Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env.Pharmacol. Life Sci., Vol 4 [5] April 2015: 124-130

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD
Global Impact Factor 0.533
Universal Impact Factor 0.9804

ORIGINAL ARTICLE

Isolation and Identification of *Salmonella Species* from Dahi Bhalay, Fruit Chaat and Fruit Juices Collected From Different Localities of Lahore

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ABSTRACT
The main objective of the present microbial study was to isolate and identify the pathogenic microorganisms from food samples Dahi bhalay (yogurt, salad, cabbage) Fruit chaat and Fruit juices to create public awareness about the health hazards resulting from these pathogenic microorganisms. To conduct this study, total 150 samples were collected from different shops of Lahore, Pakistan. All the samples were first cultured on nutrient broth and nutrient agar, which ensured the presence of certain pathogenic microorganisms. For further confirmation and identification, the culture from the nutrient agar was streaked on different selective media and the presence of pathogenic bacteria like Salmonella sp was confirmed. It may be S. typhi, S. typhimurium, and S. enteritidis which could be further confirmed separately by different chemical tests.
Keywords: *Food Items (Dahi Bhalay, Fruit Chaat, Fruit Juices)*

Received 09.01.2015 Revised 21.03.2015 Accepted 02.04.2015

INTRODUCTION

Food quality and safety is an increasingly important public health issue. Nowadays, the topics "food quality" and "food safety" are very close and two important issues in the food sector, due to the globalization of the food supply and the increased complexity of the food chain. The consumers need to purchase safe products that do not involve any kind of risk for health. On one hand, the aim of the "food safety" is to avoid health hazards for the consumer: microbiological hazards, pesticide residues, misuse of food additives and contaminants, such as chemicals, biological toxins and adulteration. On the other hand, "food quality" includes all attributes that influence the value of a product for the consumer; this includes negative attributes such as spoilage, contamination with filth, discoloration, off-odors and positive attributes such as the origin, color, flavor, texture and processing method of the food [1].

The contamination of food products with microorganisms presents a problem of global concern, since the growth and metabolism of microorganisms can cause serious foodborne intoxications and a rapid spoilage of the food products. Thus, the acceptance and safety of a food product for the consumers depends in great part on the presence and nature of microorganisms. Besides molds and yeasts, bacteria are the principle responsible for various types of food spoilage and foodborne intoxications. It has to be mentioned that a food product naturally contains an indigenous microbiota that can include spoilage and/or pathogenic bacteria species. Depending on the preservation method these species can proliferate and adulterate the product. However, most bacterial contamination occurs during processing and manipulation of the food products. [2].

The presence of *Salmonella* in foodstuffs (Dahi bhalay, fruit chaat and fruit juices) represents an internationally accepted human health concern. Although *Salmonella* causes many foodborne disease outbreaks, there is little evidence to support cross-contamination as a major contributing factor. However, the paramount importance of preventing cross-contamination and recontamination in assuring the safety of foodstuffs is well known. Sources and factors linked to cross-contamination and recontamination of *Salmonella* in foods are reviewed in detail. Those foods which are not submitted to lethal treatment at the end of processing or which do not receive further treatment in the home deserves special attention. *Salmonella* cross-contamination and recontamination episodes have been connected to the following factors: poor sanitation practices, poor equipment design, and deficient control of ingredients.

Cross-contamination and recontamination events at factory level evidence the difficulty encountered for eradicating this pathogen from the environment and facilities, highlighting the need to reinforce industry preventive control measures such as appropriate and standardized sanitation. Also, at consumer level, Public Health Authorities should install hygiene education programs in order to raise consumer awareness of the risks of cross-contamination in the home and their role in its prevention. Finally, a review on cross contamination models of *Salmonella sp*. is presented [3].

Fruit juices are an important part of the modern diet in many countries. However, few data are available concerning the microbiological quality of the fruit juices sold in Greece. Using standard microbiological procedures, we conducted a bacteriological survey of commercially sold, pasteurized, shelf-stable fruit juices from retail markets. A total of 120 samples of fruit juices sold in various retail markets were examined for their bacteriological quality. The pH of the tested juices was 2.4–4.8. Bacteria were isolated from 51 samples (42.5%) and fungi from 78 samples (65%). *Escherichia coli* O157:H7 was detected in four of the analyzed samples (3.34%), and *Staphylococcus aureus* was detected in four different samples (3.34%). In 11 samples (9.1%), the total number of microorganisms detected was as high as 125 colony-forming units (CFU). Acidophilic microorganisms were isolated from 26 samples (21.7%) and *Blastomyces* was detected in 46 samples (38.3%). All samples were negative for *Lactobacillus*, *Clostridium perfrigens*, *Salmonella* spp., *Bacillus cereus*, total coliforms, *E. coli*, and *Listeria monocytogenes* [4].

Contamination of fruit can occur anywhere in the growing, harvesting, cleaning and transportation chain from orchard to processor. Water used in orchards for diluting pesticides, irrigation, and washing apples represents a possible source of contamination. Fruits are raw agricultural commodities and can also be exposed to contamination from animals, birds, insects, and from domestic and agricultural waste. The harvesting and use of drop fruit can increase the risk of contamination. The contact surfaces of equipment used in harvesting, storage, and packing of the fruit may also be contaminated with rodent or animal manure. Other possible sources of contamination include workers harvesting and handling the fruit, and the conditions under which it is stored and shipped.

In addition, juice processing establishments may be sources of contamination. Pathogens introduced into a facility via contaminated fruit could persist if proper sanitation standards are not followed. Information on typical levels of *E. coli*, *Salmonella sp*., *Cryptosporidium sp*. and other pathogens on fruit destined for juice are lacking. Producers of unpasteurized juice should consider that any fruit entering a facility might carry pathogens. Other potential sources of juice contamination are water, insects, contaminated equipment, and poor worker hygiene practices.

The ability of *E. coli* O157:H7 or *Salmonella sp*. to survive on fruit surfaces during juicing and storage raises concerns about the way fruit and juice are handled. Contamination of the interior can occur through surface bruises, cuts or orifices. Because handling is unavoidable, the extent of microbial attachment will depend on the sanitation conditions in the manufacturing environment that reduce microbial build-up. However, the likelihood of actual growth upon intact fruit surfaces (peels) is minimal, provided procedures to process fruits after harvesting are immediate or storage conditions are adequate. For example, the decision to use drop-fruits (apples or oranges, for example) carry the risk of contamination by *Salmonella sp., E. coli* and other pathogens, directly from raw or improperly composted manure, contaminated irrigation water, soil or contact with animals and insects. Therefore, drop-fruits tend to be processed immediately, and before any surface bacterial growth can occur [5].

Salmonella sp. has been recognized as human and animal pathogens for over a century. Numerous serotypes have been described, but seven of these were responsible for 61.6% of human cases in the U.S. in 2007. According to recent data from Food Net, the incidence of salmonellosis has not diminished significantly over the past ten years but some serotypes have increased or decreased in importance [6]. Most serotypes are not host- specific, but a few species are restricted to one kind of animal such as *S. Pullorum* in chickens and *S. Typhi* (causative agent of typhoid fever) in humans. With the exception of typhoid and paratyphoid fevers, cases of salmonellosis generally involve mild to moderate symptoms of gastroenteritis lasting for about five days. However, the very young, the old, and the immunocompromised may contract more severe infections [7]. *Salmonellae* may also cause urinary tract infections and sometimes migrate out of the intestine and cause septicemia and reactive arthritis [8, 9, 10]. Several large outbreaks of salmonellosis have occurred during the past year. *S. Typhimurium* present in municipal tap water affected over 400 people in Colorado [11] and *S. Typhimurium*, believed to be carried in pork, has made more than 1000 people ill in Denmark [12]. Perhaps the most noteworthy outbreak in 2008 was the nationwide illness caused by *S. Saintpaul* that extended for several months while investigators sought to pinpoint the fresh produce vehicles of infection [6].

Salmonella sp. is estimated to cause about 1.4 million non-typhoidal infections in humans per year in the U.S., and it has been estimated that 95% of these cases were due to consumption of contaminated food

[13](Mead, 1999). Additionally, 300–400 cases of typhoid fever are reported yearly in the U.S. Although salmonellosis is typically not fatal, the large number of cases that occur annually does have a significant economic impact on individuals, the health care system, and businesses associated with outbreaks. *Salmonellae* naturally live in the intestines of humans and other animals; therefore, faecal material is the ultimate source of these bacteria. Of the ten *Salmonella* serotypes detected most frequently in human infections, six are also among the most common isolates from swine and poultry. *Salmonella* serotypes in farm animals have changed over time as husbandry practices have become more intensive and as the international trade in feed has increased [14].

In addition to human health implications, *Salmonella* is a pathogen of significant importance in worldwide animal production and the emergence of antibiotic-resistant strains, due principally to the therapeutic use of antimicrobials in animals, is a further threat to human and animal health. Increasing attention has been focused on the prevention and control of *Salmonella* in animal production, as this is the main source of outbreaks in humans [15, 16]. The need for global co-operation in controlling salmonellosis was emphasised at an early stage by the World Health Organization (WHO). This is readily understandable since *Salmonella* infections are also spread through international trade in animal feed, live animals and food. The control of *Salmonella* is thus an urgent challenge confronted by Veterinary Services and producers as they seek to produce safe foods of animal origin [17].

Salmonellae cause disease in both humans and animals. The serovar *S. Typhi* and most *S. Paratyphi* strains (A, B and C), which cause serious systemic infections in humans, are specific human pathogens. These pathogens have no animal reservoir and so are not dealt with in this paper. Instead, the authors focus on the remaining serovars, usually known as the 'zoonotic *Salmonella spp.*', which cause so-called non-typhoidal salmonellosis in humans and sometimes also in animals. Understanding the mechanisms behind the survival of *Salmonella* bacteria, as they invade an exposed animal, and their ability to cause disease would enable researchers to prevent much of the suffering and economic losses caused by this pathogen. However, despite substantial research efforts, progress has been limited.

MATERIAL AND METHOD

The research was focused on the microbial studies of pathogenic bacteria (*Salmonella sp*) in food samples (dahi bhalay, fruit chaat, fruit juices) collected from different localities of Lahore, Pakistan. Total 150 samples were collected from different shops of Lahore to test the presence of pathogenic bacteria. First group of 75 samples were collected from December 2013 to February 2014. And second group of 75 samples were collected from April 2014 to June 2014.

Isolation of bacterial contaminants from these three food items was performed through standard techniques. All the samples were tested at Zoology Research Lab at Lahore College for Women University, Lahore (LCWU). The lab was fully equipped with necessary apparatus for required analysis.3.25g of nutrient broth medium was added into the 250ml of distilled water and mixed well with stirrer. After complete mixing poured into the conical flask. Mouth of the conical flask was covered with a cotton plug and aluminium foil and autoclaved it at 15psi pressure and temperature of 121 ºC for at least 30 minutes. 6.8 g of nutrient agar were added in the 250ml of distilled water and mix these ingredients by using the stirrer. When the components of the media are completely dissolved, poured the media in the conical flask. Covered the mouth of the conical flask with cotton plug and aluminium foil and autoclaved it at 15psi pressure and temperature of 121 ºC for at least 30 minutes.

For morphological identification of bacterial colonies, nutrient agar plates were prepared. Small amount of culture was taken with the help of inoculating loop from the nutrient broth medium and were streaked on the agar plates. Different types of bacterial colonies appeared on the Nutrient agar plates. The samples collected were cultured with Nutrient Broth, Nutrient Agar and for further identification they were cultured on the selective media. Pure colonies of isolates were identified and characterized using standard microbiological technique. All 150 samples were found contaminated with pathogenic bacteria (*E.coli, Salmonella, Shigella,* and *Staphylococcus*). But only *Salmonella sp.* was identified by using selective media.

RESULTS AND DISCUSSION

The present study was conducted to isolate and identify pathogenic microorganisms in food samples. For this purpose, total 150 food samples were collected from different localities of Lahore, Pakistan. The samples collected were cultured with Nutrient Broth, Nutrient Agar and for further identification they were cultured on the selective media. All 150 samples were found contaminated with pathogenic bacteria (*E.coli, Salmonella, Shigella,* and *Staphylococcus*).

The main objective of the present microbial study was to isolate and identify the pathogenic microorganisms from food samples (Dahi bhalay, Fruit chaat, and Fruit juices) to create public awareness about the health hazards resulting from these pathogenic microorganisms. To conduct this study, total 150

samples were collected from different shops of Lahore, Pakistan. All the samples were first cultured on nutrient broth and nutrient agar, which ensured the presence of certain pathogenic microorganisms. For further confirmation and identification, the culture from the nutrient agar was streaked on different selective media and the presence of pathogenic bacteria like *Salmonella sp* was confirmed. It may be *S.typhi, S.typhimurium, and S.enteritidis* which could be further confirmedseparately by different chemical tests.

GROWTH IN NUTRIENT BROTH MEDIUM

To check the presence of pathogenic bacteria, nutrient broth medium was prepared and transferred into the test tubes. All the 150 samples were dipped separately into the nutrient broth and were left in the shaker for overnight. After 18-24 hours of inoculation, the nutrient broth medium become turbid, turbidity ensured the presence of certain bacteria in the samples.

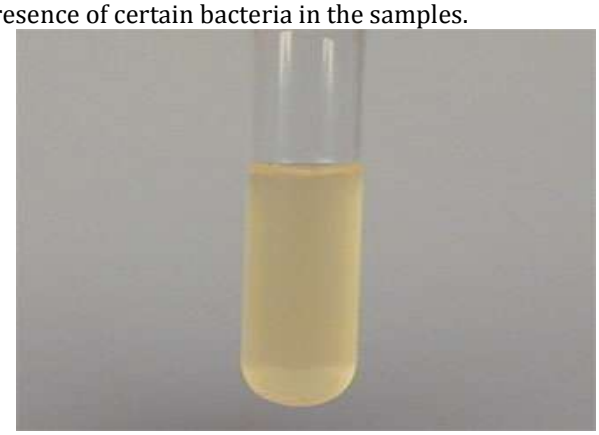


FIG 1: Growth of pathogenic bacteria in Nutrient Broth

GROWTH ON NUTRIENT AGAR

For morphological identification of bacterial colonies, nutrient agar plates were prepared. Small amount of culture was taken with the help of inoculating loop and were streaked on the agar plates. Different types of bacterial colonies appeared on the plates and are as follows:

- The opaque, smooth, and glistening colonies with grape like clusters appeared on the nutrient agar plate which detected the presence of *Staphylococcus spp.*
- Small, discrete, circular, smooth, translucent and colorless colonies appeared on the nutrient agar plate which detected the presence of *Salmonella spp*.
- Small, smooth, round and colorless colonies appeared on the nutrient agar plate which detected the presence of *E.coli.*

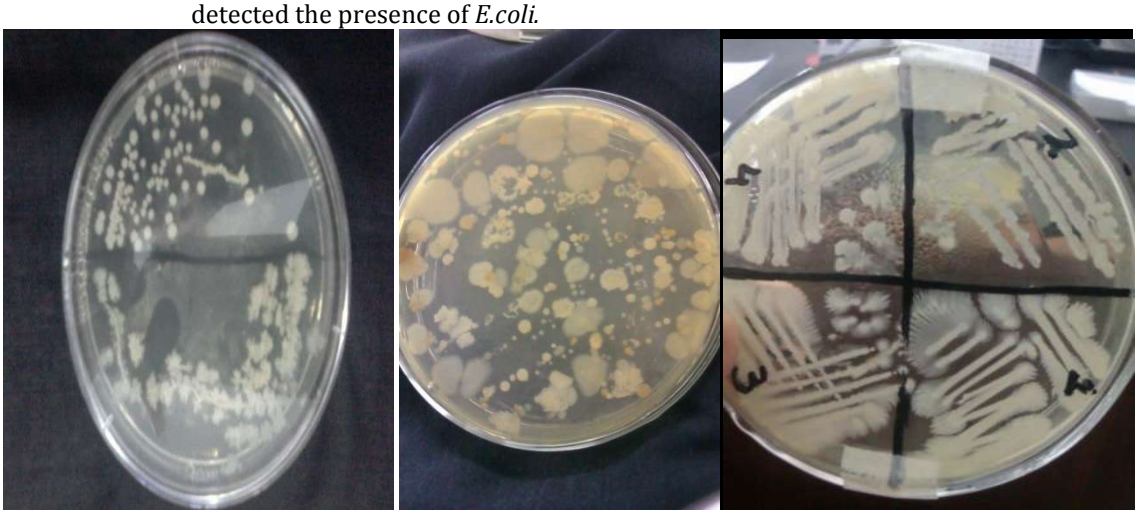


FIG 2: Growth of bacteria on Nutrient agar.

Bacterial colonies (*Salmonella*) detected on the nutrient agar plates were further confirmed on the selective media and are as follows:

GROWTH ON HEKTOEN ENTERIC AGAR (HEA)

Salmonella appeared as green colonies with black centers.*S. typhi* do not produce transparent colonies on HE agar. *S. typhi* is a weak H_2S producer, and rare strain of lactose fermenting *Salmonella*. Results of Hektoen enteric agar are show in figure **(3).** Cultural response on Hektoen Enteric Agar at 35±0.2°C after 18-24 hours incubation is as follows:

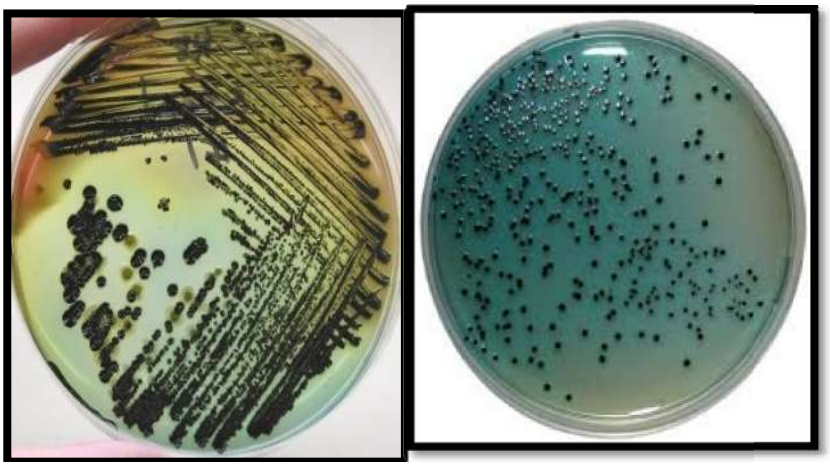


FIG 3: Hektoen Enteric Agar plates showing growth of pathogenic *Salmonella* (Green and blue green colonies with black center)

GROWTH ON SALMONELLA SHIGELLA AGAR (SSA)

SS Agar is a moderately selective medium in which gram positive bacteria are inhibited by bile salts, brilliant green, and sodium citrate. On fermentation of lactose by few lactose-fermenting normal intestinal flora, acid is produced which is indicated by change of colour from yellow to red by the pH indicator-neutral red. Thus these organisms grow as red pigmented colonies. Lactose non-fermenting organisms grow as translucent colourless colonies with or without black centres. *Salmonella* species appears as colourless colonies with black centres resulting from H 2S production.

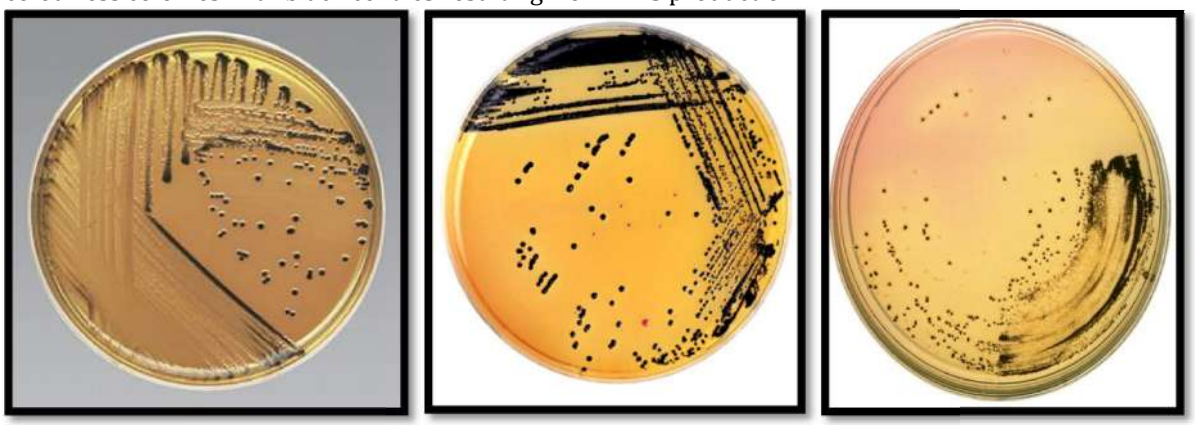
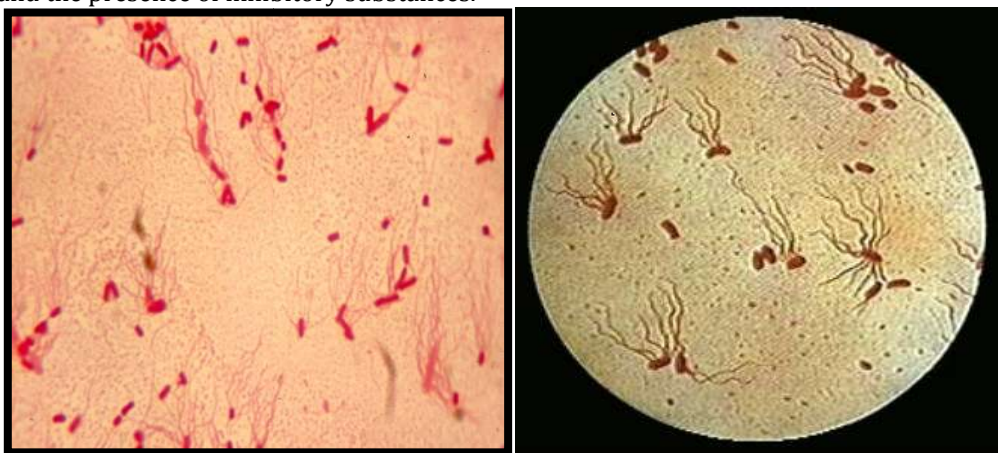


FIG 4: *SalmonellaShigella* Agar plates showing growth of pathogenic *Salmonella* (Colourless colonies with black centers)

GRAM STAINING RESULTS

Analysis of gram-stained smears involves consideration of staining characteristics and cell size, shape and arrangement. These properties can be influence by culture age, media, incubation, atmosphere, staining methods and the presence of inhibitory substances.



Salmonella sp. Gram-ve

Confirmation of pathogenic bacteria:

Results indicate that a wide spectrum of food is contaminated by large number of *Salmonella sp.* It may be *S.typhi, S.typhimurium,*and *S.enteritidis*. Presence of pathogenic bacteria in all 150 samples was analyzed microbiologically in foodstuffs.

Salmonellatyphi, has been a major human pathogen for thousands of years, thriving in conditions of poor sanitation, crowding, and social chaos. It may have responsible for the Great Plague of Athens at the end of the Peloponnesian War [18].The name *S typhi* is derived from the ancient Greek typhos, an ethereal smoke or cloud that was believed to cause disease and madness. In the advanced stages of typhoid fever, the patient's level of consciousness is truly clouded. Although antibiotics have markedly reduced the frequency of typhoid fever in the developed world, it remains endemic in developing countries [19] (Chao *et al*., 1987).

S. typhi has no nonhuman vectors. The following are modes of transmission: (1) Oral transmission via food or beverages handled by an individual who chronically sheds the bacteria through stool or, less commonly, urine. (2) Hand-to-mouth transmission after using a contaminated toilet and neglecting hand hygiene. (3) Oral transmission via sewage-contaminated water or shellfish (especially in the developing world). (4) An inoculum as small as 100,000 organism's cause's infection in more than 50% of healthy volunteers [20].

In contrast to the non-typhoidal *salmonellae*, *S. typhi* enters the host's system primarily through the distal ileum. *S. typhi* has specialized fimbriae that adhere to the epithelium over clusters of lymphoid tissue in the ileum (Peyer patches), the main relay point for macrophages traveling from the gut into the lymphatic system*. S typhi* has a VI capsular antigen that masks PAMPs, avoiding neutrophil-based inflammation. The bacteria then induce their host macrophages to attract more macrophages [21].

The gallbladder is then infected via either bacteremia or direct extension of *S. typhi* –infected bile. The result is that the organism re-enters the gastrointestinal tract in the bile and reinfects Peyer patches. Bacteria that do not reinfect the host are typically shed in the stool and are then available to infect other hosts [22].

CONCLUSION

The objective of this study was to determine the contamination of food samples (Dahi bhalay, Fruit chaat and Fruit juices). A wide spectrum of food was contaminated by large number of *Salmonella sp*. But it is not confirmed that either it is *S.typhi*, *S.typhimurium* or *S.enteritidis*. The *salmonella* contamination observed in present study may indicate that food that we use daily cause severe diseases such as salmonellosis. The present research was conducted during the period of December 2013 to June 2014. All the 150 samples of food were collected from different localities of Lahore; Pakistan. Very effective procedures were used for the isolation and identification of bacterial colonies. Different selective and differential media were used for the isolation and identification of bacterial colonies. Present research indicates that all the samples of food were contaminated with pathogenic bacteria.

REFERENCES

1. FAO (2003). "Assuring food safety and quality: guidelines for strengthening national food control systems." *FAO food and nutrition paper*, 0254-4725
2. Blackburn, C. d. W. (2006). "*Food spoilage microorganisms*." Woodhead Publishing.
3. Elena Carrasco, Andrés Morales-Rueda, Rosa María García-Gimeno, *food research international*, (2012) 45: 545-556
4. Vantarakis, A., Affifi, M., Kokkinos, P., Tsibouxi, M., Papapetropoulou, M. (2011) Anaerobe,17: 288-291.
5. Senkel Jr A., Henderson, R. A., Jolbitado, B., and Meng, J. (1999) Use of hazard analysis critical control point and alternative treatments in the production of apple cider,J. Food Prot., 62: 778-785.
6. Centers for Disease Control. 2008. Summary of notifiable diseases United States, 2006. Morbid Mortal Weekly Rep 55:77–81.
7. Gordon M. A. 2008. *Salmonella* infections in immunocom- promised adults. J Infect 56:413–422.
8. Rohekar, S, Tsui, F. W., Tsui, H. W., Xi N, Riarh R, Bilotta R, Inman, R. D. 2008. Symptomatic acute reactive arthritis after an outbreak of *Salmonella*. J Rheumatol 35(8):1599–1602.
9. Ternhag A, Torner A, Svensson A, Ekdahl K, Giesecke J. (2008). Short and long term effects of bacterial gastrointestinal infections. Emerg Infect Dis 14:143–148.
10. Wilson I. G., Whitehead E. 2006. Emergence of *Salmonella* Blockley, possible association with long-term reactive arthritis, and antimicrobial resistance. FEMS Immunol Med Microbiol 46:3–7.
11. Berg R. 2008. The Alamosa *Salmonella* outbreak; a gumshoe investigation. J. Environ Health 71:54–55.
12. Ethelberg S, Wingstrand A, Jensen T, Sørensen G, Müller L, Lisby M, Nielsen EM, Mølbak K. 2008. Large outbreaks of *Salmonella typhimurium* infection in Denmark in 2008. 13(44):19023.
13. Mead P.S., Slutsker L., Dietz V., McCaig L.F., Bresee J.S., Shapiro C., Griffin P.M. & Tauxe R.V. (1999). Food-related illness and death in the United States. Emerg. infect. 5 (5), 607-625.

14. Foley S. L., Lynne A. M. (2008). Food animal-associated *Salmonella* challenges: pathogenicity and antimicrobial resistance. J Anim Sci 86:E173–E187.
15. Wierup M. (1994). Control and prevention of salmonellosis in livestock farms. In Comprehensive report on technical items presented to the International Committee or to Regional Commissions. World Organisation for Animal Health (OIE), Paris, 249-269.
16. World Health Organization (WHO) (1993). Report of the WHO Consultation on control of *Salmonella* infections in animals: prevention of foodborne *Salmonella* infections in humans, Jena, Germany, 21-26 November. WHO/CDS/VPH/93.129. WHO, Geneva.
17. Bögel K. (1991). Global cooperation in the control of salmonellosis. In Proc. Symposium on the Diagnosis and Control of *Salmonella* (G.H. Snoeyenbos, ed.), San Diego, California, 29 October. United States Animal Health Association, Richmond, Virginia, 1-5.
18. Papagrigorakis, M. J., Synodinos, P. N., Yapijakis, C. 2007. Ancient typhoid epidemic reveals possible ancestral strain of *Salmonella enterica* serovar Typhi. *Infection, Genetics and Evolution*, 7(1):126-7.
19. Chao, W., Ding, R., Chen, R. (1987). 'Survival of pathogenic bacteria in environmental microcosms'.Chinese J. Microbial Immun. Vol 20: pp 339-348.
20. Levine, M. M., Tacket, C. O., Sztein, M. B. 2001. Host-*Salmonella* interaction: human trials. *Microbes and Infection*, 3(14-15):1271-9.
21. Raffatellu, M., Chessa, D., Wilson, R. P., Tükel, C., Akçelik, M., Bäumler, A. J. 2006. Capsule-mediated immune evasion: a new hypothesis explaining aspects of typhoid fever pathogenesis. *Infections and Immunity*, 74(1):19-27.
22. Parry, C. M. (2006). Epidemiological and clinical aspects of human typhoid fever. In Matroeni, P. and Maskell, D. (Eds.). *Salmonella* infections: Clinical, immunological and molecular aspects, p. 1-18. New York: Cambridge University Press.

CITATION OF THIS ARTICLE

Kausar M, Noreen A, Idrees K, Maria M and Sidra N. Isolation and Identification of *Salmonella Species* from Dahi Bhalay, Fruit Chaat and Fruit Juices Collected From Different Localities of Lahore.Bull. Env. Pharmacol. Life Sci., Vol 4 [5] April 2015: 124-130