BACTERIOLOGICAL EXAMINATION OF DRINKING WATER OF KARACHI AND ISOLATION OF ENTERIC PATHOGENS

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A bacteriological examination of drinking water from various localities of Karachi was carried out and the total count as well as the most probable number of coli-aerogenes was determined for the samples collected. A seasonal variation was observed in the T.C. and M.P.N. Each of the samples was further examined for the presence of any traceable pathogen.

Introduction

Higher incidences of typhoid, gastroenteritis and diarrhoea have become common problems of public health in Karachi. The origin seems to lie in the defective water and sewage pipelines, most of which happen to be old and rusted. At some places they have been laid very closely together, sometimes even crossing each other. Bursting of pipelines is a common occurrence, particularly in some of the older localities of Karachi, causing pollution of the drinking water. This condition is further worsened where there is a combined sanitary system and the sewage is let down in storm-water drains.

Among the measures necessary for relieving the situation, it was considered of importance to develop a low cost filtering device from indigenous raw material and the work carried out in this direction is being reported elsewhere. The present paper deals with the bacteriological examination of drinking water from various localities of the city in order to find out whether the pathogens were originally present in the drinking water or they were introduced during its journey from the treatment plant to the water taps. Hussain and Sethna[1] while working on the incidences of enteric fever had reported earlier the presence of a few pathogens in the drinking water being supplied to the Civil Lines area of Karachi.

A total of 125 samples was collected from various parts of the city at different distances from the filter plant and subjected to presumptive-coli tests. Each of these samples was further examined for the presence of any traceable pathogen. The results obtained are shown in Table 1.

Material and Methods

In order to obtain samples from every locality of Karachi, the city was divided into sectors and a few neighbouring areas were also included in the survey. Samples were collected from public taps and also from storage tanks, underground reservoirs of private buildings and a few hand-pumps fitted at different places.

Methods adopted for testing were in accordance with those laid down in the 'Standard Methods for the Examination of Water and Sewage.[2] Glass stoppered flasks of 500 ml. capacity were used to collect samples. For presumptive-coli tests three sets each consisting of 5 tubes with 10 ml., 1 ml. and 0.1 ml. of the sample respectively, were used and "Most Probable Numbers" were ascertained from McCrady's table. Serial dilutions were made and inoculated in agar plates in duplicate. A temperature of 37°C. was maintained for the incubation and colonies were counted after 48 hours. In addition to agar, other differential and selective media were also inoculated for the isolation of organisms. Lactose broth tubes were incubated at 37°C. and checked after 18,24 and 48 hours. The M.P.N. was determined from the 48 hours reading.

In the case of *E. coli* only those organisms are being reported, which grew in brilliant green bile 2% broth, producing acid and gas at 44°C. It has however to be noted that according to Raghavachari and Iyer,[3] organisms other than *E. coli* may also account for acid and gas at this temperature under the climatic conditions of the sub-continent. Peptone water-tubes were therefore incubated at 44°C. and checked for indol production after 48 hours.[4] This was further confirmed by the production of acid and clot in litmus milk, nonutilisation of citrate and uric acid, reduction of nitrates to nitrites, negativity of V.P. test and positivity of M.R. test. Only members of the enteric group were identified upto species and the identification was based on biochemical tests according to Burgey's Manual of Determinative Bacteriology.[5] In the identification of organisms, the results were further checked up by following the procedures laid down in Kauffmann's Enterobacteriacea.[6]

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TABLE 1.—SHOWING BACTERIAL FLORA OF DRINKING WATER WITH REGARD TO INTENSITY AND LOCALITY.

Sample Nos.	Source	Locality	Organisms isolated*	Average total count of Organisms/ ml. of water	M.P.N. of coli-ærogenes/ 100 ml. of water
1.	Hand pump	Achchi Qabar	1,2,3	700	225
2.	Drum (Malabari Tea Shop)	Rampart Row	1,2,3,5	600	350
3.	Water pitcher (Shop)	Kharadar	3,6,7	660	250
4.	Water tank (Habib Bank)	Rampart Row	1,2	900	425
5.	Public tap	,,	3,8	260	0
6.	,,	,,	3,7	350	70
7.	Water pitcher	,,	3,6,9	720	130
8.	Public tap	Khori Garden	3,6	590	0
9.	Open drum (Tea-Shop)	,,	3,6	620	0
10.	,,	Rampart Row	2,3,9	585	85
11.	Water pitcher (Sharif Manzil)	A.A. Rehman Street	3	665	0
12.	Hand pump (Under ground tank)	,,	2,3	850	420
13.	Water pitcher	Mithadar	3	1200	0
14.	Public tap	,,	3	640	0
15.	,,	Fish market Khadda	6,10,11	710	0
16.	,,	,,	1,7,10	350	250
17.	,,	Moosa Lane Khadda	2,3	980	425
18.	Public tap	Moosa Lane (Khadda)	1,7	430	350
19.	,,	Shidi Village Road (Lyari)	2,10	665	115
20.	,,	Kalri off lane	2,3,12	565	170

(*Table continued*)

(*Table 1 continued*)

21.	Public tap	Masan Road (Bihar Colony)	1,3	1000	0
22.	,,	Tannery Road (Bihar Colony)	6,7	1000	250
23.	,,	Usman Park (Rangiwara)	2,3,12	220	200
24.	,,	Off Tannery Road	7	350	40
25.	Underground tank	Shershah Colony	3,6,13	1100	14
26.	,,	Atmaram Road	2,3,14	240	225
27.	,,	Miranaka Chakiwara	3,12	980	0
28	,,	Chakiwara Road	2,8	550	95
29.	Public tap	Karabhai Karimjee Road	3,13	750	20
30.	Storage tank (Tea-shop)	Allama Iqbal Road	3	830	0
31.	,,	,,	6,7,10	960	55
32.	Public tap	Pir Ghaib Shah Road	2,3	1600	150
33.	Metallic drum (Shop)	Keamari	9,15	830	25
34.	Cement tank (Hotel)	Keamari Harbour	2,3,6	4000	85
35.	,,	Japani Road	10,16	1000	0
36.	Public tap	,,	1,3,7	550	65
37.	Cement tank (Hotel)	Baba-e-Urdu Road	3,6,9	600	50
38.	Underground tank	Baba-e-Urdu Road	2,3	880	15
39.	,,	Princess Street	3,12,13	700	0
40.	Underground tank (Private Bldg.)	,,	2,3	960	11
41.	Water tap (Private Bldg.)	,,	3	980	0
42.	Public tap.	Bohrapir	2,3,6,16	980	117
43.	,,		7,14	410	170

(*Table continued*)

(*Table 1 continued*)

44.	Water tap (Hotel Alfaruq)	Ranchore Lines	3,7	940	110
45.	Cement tank	Ramaswami	2,7	860	95
46.	Metallic tank	,,	1,9	870	85
47.	Metallic tank (Hotel)	,,	2,3	760	70
48.	Cement tank (Hotel)	Lawrence Road	2,15	1100	25
49.	Water tap (Private Bldg.)	,,	1,7,14,16	790	135
50.	Water tap (Hotel)	,,	2,3	780	80
51.	Public tap	Soldier Bazar	1,3,9	640	50
52.	,,	,,	3	540	0
53.	,,	,,	3,6	480	0
54.	Water tank (Hotel)	,,	3	1200	0
55.	Water tap	Kutchery Road	2,15	1010	85
56.	,,	Artillary Maidan	1,7	650	85
57.	Water tank (Hotel)	Bunder Road Burns Road	2,3	1400	225
58.	Water tank (Hotel)	Bunder Road Victoria Road	2,3,6	1200	70
59.	,,	Bunder Road Baba-e-Urdu Road	6,7,16	1010	140
60.	Water tap (Private Bldg.)	Bunder Road Nappier Road	7	840	50
61.	Cement tank (Hotel)	Mohammadi Building	2,10	1500	85
62.	,,	City Station	3,10	1200	0
63.	Metallic drum	Ispahani Bldg. McLeod Road	3,10	840	0
64.	Water tap (Hotel)	McLeod Road	14	450	0
65.	Kitchen tap	Naval Club	No growth	0	0
66.	Cement tank (Hotel)	Frere Road	3	940	0
67.	,,	Frere Road Nappier Road	2,7	1100	45

(*Table continued*)

(*Table 1 continued*)

68.	Cement tank (Hotel)	Burns Road Women's College	2,3	1300	70
69.	Storage tank (Tea-Shop)	Behind Fredrick's Cafeteria	1,9	1300	25
70.	Water tank (Canteen)	Pak. Sectt.	6	1600	0
71.	,,	,,	3,6	1010	0
72.	Water tap (Hotel)	Victoria Road	2,14	980	40
73.	Drum (Tea shop)	Korangi Road P.N.H. Area	2,3,15	1080	17
74.	Water pitcher (Hotel)	,,	3,6,7	1250	20
75.	,,	,,	3,7,15	1600	20
76.	Tank (Hotel)	Delhi Colony	3,6	935	0
77.	,,	,,	8	150	0
78.	,,	,,	1,2	1100	25
79.	Water storage tank	Delhi Colony	2,3	1600	30
80.	,,	,,	3,17	1600	0
81.	Mashk (Leather Bag)	,,	6,7	1100	85
82.	Tank (Hotel)	Gizri Road	3,8	960	0
83.	Water tap (Hotel)	,,	3,9,12	840	25
84.	Water tap (Private House)	Bath Island	No growth	0	0
85.	,,	,,	No growth	0	0
86.	Water tank (Teashop)	,,	2,3	1010	30
87.	Water tap (House)	,,	1,3	450	0
88.	Water pitcher (Teashop)	Clifton Beach	1,3,7	955	12
89.	,,	,,	2,15	840	65
90.	,,	,,	8	720	10
91.	K.D.A. filter plant	Country Club Road	No growth	0	0
92.	,,	,,	No growth	0	0
93.	,,	,,	No growth	0	0

(*Table continued*)

(*Table 1 continued*)

94.	,,	,,	No growth	0	0
95.	Main water tank	University Campus	7	580	8
96.	Hostel kitchen	,,	7,9	550	40
97.	Cemennt water tank	,,	2,3	660	40
98.	Main water pipe	Pak. National Exhibition	2,15	280	65
99.	Tank (Hotel)	,,	1,2	140	35
100.	Domestic water Filter (Private House)	P.E.C.H.S.	No growth	0	0
101	Water tap (Private House)	,,	3,15	720	0
102.	Cement tank (Hotel)	Bahadurabad	6,7	730	85
103.	Cement tank (Hotel)	Bahadurabad	14	960	0
104.	Water tap (Private House)	Alamgir Road P.E.C.H.S.	2,3	840	85
105.	,,	Liaqatabad	7	655	80
106.	Cement tank (Hotel)	,,	2,15	965	75
107.	,,	,,	1,6	1100	0
108.	Water tap (Private House)	,,	7,17	450	175
109.	Storing drum (Private House)	,,	2,4	880	0
110.	Water tap (Hotel)	Nazimabad (Chowrangi)	No growth	0	0
111.	Tubewell	Malir City	3	840	0
112.	Cement tank (Hotel)	,,	9,14	550	15
113.	Water tap	Saudabad	1,7	485	110
114.	,,	,,	1,15	540	0
115.	Metallic tank (Hotel)	Malir Road	3,6	825	35
116.	Water tap	,,	2,7	805	130
117.	,,	,,	7	525	225
118.	,,	Landhi	3,8	480	0

(*Table continued*)

(*Table 1 continued*)

119.	,,	,,	7,9	340	115
120.	,,	,,	3,7	455	200
121.	Cement tank (Hotel)	,,	12,15	600	0
122.	,,	Malir Halt	1,12	550	0
123.	Water tap	,,	2,7	640	215
124.	,,	,,	1,7	815	175
125.	,,	,,	7,8	880	105

* 1, *Salmonella typhosa;* 2, *E. coli;* 3, *Staphyloccoccus Spp.* 4, *Shigella sonnei;* 5, *Gaffkya tetragena;* 6, *Shigella dysenteriae;* 7, *Aerobacter aerogenes;* 8, *S. paratyphi;* 9, *Paracolobactrum aerogenoides;* 10, *Sarcina lutea;* 11, *klebsiella coloacae;* 12, *Shigella sonnei;* 13, *Aerobacter coloacae;* 14, *Shigella flxneri;* 15, *Streptococcus faecalis;* 16, *Xanthomonas* Spp.; 17, *Alkaligenes faecalis.*

Results and Discussion

Out of the total number of 125 samples, 80 gave presumptive-coli positive tests. Only in 39 cases *E. coli* 'type I' was found while the remaining ones contained other members of coli-ærogenes group. A noteworthy fact was that in 7 cases, no member of coli-ærogenes was detected but they gave positive presumptive-coli tests. The samples, however, contained members of *paracolon* It may be assumed that these organisms attacked the lactose a little earlier and gave false presumptive-coli tests.

Although the presence of coli-ærogenes indicates that the water had contact with sewage, a large number of positive presumptive-coli tests further suggested the possibility of other enteric pathogens being present, and subsequent studies revealed that a number of samples which gave negative coli tests did contain organisms belonging to groups *Salmonella*, *Shigella* and *Streptococci.*

When various sources were compared with respect to the total number of all organisms and the probable number of coli-ærogenes which the samples contained, it was found that water collected from the containers of hotels, restaurants and public canteens had higher total count and lesser probable number of coli-ærogenes in comparison to water collected directly from taps. The same was the case with water from underground reservoirs and tanks. It also showed higher total count but lesser probable number of coli-ærogenes in comparison to that of taps. Higher total count may be due to uncleanliness of containers and tanks. Algal and fungal growth was observed in some underground reservoirs. The reason for showing lesser M.P.N. of the stored water could be attributed to the fact that on long storage members of coli-ærogenes being less resistant to unsuitable conditions, disappeared while the other organsims survived. Even among the coli-ærogenes group, *E. coli* has been found to be most senstive to the climatic conditions of the subcontinent. Clemesha [7] while working on the resistance of coli-ærogenes organisms reported that *E. coli* was rapidly destroyed under the conditions of indian sunshine. Water collected directly from taps was found to harbour a large number of E. coli 'type I' and showed higher probable number of coli-ærogenes, though it gave lesser total count. Greater number of *E. coli* and its comparative lower total count is an indication of recent fæcal pollution. Gray [8] found that the number of *E. coli* is largest in recent pollution whereas it gradually decreases on storage over a long period. The higher total count and lesser M.P.N. of stored water and higher M.P.N. and lower total count of tapwater in the present study conforms to his findings.

In agreement with Howards [9] we also found seasonal variation in total count and most probable number of coli-ærogenes. In the winter season (October-March), the M.P.N. was found to be very high and the total count very low. In the following months (April-September), the M.P.N. gradually decreased with the rise of temperature and the total count was found to be on the increase. Again, with the advent of winter, the total count gradually came down with temperature and M.P.N. went up.

One striking fact was the presence of soil bacteria, Xanthomonas in some samples. Intermittent supply of water seems to be the cause for the presence of such bacteria as well as for its contamination with some of the other organisms introduced into the pipelines during its journey, for the water collected directly from the filter plant did not show any growth. Most of the areas of Karachi get water twice in 24 hours. As the supply is stopped, a vacuum is created in the pipelines, and if there is any leakage due to rupture or at the joints, the water or the soil is sucked into the pipelines from the surrounding area.

Conclusion

125 samples of water were collected from the different localities of Karachi; out of this number 81 i.e. 64.0% gave presumptive-coli positive tests. Only 39 i.e. 31.2% samples contained *E. coli* 'type I'. Water stored in reservoirs, gave higher total count and lesser M.P.N. in comparison to that collected directly from taps. Tap water was found to harbour a large number of *E. coli* 'type I'. Seasonal variation was observed in the M.P.N. as well as in the total count. In the winter season the Total count was found to be less and the M.P.N. high but a depreciation occured in the M.P.N. with rise of temperature in the summer months. As winter approached, the M.P.N. showed a gradual rise and reached its peak in the coldest months.

Soil flora *Xanthomonas* was found in some samples collected from water pipe lines in which the supply is not constant. The soil or water found in the vicinity of leaking pipelines are probably sucked into the pipe lines due to vacuum created when the supply is cut-off.

The organisms isolated from water include *Streptococcus fæcalis*, *Shigella sonnei*, *Shigella dysenteriæ*, *Shigella flexneri*, *Salmonella paratyphi*, *Salmonella typhosa*, *Aerobacter ærogenes*, *Klebsiella cloacae*, *Paracolobactrum aerogenoides* and *Escherichia coli*.

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