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Appraisal of Microbiological Quality of Food with Respect to Drinking Water in Punjab

KEYWORDS

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ABSTRACT *Foodborne diseases comprise a large group of illnesses that are caused by the ingestion of contaminated food.Water is a critical raw material in many street food vending operations. Keeping this in view a total of 127 drinking water samples (Moga 19, Ludhiana 15, Jalandhar 20, Patiala 15, Tarn-Taran 20, Amritsar 17 and Sangrur 20) were analyzed for emerging pathogens and indicator organism using Bacteriological water testing kit (Sahota et al 2010). It was found that 73.7% of drinking water samples from Moga were bacteriologically contaminated. Similarly 60% from Sangrur,65% Jalandhar ,66% Patiala and 94% from Amritsar were bacteriologically contaminated. Out of 127 samples in total E.coli was present in 29.92% samples, Aeromonas hydrophila in 24.41%, Listeria sp.27.55 %, and Yersinia enterocolitica in 57.0% . Contaminated water can create a public health risk when it is used for drinking, washing of foods, incorporated in the food as an ingredient and used in the processing of food or used for washing equipment, utensils and hands. A logical step towards reducing the risks of food borne illness from street foods would be controlling drinking water contamination and awareness among street vendors and public.*

Introduction:
Foodborne diseases comprise a large group of illnesses that are caused by the ingestion of contaminated food (WHO, 2013). It represents a major concern in developing countries. The data regarding foodborne illnesses remain scarce (WHO, 2007). However, according to the World Health Organization (WHO), diarrheal diseases were the third leading cause of death in low income countries resulting in 1.8 million deaths around the world in 2005 alone (WHO, 2013). In general most of the cases result from the consumption of contaminated food and water (WHO, 2013).

Water is a critical raw material in many street food vending operations. It may also be contaminated with biological, chemical or physical hazards. As such contaminated water will create a public health risk if it is: when used used for drinking purposes, washing of food, incorporated into food as an ingredient and in the processing of food; or washing of equipment, utensils and containers. Contaminated ice may introduce hazards to food and beverages with which it is in contact.

The ambulant vendor can carry only limited supplies and even stationary food stalls may not have direct access to a water supply.Therefore, water supply needs close attention in street food operations. This is particularly true if contaminated water may be added to a food or applied to utensils without a subsequent step (eg heating or chemical sanitizing) to eliminate or reduce the potential hazards to an acceptable level. Keeping this in view a total of 127 drinking water samples (Moga 19, Ludhiana 15, Jalandhar 20, Patiala 15, Tarn-Taran 20, Amritsar 17 and Sangrur 20)were analyzed for emerging pathogens and indicator organism using Bacteriological water testing kit (Sahota et al 2010).

Material and Method
Research site description
Sample collection, transport and storage:
The drinking water samples for analysis were collected aseptically in sterilized glass bottles (1 L). The samples were treated with sodium thiosulfate to inactivate any residual halogen compound present in the sample ($Na_2S_2O_3$ concentration of 18mg/litre neutralizes upto 5mg of free (residual) chlorine per litre). The samples containing high concentration of zinc and copper were treated with EDTA at concentration of 372mg/litre to reduce metal toxicity (APHA 1989). The samples were analyzed within 24hrs of collection by transporting in refrigerated container at 4°C.

Microbiological analysis of water samples:
A total of 127 drinking water samples (Moga 19, Ludhiana 15, Jalandhar 20, Patiala 15, Tarn-Taran 20, Amritsar 17 and Sangrur 20) were analyzed by Bacteriological water testing kit (Sahota et al 2010) for bacteriological quality of drinking water.

MPN Analysis:
The MPN tests (APHA 1989), a statistical method is based on probability dispersion analysis.

Bacteriological analysis
Isolation, identification and biochemical characterisation of *E.coli*
From BWTK *E.coli* was isolated after primary enrichment, followed by streaking onto Eosine methyl blue agar. Typical *E.coli* colonies with green metalic sheen were isolated, restreaked to ensure purity and biochemically confirmed by Indole, methyl red,Voges Proskauer and Citrate (IMViC) test using KB002 HiAssorted TM biochemical test kit (Himedia Laboratories Pvt. Ltd., Mumbai).

Isolation of *Aeromonas hydrophila*
From positive BWTK, an inoculum was streaked onto Aeromonas Selective Supplement Medium (Ampicillin 2.50mg/500ml Medium), incubated at 37 °C for 24 h. Presumptive *Aeromonas* colonies, with dark green, opaque with darker centre, were further streaked on Brain Heart Agar (BHA; Himedia Laboratories Pvt. Ltd., Mumbai) or Aeromonas Selective Supplement Medium (Himedia Laboratories Pvt. Ltd., Mumbai) and biochemically confirmed by biochemical test kit (Himedia Laboratories Pvt. Ltd., Mumbai).

Isolation of *Yersinia enterocolitica*
The pre-enriched water samples from BWTK were streaked on Yersinia Selective Supplement Medium (Cefsulodin7.50mg/500ml Medium, Triclosan 2.0mg/500ml Medium, Novobiocin 1.25mg/500ml Medium) (Himedia Laboratories Pvt. Ltd., Mumbai) and incubated at 37 °C for 24-48 h. Colonies with characteristic bull's eye morphology with deep red centres and white to translucent periphery, were isolated, restreaked to ensure purity and biochemically confirmed by using biochemical test kit

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(Himedia Laboratories Pvt. Ltd., Mumbai).

Isolation of *Listeria* spp.
Aliquots of samples from BWTK were streaked on Gum-Listeria medium. HiListeria Latex test kit (HiMedia, Mumbai, India) was used for rapid identification of *Listeria* spp. from solid agar. Place the sensitised latex particles in solution/suspension containing antigen against which the anti-serum was directed, and observe for agglutination of the particles and compare with positive and negative control.

Results and Discussion
Global health experts are recognizing that food security challenges cannot be met until safe drinking water, sanitation, and hygiene (WASH) are available in the world's poorest communities . Without access to safe drinking water, proper sanitation, and proper hygiene, food is easily contaminated through exposure to unsafe drinking water and pathogens on hands, flies, and unclean surfaces. This can cause diarrhea, environmental enteropathy, other intestinal diseases and eventually undernutrition. It is a vicious cycle: intestinal diseases contribute to undernutrition through decreased nutrient absorption, (Braghett *et al* 2006) while undernutrition reduces the body's ability to fight off further infections (Guerrant *et al* 2006)

Surface and groundwater resources provide drinking water supply in the state of Punjab. The south-western district of the state are served by surface water whereas central and northern districts of the state receive drinking water supplies from tubewells which tap shallow and deeper aquifer system. Quality of surface water is getting deteriorated due to waste disposal, municipal waste waters and surface runoff containing agro-chemicals from agricultural fields.

Keeping this in view a total of 127 drinking water samples (Moga 19, Ludhiana 15, Jalandhar 20, Patiala 15, Tarn-Taran 20, Amritsar 17 and Sangrur 20) were analyzed by Bacteriological water testing kit (Sahota et al 2010) for bacteriological quality of drinking water. It was found that 73.7% of drinking water samples from Moga were bacteriologically contaminated. Similarly 60% from Sangrur,65% Jalandhar ,66% Patiala and 94% from Amritsar were bacteriologically contaminated. Occurrence of pathogens in water may be due to deficiency in: plumbing lines, inadequately laid plumbing lines, cross connections, treatment deficiency, biofilm growth problems, leakage point or gap in the piping system with high external pressure, low pressure conditions in the distribution system allow a flow reversal or backflow of non-potable water, hydraulic disturbances that allow biofilm material on pipe surfaces or sediments to enter the bulk water and integrated problems.

Out of 127 samples in total *E.coli* was present in 29.92% samples, *Aeromonas hydrophila* in 24.41%, *Listeria sp.*27.55 %, and *Yersinia enterocolitica* in 57.0% . The pathogens may also enter the distribution system either through the source water, or at any point within the distribution system. Treated water may also have ample recontamination possibilities based on the construction characteristics, operation, and maintenance of the water distribution system. Additional reason of contamination include contamination through uncovered storage facilities, penetrations in covered storage facilities, water main installation and repair sites, or during transitory contamination events.

The order of occurrence of *E.coli*, *Aeromonas hydrophila*, *Yersinia enterocoitica* and *Listeria* spp. in Moga was ; *Yersinia enterocoitica* 78.94% followed by *Aeromonas hydrophila* 26.3% , *Listeria* spp. 21% ,and *E.coli* 21% respectively.

In Ludhiana and Jalandhar water supply ; *Listeria* spp. 46.66%, > *E.coli 46.6%* > *Aeromonas hydrophila* 40% > *Yersinia enterocoitica* 20% and *Listeria sp.*65% , *Yersina enterocolitica* 55%, *Aeromonas hydrophila 30%*and *E.coli 15%.*

In Patiala, Tarn-Taran, Amritsar and Sangrur order of occurrence was Yersinia enterocolitica 86%, Listeria sp. 40%, *E.coli* 33.33% , Aeromonas hydrophila 6% and Yersinia enterocolitica 100%, Aeromonas hydrophila 30%,*E.coli* 15% and Yersinia enterocolitica. 47.05%, *E.coli* 47.05% , Aeromonas hydrophila 11.76% and *E.coli* 40%, Aeromonas hydrophila 25%, Listeria sp.25%, and Yersinia enterocolitica 15% respectively.

Over all study reveal that Y.enterocolitica occur in highest percentage in drinking water samples. Hydrophobicity of Y.enterocolitica plays a role in its adhesion to surfaces. Bacteria have lipid side chains, as well as portions of surface proteins that are hydrophobic. Usually, protein fold in such a way that hydrophilic amino acid regions are exposed, they play a role in hydrophobic interactions (Newby 2000). Pandove et al (2013) also analyzeda total of 418 drinking water samples from various water utilities were analyzed for occurrence of Y. enterocoliitca and faecal coliforms (Escherichia coli). Y. enterocolitica was detected in 78.09% of Municipal Corporation (MC) drinking water samples, 59.02% Submersible pump drinking water samples and 53.12% of Hand pump samples where as E. coli was found in 53.71% of Municipal Corporation (MC) drinking water samples, 29.16% Submersible

Water is a critical raw material in many street-vended operations. Contaminated water can create a public health risk when it is used for drinking, washing of foods, incorporated in the food as an ingredient and used in the processing of food or used for washing equipment, utensils and hands. It is a well known vehicle for enteropathogens such as *E. coli*, *Salmonella* spp. and *Campylobacter* spp. amongst others (Angulo et al 1997) Studies carried out in different regions of Asia, Africa and South America have frequently pointed the unavailability of potable water for various activities at the vending site as a major concern. Due to the shortage of clean potable water, many vendors tend to re-use the water, especially for cleaning utensils and used dishes.

Studies done to find out the bacteriological quality of the water used by some street vendors have revealed frequent contamination with coliforms and fecal coliforms. When the street foods in Trinidad and Tobago were analyzed, it was reported found that 35% of foods were contaminated by *E. coli* while 57.5% of water used by vendors were contaminated by coliforms (mankee et al 2003) . When water samples from storage tanks used by some vendors were checked at different localities in Pune, India, it was revealed that 29.6% of the water samples were not conforming to the WHO standards of potability and had coliform counts of more than 16/100 ml, while fecal coliform counts were more than 16/100 ml in 15.5% of water samples, 4.5% of samples were positive for *E. coli* and 2.7% for enteropathogenic *E. coli* (Bhat et al 2000).

Conclusion: A logical step towards reducing the risks of food borne illness from street foods would be controlling drinking water contamination and awareness among street vendors and public.

Acknowledgement
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Figure 1.MPN index/100ml in different areas of Moga

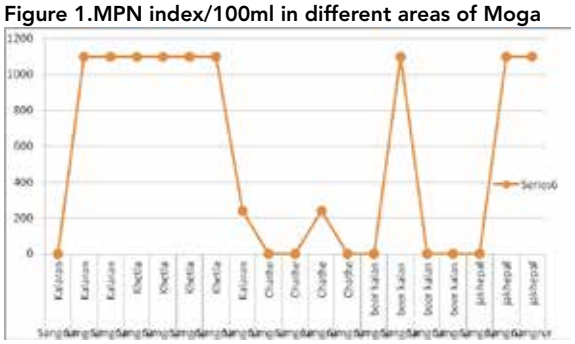

Figure 2.MPN index/100ml in different areas of Sangrur

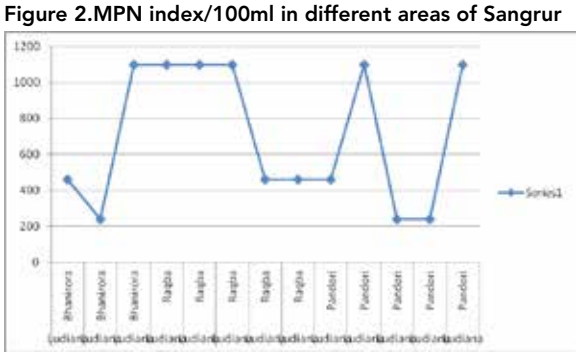

Figure 3.MPN index/100ml in different areas of Ludhiana

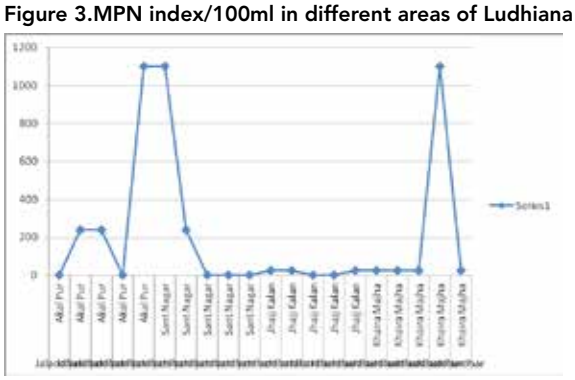

Figure 4.MPN index/100ml in different areas of Jalandhar

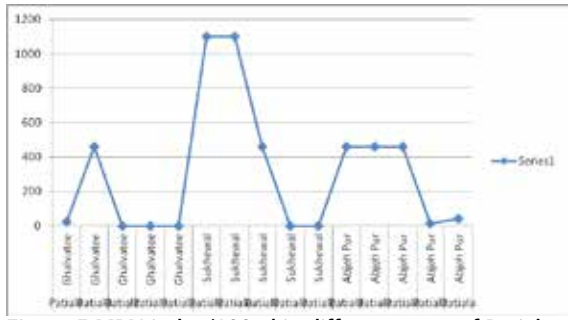

Figure 5.MPN index/100ml in different areas of Patiala

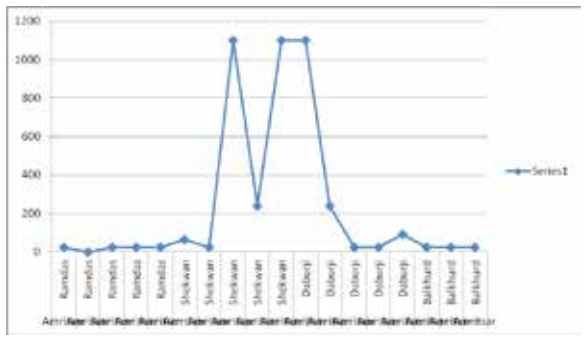

Figure6.MPN index/100ml in different areas of Amritsar

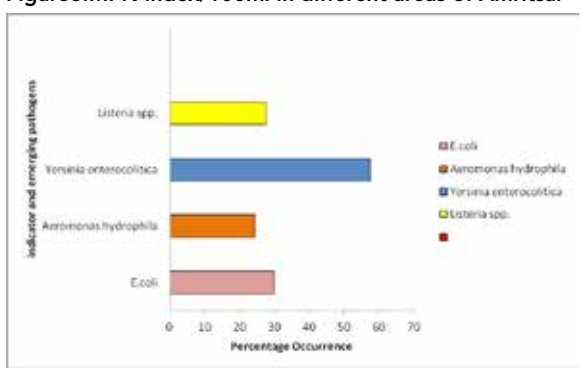

Figure 7: Overview of conventional and emerging pathogens in Punjab

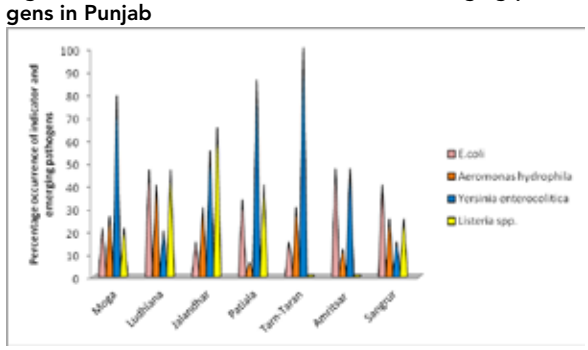

Figure 8: Occurrence of conventional and emerging pathogens in different regions of Punjabt

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