from others work on contaminated drinking water.

Additionally, by the help of our study, we came to know that what are the sources of fecal contamination of water and how we reduce these sources of contamination of water, by simple following good hygienic conditions and keep checking and also maintaining the good quality of water.

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