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INDICATION OF COLIFORMS AND OTHER BACTERIA FROM A POPULAR STREET FOOD "DAHI BHALLEY" FROM DIFFERENT AREAS OF LAHORE

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Abstract: Ten samples of a popular street food "dahi bhalley" and 5 dishwater, used repeatedly for washing the plates, were collected from different areas of Lahore. They were processed on different media for the detection of coliforms and certain pathogenic bacteria. All the food samples showed growth on Streptococcus selective agar, 80% on MacConkey agar and mannitol salt agar media, while 70% indicated the presence of coliforms as assessed by growth on brilliant green bile broth and EMB agar. In case of dishwater all the samples showed growth on the five media used. These results indicate that the street-food is unsafe for human consumption as it may lead to food poisoning and enteric infections by the pathogens it harbours.

Key words: Food microbiology; microbial contamination of food; Ready-to-eat food and public health.

INTRODUCTION

The incidence of cases and out-breaks of food borne diseases and food poisoning is still very high in spite of much awareness of the problems. Inadequate cooking or improper storage of food, as well as poor sanitary conditions in food preparation at home, restaurants, hospitals and other institutions, can cause uncomfortable and even serious diseases due to presence of coliforms and pathogenic microorganisms. Pathogenic bacteria either infect people directly when ingested or toxins present in the food cause illnesses on eating. They can also produce toxin in the person's intestine after the food is eaten (Pleczar *et al.*, 1993; Benson, 1994; Collins *et al.*, 1995).

Milk and various other foods provide excellent growth media for bacteria when suitable temperatures exist. This is in contrast to natural waters, which lack the essential nutrients for pathogens. Contamination of food or milk products with a few pathogens becomes a much more serious problem because of the ability of these substances to support tremendous increase in bacterial numbers. Many milk-borne epidemics of human diseases have been spread by contaminations of milk by soiled hands of dairy workers, unsanitary utensils and polluted water supplies. Flies and cockroaches also act as carriers of pathogens (Agbodaze and Owusu, 1989; Pelczar *et al.*, 1993; Benson, 1994).

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Pathogens like *Escherichia coli, Salmonella, Shigella, Streptococcus, Staphylococcus, Clostridium* and *Listeria* are responsible for many food and water-borne diseases (Sterritt and Lester, 1988; Sliegh and Timbury, 1994; Collins *et al.*, 1995). Foods including ready-to-eat, street vended and water samples are routinely assessed for the presence of pathogens and coliforms (Gawthorn *et al.*, 1996; Calvo *et al.*, 1998; de Simon and Ferrer, 1998; Warburton, 1998; Kaneko *et al.*, 1999; Mosupye and von Holy, 1999; Tallis *et al.*, 1999; Muleta and Ashenafi, 2001; Pingulkar *et al.*, 2001; Riva *et al.*, 2001). Many methods and media exist for the detection and enumeration of coliforms and pathogens such as MPN technique, membrane filter method, MacConkey agar and mannitol agar, etc. (Ward *et al.*, 1981; Benson, 1994; Collins *et al.*, 1995;Merck, 1996; de Boer, 1998).

The present study was planned to screen a ready-to-eat street food "dahi bhalley" and dishwater samples for the presence of coliforms and certain pathogens by using MPN technique and different selective media. Results of this study will be helpful in assessing hygienic conditions related to the food preparation, storage and serving.

MATERIALS AND METHODS

Samples of "dahi bhalley" and dishwater were collected in sterile glass bottles, from different areas of Lahore (Table I). The samples were processed on the same day for bacteriological examinations.

Qualitative analysis of the samples for coliform bacteria

Samples were analyzed by multiple tube most probable number (MPN) fermentation technique (Benson, 1994; Collins *et al.*, 1995). For the presumptive test single strength (SSB) and double strength (DSB) brilliant green bile broth, were prepared according to Merck (1996). Three sets, each of three test tubes, fitted with Durham tubes were taken for a given sample. One set of the test tubes was dispensed with 10 ml of DSB while the other two with the same amount of SSB. Following autoclaving at 121°C for 15 minutes, DSB test tubes were inoculated with 10 ml, one set of SSB with 1 ml and the other with 0.1 ml of 10 times diluted suspension of a sample. Dishwater samples were inoculated in the same manner but without the dilution. Gas production was noted between 24 to 48 hours of incubation at 37°C and MPN of coliforms were determined from MPN table (Benson, 1994). For confirmed test, EMB agar (Merck, 1996) was prepared, autoclaved and poured in presterile petriplates. Culture from brilliant green bile broth was streaked and the plates were incubated for 24 hours at 37°C for appearance of growth of coliforms' colonies. The bacterial colonies from the EMB agar plates were sub cultured into single strength brilliant green bile broth for the complete test and on nutrient agar slopes. Following incubation at 37°C for 24 hours, gas production was noted and growth on nutrient agar slants processed for Gram's staining.

Quantitative analysis of samples for pathogenic bacteria

For the detection and estimation of pathogenic bacteria three media were used. 51.5 gms of MacConkey agar (Oxoid), 111 gms of mannitol salt agar (Biolife), 45.6gms of streptococcus selective agar (Biolife) were dissolved per liter of distilled water and autoclaved at 121°C for 15 minutes. After cooling down to 50°C, media were poured in presterile petriplates. After solidification 0.1 ml of 10^2, 10^3 and 10^4 times dilutions of a sample were spread on each of the three media and incubated at 37°C for 24 hours. The plates were then observed for bacteriological enumeration; colony forming units (CFU)/ml. Colour and other features of bacterial colonies on the media used were noted and the growth identified according to Merck (1996).

RESULTS

Coliform bacteria

Results of presumptive test showed that both food as well as dishwater of samples 2, 4 and 5 were positive for gas production. Samples 1, 3, 6 and 8 also produced gas, while 7, 9 and 10 did not. However, dishwater of sample 9 and 10 showed positive results. It was noticed that in case of brilliant green bile broth tubes inoculated with 10 ml, the Durham tubes filled completely with gas after 24-hours of incubation in almost all the samples. Most probable numbers of coliforms for these samples are given in Table I.

All samples, which showed gas production in presumptive test yielded, nucleated colonies on EMB agar, which confirmed the presence of coliform bacteria. Appearance of colourless translucent colonies for sample 2 on the agar plates indicated the presence of pathogens such as *Salmonella* and *Shigella*, while dishwater of samples 2 and 5 formed violet colonies on EMB agar, with metallic sheen. Thus *E.coli* were present in these samples. Inoculation of colonies from EMB agar to brilliant green bile broth tubes showed results similar to those obtained in presumptive test for all the samples. All cultures, streaked on nutrient agar from EMB agar were found Gram-ve rods (Table I).

MacConkey agar test

Results of this test have been presented in Table II. As can be seen from the table almost all samples contained pathogenic bacteria. As red, pink and colourless bacterial colonies on this medium indicate presence of *E. coli*, other colifonns and *Salmonella* or *Shigella*, respectively. Food samples 1, 3 and 7-9 produced only red colonies on the MacConkey agar. Red, pink and colourless bacterial colonies appeared in case of sample 2, 5 and 6. Samples 4 and 10 did not show any growth. Dishwater of samples 2 and 10 produced red, pink and colourless, sample 4 pink and colourless, sample 5 red and pink while, sample 9 only colourless bacterial colonies. For remaining samples dishwater was not collected. Colony forming units (CFU)/ml of samples for each type of colony are

given in Table II. Some samples gave rise to too abundant growth for all dilutions to count CFU.

Table I: Indication and most probable (MPN) of coliforms in a popular street-food (dahi bhalley) from different localities of Lahore.

Sample No.	Area and date of collection	Weight of 1 ml of sample		MPN/100 ml of sample		Confirmed test		Completed test			
								Gas production		Grams' staining	
		f	w	f	w	f	w	F	w	f	w
1	Samanabad 24/2/2001	1.12	---	$11x10^{3}$	---	Nu	---	+	---	b-	---
2	Singhpura 27/2/2001	1.35	1.01	$11x10^{3+}$	1100+	Vi, Tr, Nu	vi	+	+	b-	b-
3	Dharampura 13/3/2001	1.14	---	$11x10^{3+}$	---	Nu	---	+	---	b-	---
4	University of the Punjab (New Campus) 14/3/2001	1.12	1.04	$11x10^{3+}$	1100+	Nu	Nu	+	+	b-	b-
5	Gulshan-e-Ravi 28/3/2001	1.00	1.00	$11x10^{3+}$	1100+	Nu	Vi	+	+	b-	b-
6	Garden Town 9/4/2001	1.07	---	$11x10^{3+}$	---	Nu	---	+	---	b-	---
7	Ichhra 10/4/2001	0.96	---	---	---	-	---	-	---	-	---
8	Yateem Khana 24/4/2001	1.04	---	1500	---	Nu	---	+	---	b-	---
9	Gulberg III 28/4/2001	1.08	1.02	-	1100+	-	Nu	-	+	-	b-
10	Qila Gujar Singh 16/5/2001	1.09	1.03	-	1100+	-	Nu	-	+	-	b-

f: food sample (10 times diluted); w: dish water; -: negative; +: positive; $11x10^{3+}$: more than 11000; Nu: nucleated colonies; Tr: translucent colourless colonies; ----------; sample not available; b-: gram –ve bacilli; Vi: violet colonies with metallic sheen.

Mannitol salt agar test

Almost all samples produced pale bacterial colonies surrounded by yellow zones as well as with no colour change in the medium, except sample 5 and 10. CFU / ml of sample for both types of the colonies are shown in Table II.

Table II: Colour and number of CFU 10^5/ml of sample screened on different selective agar media.

Sample No.	MacConkey		Mannitol salt		Streptococcus selective	
	f	w	f	w	F	w
1	Red=0.7	---	Py=2.1 Ow=0.67	---	+	---
2	Red=0.3 Pink=un Trns=un	Red=un Pink=un Trns=un	Py=3.0 Ow=2.29	Py=0.02 Ow=un	+	+
3	Red=47	---	Py=2000 Ow=30	---	+	---
4	-	Pink=0.02 Trns=un	Py=8 Ow=5	Py=0.03 Ow=0.04	+	+
5	Red=0.3 Pink=1.4 Trns=un	Red=.004 Pink=.004	-	Py=0.01	+	+
6	Red=0.2 Pink=0.2 Trns=0.3	---	Py=33 Ow=4.3	---	+	---
7	Red=11.4	---	Py=10.6 Ow=1.5	---	+	---
8	Red=1.68	---	Py=2.5 Ow=2.23	---	+	---
9	Red=0.4	Trns=un	Py=13 Ow=0.3	Py=0.8 Ow=0.05	+	+
10	-	Red=0.5 Pink=un Trns=un	-	Py=1.22 Ow=0.25	+	+

f: food sample; w: dish water;Py: pale colonies with yellow zones; Ow: off-white colonies without yellow zones; ---: sample not available; +: abundant uncountable growth of *Streptococci*; Trns: translucent colourless colonies; un: uncountable; -: negative for growth.

Streptococcus selective agar test

All samples showed so abundant growth on streptococcus selective agar medium, that the colony count was not possible (Table II).

DISCUSSION

Bacteriological examinations showed that 70% of foods while all dishwater samples were contaminated with coliforms. Most probable numbers of coliforms for the food and water samples were found in the range of 1400 to >11000 and 10000 to >11000/gm, respectively. Food as well as dishwater of sample 2 and dishwater of 5 also indicated presence of *E. coli*. In confirmed test food sample 2 also produced colourless colonies on

EMB agar. Appearance of such colonies is considered to indicate the presence of *Salmonella* or *Shigella* (Merck, 1996). Sample 2 and 5 representative of Singhpura and Gulshan-e-Ravi, respectively throw light on the sanitary conditions of these heavily populated areas. Results of the present study showed that 20% of all the samples were contaminated with *E.coli*, while 6.67% also with *Salmonella/Shigella*. While, in a similar study Mosupye and von Holy (1999) found *Salmonella* spp., in 2% and *E. coli* in 6% of food samples in Johannesburg.

On MacConkey agar 80% food and all dishwater samples showed extensive growth (Table II). Among the food eight, three and three samples, gave rise growth of red with turbid zones, pink and translucent colourless bacterial colonies, respectively. This indicated the presence of *E. coli*, other coliforms (such as *Enterobacter* and *Klebsiella*) and *Salmonella* or *Shigella* in the 80, 30 and 30% of the samples, respectively.

When the samples were analyzed on mannitol salt agar it appeared that 80% of the food and dishwater samples contained *Staphylococcus aureus*. These results show that either the water used to prepare the food or other sources of contaminations had been prevailing. Further more nature of the ingredients of the food, such as raw salad might be the sources of contamination, whereas boiled potatoes, etc., may act as medium to enhance the growth of the microbes. Kaneko *et al.*, (1999) have shown while studying "bacterial contamination of ready-to-eat foods and fresh products in retail shops and food factories" that raw vegetables cut for salad were the most heavily contaminated with coliforms. In fact testing for 'total' Enterobacteriaceae, coliforms and *E. coli* as marker organisms in foods and detection of specific pathogens of the family Enterobacteriaceae, including pathogenic *E. coli, Salmonella, Shigella* and *Yersinia* spp., is widely applied in many food control laboratories (de Boer, 1998; Mosupye and von Holy, 1999; Pingulkar *et al.*, 2001).

Viable counts of *S.aureus* on the selected medium ranged from $2.1x10^5$ to $2x10^8$ and 10^3 to uncountable for food and dishwater samples, respectively. These figures once again indicate the highly unhygienic handling, preparation and preservation of the food. It is pertinent here to mention that some strains of *Staphylococcus* are known to cause mild food poisoning (Pelczar *et al.*, 1993; Collins *et al.*, 1995). Abundant growth for all the samples was noticed on streptococcus selective agar. Problems of throat infections may be prevailing due to eating of such foods. In fact, presence and magnitude of such bacteria in food samples is a good indicator of the microbial safety of food and hygienic conditions.

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