BACTERIAL ANALYSIS OF WATER SOURCES IN LYALLPUR

NASSIM ABD-EL-AZIZ NEWEIGY AND RIAZ-UR-RAHMAN*

The bacterial flora in water samples obtained from tap and pump water from various localities in Lyallpur during October and November were determined. Each sample of water was tested for total count and coliform count. The standard plate count of water from all sources used in Lyallpur city was higher than permissible for potable water. The standard plate count of water at 37°C. and 22°C. did not differ significantly and either temperature may be used as the incubation temperature. From epidemiological point of view, most of the tap water samples had a high coliform count. The presence of typical *E. coli* with great probability of the presence of enteric pathogens was also evident. Possibility of contamination from an adjacent sewer age system cannot be ruled out. The coliform count in pump water was lower than in tap water, and the difference was highly significant.

INTRODUCTION

Tap and hand pumps are the main water sources used in washing milk utensils in Lyallpur city. A study aiming to elucidate the bacteriological quality of water in these sources is of great value to all interested in the bacteriological potability of water.

Taylor (1942) stated that bacterial flora of water contained many types of organisms. These included psychrophilic and other bacteria generally associated with spoilage in dairy products. Thomas (1949) classified the bacteria found in the water supplies for farm and creamery into two main groups. The first group comprised organisms which normally existed in water or in the surface layer of the soil and the second group of organisms of faecal origin. The organisms producing gelatinous slimy or tapioca curd defects on the surface of cottage cheese were found by Parker *et al.* (1950) and Davis and Babel (1954) to be typical water types and members of the psychrophilic group of *Pseudomonas* and *Alcaligenes*.

Wagenaar (1952) and Wagenaar and Jezeski (1952) reported that surface taint in butter was due to psychrophiles, especially *Pseudomonas putrefaciens* or other members of *Pseudomonas* which were normally water-borne micro-organisms. Burgwald *et al.* (1952) and Olson *et al.* (1953) attributed the poor keeping quality of recombined milk to psychrophilic bacteria which were traced to water sources. Thomas (1953) and Thomas *et al.* (1954) traced

*Department of Food Technology, West Pakistan Agricultural University, Lyallpur.

the psychrophiles causing ropiness in milk to water supplies. While examining samples of water supplies used in 14 butter and 12 cottage cheese plants, Harmon (1957) observed that most of the samples were free from coliform bacteria but contained psychrophilic proteolytic and lipolytic organisms indicating that the coliform test alone did not afford sufficient information about the microbiological condition of water supplies intended for dairy plant use.

The Municipal Committee of Lyallpur (West Pakistan) during 1965 sent 82 tap and pump water samples from different localities of Lyallpur city in different seasons of the year to the Epidemiological Laboratory in Lahore (West Pakistan) for microbiological examination. The presumptive coliform test using MacConkey broth and the Eijkman test at 44°C were made. Forty two samples were potable, 33 samples were contaminated with E. *coli* and six samples (from factories using their own water sources) were super-chlorinated and unfit for drinking. From the data supplied, it appeared that the seasons of the year had no significant effect on the contamination of water.

MATERIAL AND METHODS

Water samples were secured in sterile bottles from the water taps and main hand pumps from different localities of Lyallpur city as well as from the tap and tubewell of the Food Technology Department of the West Pakistan Agricultural University at weekly intervals during the months of October and November. Water was allowed to run for five minutes before taking the sample. Samples were analysed immediately after reaching the laboratory.

Plate Count. The procedure outlined by American Public Health Association (1960) was followed. Tryptone dextrose yeast extract agar was used for the plate count of water. A series of dilutions was prepared to result in countable plates with colonies ranging between 30 and 300. The dilutions ranged from no dilution to 1 : 100 in phosphate buffered solution. The plating was quadruplicate for every dilution. Two plates were incubated at 22°C. for 72 ± 3 hours and the other two at 37°C. for 48 ± 3 hours and the counts were recorded.

Coliform. The multiple fermentation tubes procedure was followed. Lactose broth was the medium used. A series of five fermentation tubes was made for each inoculum of 0. 1, 1, 10 and 100 ml. according to Difco Laboratories (1953). The incubation temperature was 32°C. for 48 ± 3 hours, during which the series of fermentation tubes were examined for gas production and the results were recorded.

In the case of doubtful gas production the culture was further examined by streaking on desoxycholate agar for the growth of typical coliform colonies

on this selective medium. The typical colonies were transferred to both lactose broth and slants and then re-examined for its ability to ferment lactose and its reaction to Gram stain.

To detect the presence of typical *E. coli* strains from the positive fermentation tubes were examined for their ability to produce gas in lactose broth at 44.5°C.

During the coliform confirmation test on desoxycholate agar some colourless colonies appeared on the plates and changed the red colour of the medium to colourless. This raised the question of the probability of the presence of enteric pathogens. These colonies were picked and streaked on Salmonella—Shigella agar. Colonies which developed on the medium were picked and streaked on Simmons citrate agar.

RESULTS AND DISCUSSION

Tap and hand pump water samples obtained from different localities of Lyallpur city as well as from the Food Technology Department (F. T.) tube-well and the Dairy Technology Laboratory (D. T.) tap during the months of October and November were analysed for the standard plate (SPC) at 22°C and 37°C and the coliform (CC) counts. The data regarding (1) the effect of the incubation temperature on the standard plate count (SPC), (2) the standard plate (SPC) and coliform (CC) counts in tap and hand pump water from different localities and (3) the standard plate (SPC) and coliform (CC) counts in F. T. tubewell and D. T. tap water are given in the tables and analysed statistically.

1. *Effect of the Incubation Temperature.* The SPC at 37°C and 22°C as number per ml. is given in ranges in Table I, where the frequencies and the percentages of samples in these ranges are shown. Maximum number of samples (53.85 per cent) ranged between 100—1000 SPC per ml. at both the temperatures. Only 33.77 to 34.61 per cent samples at both the incubation temperatures ranged over 1000 SPC per ml. Statistically the results were non-significant.

2. *Standard Plate Count (SPC) at 37°C. of Tap and Hand Pump Water Sources.* On comparing the SPC of both water sources (Table 2), it would be observed that 66.67 per cent of the tap water samples and 44.45 per cent of pump samples showed a range of 100 to 1000 SPC per ml. A higher percentage of tap water samples (33.33 per cent) were over 1000 SPC per ml. as compared to the corresponding range in the pump water samples which was only 22.22 per cent. The results from the two water sources showed non-significant differences.

TABLE 1.—*Effect of temperature of incubation on the standard plate count (SPC) in water*

Ranges of SPC per ml.	37°C.		22°C.	
	Frequency	Per cent number of samples	Frequency	Per cent number of samples.
0 — 20	0	0.00	1	3.84
20 — 100	3	11.54	3	11.54
100 —1000	14	53.85	14	53.85
Over 1000	9	34.61	8	30.77
Total	26	100.00	26	100.00

S. E. = 139 ; t = 0.8 N. S.

TABLE 2.—*The standard plate count (SPC) at 37°C of tap and hand pump water*

Ranges of SPC per ml.		Tap water		Pump water	
		Frequency	Per cent number of samples	Frequency	Per cent number of samples
0 — 20	..	—	—	—	—
20 — 100	..	—	—	3	33.33
100 —1000	..	6	66.67	4	44.45
Over 1000	..	3	33.33	2	22.22
Total	..	9	100.00	9	100.00
Arith. Mean			2,000		850
Log. Mean			630		250

S. E. = 0.08 ; t = 0.9531 N. S.

The results on SPC for Food Technology tubewell and Dairy Technology tap water samples in Table 3 indicated that F. T. tubewell water samples contained 1,860 total bacterial count which were about six times more than other source. The differences were highly significant.

3. *The Number of Coliform Count (cc) in Tap and Hand Pump.* The most probable number of coliform count per 100 ml. water is given in ranges where the frequencies and the percentages of samples in these ranges are shown in Table 4. The data showed that 64.28 per cent of the tap water samples contained between 10-100 cc per 100 ml. while 42.86 per cent of hand pump water samples were within this range. A greater percentage of the samples in the later case contained 2-10 cc per 100 ml. The tap water as compared to tubewell water samples (Table 5) showed a higher coliform count (30.5 per 100 ml.). The difference in both the cases was highly significant.

Tap water of Lyallpur city from bacteriological view-point was neither suitable for drinking nor for use in manufacturing purposes. All samples examined showed a high coliform count and the presence of typical *E. coli*. This indicated a possibility of the presence of enteric pathogens. The possible presence of these enteric pathogens was indicated when the coliform cultures of the positive fermentation tubes were streaked on Salmonella-Shigella agar. The bacterial growth caused the colour of the medium to change from red to colourless and a black precipitation in the centre of many colonies. When these colonies were streaked on Simmons citrate agar, there was no growth.

The hand-pump water from Lyallpur city contained less coliforms than tap water. The coliforms present in hand pump water were of non-typical types. The increased coliform-content as well as the presence of typical *E. coli* in tap water may be due to the great distances water travels from the city waterworks, through water pipes, to different areas of city and also sewerage disposal tunnels running adjacent to water pipes. The SPC for water from the Dairy Technology Laboratory water tap was lower and CC was higher than the water samples from Food Technology tube-well. The higher SPC in the case of Food Technology tube-well water may be due to the presence of groups other than coliforms, since inoculated fermentation tubes containing lactose broth held at 20°C for 5-7 days changed into bluish green colour. This might have been due to the presence of *Pseudomonas*.

TABLE 3.—*The standard plate count of the Dairy Technology Laboratory tap and Food Technology Department tubewell waters*

Sample number	Standard plate count per ml. at 37°C	
	F. T. tube-water	D.T. tap water
1.	1,550	390
2.	2,400	450
3.	1,800	200
4.	1,700	310
Total	7,450	1,350
Arith. Mean	1,860	340
Log. Mean	2,000	320

S. E. = 0.027 ; t = 9.1 Highly significant.

TABLE 4.—*The most probable number of coliform count of tap and hand pump water samples from Lyallpur city*

Ranges of coliform count per 100 ml.	Tap water		Hand pump water	
	Frequency	Per cent number of samples	Frequency	Per cent number of samples
0 — 2 ..	..	..	4	28.57
2 —10 ..	2	14.28	4	28.57
10 —100 ..	9	64.28	6	42.89
More than 100 ..	3	21.44	—	—
Total ..	14	100.00	14	100.0
Arith. Mean	62		11	
Log. Mean	36		8.5	

S. E. = 0.04 ; t = 4.178 Highly significant.

TABLE 5.—*The most probable number coliform count in Dairy Technology Laboratory tap and Food Technology Department tube-well Water*

Sample number	Most probable number coliform per 100 ml. D. T. tap water	F. T. tube-well water
1.	33	2.3
2.	33	2.3
3.	23	4.5
4.	33	2.3
Total	122	11.4
Arith. Mean	30.5	2.85

S. E. = 0.9 ; t = 10.8 Highly significant.

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