The Journal of Rural and Agricultural Research Vol. 16 No. 2, 28-32 (2016)
Received August 2016; Acceptance Novemver 2016

Microbiological quality of water used in infant foods with special reference to Escherichia coli

MONIKA SHARMA* AND YOGENDRA KUMAR
Deptt. of Animal Husbandry and Dairying, Kisan (P.G.) College, Simbhaoli, Hapur

Abstract

Water is important for infant and should supplement breast or formulas feeding. A nursing mother must maintain a high intake of water (2-3 liters/days) to provide adequate water dilution of her milk. Thus, the quality of water used for as reconstitution of infant milk food are important role in determining the quality of infant food may be converted to a poor quality feed, if bed quality water is used for reconstitution purpose. A total number of 150 water samples were collected from ten villages surrounding the Meerut district in the year 2009-10. Village water samples were collected aseptically from handpumps and storage pots in sterilized glass. Forty six water samples were also collected from the two supplied by municipal supply system. Collected water samples were analysed for their bacterial counts yeast and mould counts, E.coli counts. During the present study 132 isolates of E.coli were obtained from water samples. All these isolates produced indole, methyl red and fermented glucose anaerobically. Based on additional characters of producting of indole and lactose fermented anaerobically. 26 strains were identified as E.coli. Based on above considerations, no single test has been evolved to monitor different species of E.coli definitely and precisely. However, due emphasis was given to characters such as indole and methyl red for likely pathogenic nature of these isolates. In village water sample contain 26 strains E.coli. Contrary of this, the town water supply had satisfactory quality with low counts and absence of coliform. Use of such water for preparation of infant feed may prove to be source of potential pathogens endangering the health of infants. There is a need to improve the quality of water samples obtained from villages attempt were to treat them by chlorination and boiling.

Keywords: Water quality, microbiological quality, infant food, E.coli

Introduction

Water is an integral component for reconstitution of baby foods. But it also act as an important vehicle for spreading various types of disease. Drinking water that is contaminated is only one of many possible source of infectious microorganism. Infact, water has been shown to be responsible for the spread of various diarrhoeal disease and poor quality of water is an important factors deteriorating the good quality of infant food. Polluted (sewage waters) contain solids and dissolved organic compounds that impart an offensive odour and serve as an excellent medium for the growth and multiplication of microorganisms. Typical organism found in different type of water belong to fungi, protozoa, algae, bacteria, actinomycetes and viruses for example the causative agents of *dysentery (Entamoeba histolytica) typhoid fever (Salomonella typhi)*, cholera *(Vibrio cholera)* etc. faecal coliform and enterococci were also found in water.

According to a WHO report, an estimated 15 million children in developing countries fall victim to water and sanitation related diseases. It was further reported that in India, diarrhea alone was responsible for the death of 1.5 million children annually. In view of these facts, a survey was conducted to examine the bacteriological quality of water with the idea that such water may likely be used for preparation of infant feed. The study obtained in the present investigation indicated unsatisfactory microbiological quality of water especially in villages. Keeping in view the microbiological quality of water used in infant foods was carried out on following objectives:-

To study the bacterial counts in water used in infant foods.

Incidence and characterization of *E.coli* in water used in infant foods.

Corresponding address:
*A-60, Defence Enclave, Kankerkhera, Meerut

Materials and Methods

A total number of 150 water samples were collected from ten villages surrounding the Meerut district in the year 2009-10. Then village water samples collected aseptically from handpumps and storage pots (Usually muds or glavanised iron buckets) in sterilized glass bottles with screw in Teflon caps and analyzed were completed within 5 hours of collection. Forty six water samples were also collected from the town supplied by municipal supply system. Collected water samples were analysed for their bacterial counts yeast and mould counts, *E.coli* counts.

The total bacterial counts was carried out by pour plate technique using total count agar. The samples collected from the city were treated before analysed with a few drops of sterilized solution of sodium thiosulphate (0.008%) to neutralize the chlorine. All the samples were analysed for total bacterial count using Nutrient Agar by serial dilution. The sample poured and mixed thoroughly the petri plates. The plates were incubated at 37°C for 24 hrs after which colonies formed were counted and expressed as colony forming unit per gm. (cfu/g)

E.coli counts was done using on Mac Conkey agar. The enrichment procedure was also followed using Mac Conkey Broth purple to recover the low populations in the product. The incubation was carried out for 24 to 48 hours at 37°C. *E.coli* were determined by the five tubes, most-probable number (MPN) technique as recommended by APHA (1976).

The water samples were inoculated at the rate of 10ml. of five tubes of double strength Mac Conkey Broth purple and 1.0 and 0.1 ml to five tubes each of single strength Mac Conkey Broth purple. The tubes were incubated for 48 hr at 37°C and formation of acid and gas indicated a positive sample. The results were read by Mac Cardy's Probability. Eijkman's test was employed to ascertain the presence of faecal *E.coli* in positive tubes. Conformation of E.coli was done by subjecting these to the following biochemical reactions indole production, lactose fermentation, methyl red, citrate utilization, urease and TSI etc.

Analysis was done using simple statistical tools like average and percentage. The CFU/g of the sample was calculated by using the following formula:

$$\text{CFU/g} = \frac{\textit{Number of colonies} \times \textit{Dilution factor}}{\textit{Dry weight of the sample}}$$

Results and Discussion

A wide variation was observed in total counts of water samples obtained from handpumps and storage pots (usually muds pots or galvanised iron buckets) table 1. The average log counts recorded was 4.1 and 4.8, respectively. The total bacterial count of tap water ranged between 0.5 to 2.5. Approximately 83.33% of the water samples from tap contain bacteria in range of 10 to 100 per ml. While, 16.67 percent had the bacterial load between 101 to 1000 per ml.

Table 1: Total bacterial counts in water samples

Source of Water	No. of Samples	Log Counts/ml. Minimum	Maximum	Average
Town Tap	46	0.5	2.5	2.2
Village				
(a) Handpump	65	1.5	4.5	4.1
(b) Pot (Stored 2-3 days)	39	1.0	5.2	4.8

On the other hand, 7.41% of the water samples from handpumps contained bacteria in the range of 1001-10,000 per ml. While, 66.67% had the bacterial load between 101 to 1000 per ml. and 25.92 percent had the bacterial load between 10-100 per ml. On the other hand, 14.81% of the stored pot water contained from the more than 10,000 per ml. Another 55.56 and 29.63% of the stored pot water also contained from 1001-10,000 and 101 to 1000 per ml. (Table 2).

It can be concluded from the table 2 that the average bacterial counts were relatively higher in stored water than in fresh water samples. Distribution of total bacterial counts in water samples also indicates that the percentage of town water supply was higher

Table 2: Distribution of total bacterial counts in water samples

Range(Counts / ml.)	Town Supply		Village Supply			
	Tap Water		Handpump Water		Pot Water	
	Positive Samples	Percent	Positive Samples	Percent	Positive Samples	Percent
10-100	25	83.33	21	25.92	0	-
101-1000	5	16.67	54	66.67	8	29.63
1001-10,000	0	-	6	7.41	15	55.56
More than 10,000	0	-	0	-	4	14.81

than village water supply in the range of 10-100 counts per ml.

Incidence of E.coli

Water samples collected from villages were generally contaminated with *E.coli*. Average log MPN was higher for stored water (2.6/100ml) than for fresh water samples obtained from hand pump (3.1/100ml) (Table 3).

Table 3: Occurrence of *E. coli* in water samples

Source of Water	No. of Samples	Log MPN/100 ml. Minimum	Maximum	Average
TownTap	46	0.0	0.5	0.3
Village				
(a) Handpump	65	0.5	3.5	3.1
(b) Pot (Stored 2-3 days)	39	0.0	3.0	2.6

While, average log MPN was obtained from tap water (0.3/100ml). As high as 75.00 percent of the samples were positive for *E.coli*. Among there, 57.77 percent contained faecal type.

Table 4: Distribution of E. coli in water samples

Range (MPN/100 ml.)	Faecal Types Positive Samples	%	Non-Faecal Types Positive Samples	%
1-10	15	57.77	45	75.00
11-100	9	34.61	10	16.67
More than 100	2	7.69	5	8.33

It can be concluded that the *E.coli* counts were maximum in handpump water than the tap water and pot water. It was due to keeping of higher percent of faecal types in the range of 1-10 per ml. On the others, kept a higher percent of non faecal types in the range of 1-10 MPN/100 ml.

Characterization of E.coli isolates

On Mac Conkey's medium, colonies were pink due to lactose fermentation. In general colonies are circular, moist, smooth with entire margin and non-mucoid. In liquid medium, growth occurs as uniform turbidity. *E.coli* is a Gram's negative bacilli measuring 1-3 µm × 0.4 - 0.7 µm. It is non-sporing and non-capsulated. Based on these tests, 132 isolates were confirmed as *E.coli*. There are indole positive and lactose fermented (Mac conkey agar).

Correlation among different characteristics of E.coli isolates

The relationship among different characteristics for the identification of *E.coli* has been illustrated in tables 5 to 10.

Fig. 1. MacConkey's agar showing pink colonies of *E.coli*

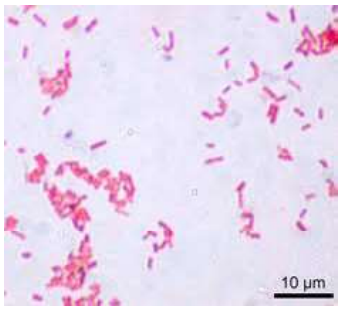

Fig. 2. Gram staining showing pink coloured Gram negative bacilli

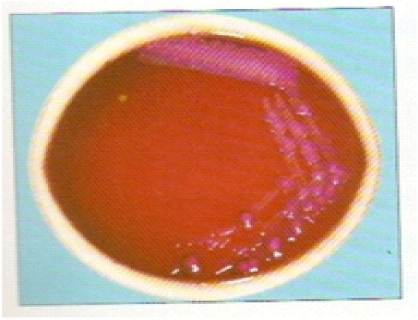

Fig. 3. MacConkey's agar showing lactose fermenting (pink) & non lactose fermenting (plae) colonies

Table 5: Lactose fermentation and other characteristics of E. coli in water samples.

Characteristics	Lactose Fermenters (113) Number	Percent	Lactose Non-Fermenters(19) Number	Percent
Indole	97	85.84	13	68.42
Methyl Red	94	83.18	16	84.21
Urease	16	14.16	8	42.11
Citrate	14	12.39	3	15.79
Triple Sugar Iron	89	78.76	12	63.16

About 85.84 percent of the lactose fermented among *E.coli* isolates were positive for indole and 83.18

percent methyl red. However, 14.16 and 12.39 percent of such cultures produced urease and citrate, respectively and 78.76 percent produced triple sugar Iron. On the other hand, none of the lactose fermenters produced 63.16 percent triple sugar iron and only 15.79

Table 6: Indole production and other characteristics of *E.coli* in water samples.

Characteristics	Indole Producers (110)		Indole Non-Producers(22)	
	Number	Percent	Number	Percent
Lactose Fermentation (anaerobic)	98	89.09	15	68.18
Methyl Red	97	88.18	13	59.09
Urease	15	13.64	9	40.91
Citrate	13	11.82	4	18.18
Triple Sugar Iron	84	76.36	17	77.27

Among the indole positive cultures, 89.09 percent fermented lactose, 88.18% produced methyl red and 76.36 percent had normal growth presence of triple sugar iron. However, 13.64 and 11.82 percent of these isolates produced urease and citrate, respectively. Contrary to this only, 40.91 and 18.18% of the indole negative isolates, produced urease and citrate. However, 68.18, 59.09 and 77.27% of indole non producer isolates produced lactose fermented methyl red and triple sugar iron, respectively (Table 6).

Table 7: Methyl red positive and other characteristics of *E.coli* in water samples.

Characteristics	Methyl red positive (110)		Methyl red negative(22)	
	Number	Percent	Number	Percent
Lactose Fermentation (anaerobic)	98	89.09	15	65.18
Indole	97	88.18	13	59.09
Urease	15	13.64	9	40.91
Citrate	5	4.54	12	54.55
Triple Sugar Iron	87	79.09	14	63.64

It may further be noted that 88.18% of the methyl red positive isolates of *E.coli* were positive for indole, and 79.09, 4.54 and 13.64% of the positive cultures produced triple sugar iron, citrate and urease, respectively. However, 89.09% of the methyl red positive isolates was produced fermented lactose. On the other hand, 63.64 and 54.55% of methyl red negative isolates also elaborated triple sugar iron and citrate, while, 40.91% of them produced urease. It was further noticed that 59.09 and 65.18% of these isolates produced indole and fermented lactose (Table 7).

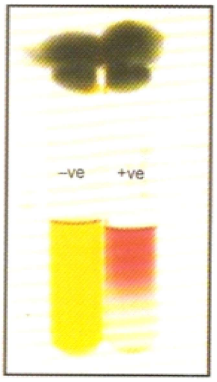

Fig.4 Methyl Red Test (MR Test)

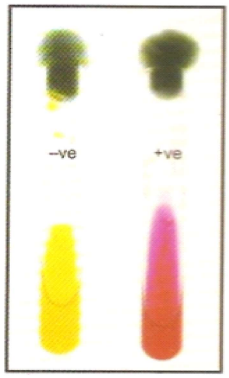

Fig. 5. Urease Test

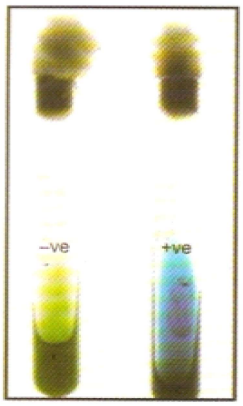

Fig.6 Citrate utilisation Test

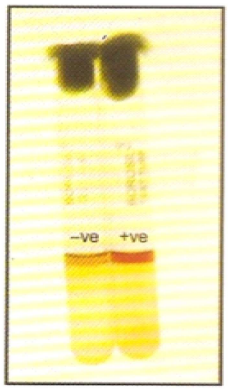

Fig.7 Indole Test

It may further be noted that 82.35% of citrate ultilizers isolates of *E.coli* were positive for lactose fermented and 76.47% produced indole. While, 64.71, percent methyl red an equal number of urease. Approximately 76.47% of citrate utilizar produced TSI. On the other hand, 76.52 and 11.30% of non citrate utilizar were produced TSI and urease, respectively.

Table 8: Urease production and other characteristics of *E.coli* in water samples.

Characteristics	Urease Producers (24)		Urease Non-Producers(108)	
	Number	Percent	Number	Percent
Lactose Fermentation (anaerobic)	22	91.67	91	84.26
Indole	21	87.50	89	82.41
Methyl Red	19	79.17	91	84.26
Citrate	13	54.17	4	3.70
Triple Sugar Iron	8	33.33	93	86.11

Among urease isolates of *E.coli,* 91.67% fermented lactose and 87.50% produced indole. About 79.17 and 54.17% of the urease isolates were observed to synthesis methyl red and citrate, respectively. While, 33.33% produced TSI. Approximately, 86.11 and 3.70 percent of non urease produced isolates also produced TSI and citrate While, 84.26, 82.41 and 84.26% of urease negative isolates produced fermented lactose, indole and methyl red, respectively (Table 8).

Table 9: Citrate utilization and other characteristics of *E.coli* in water samples.

Characteristics	Citrate Utilizers (17)		Citrate Non-Utilizers(115)	
	Number	Percent	Number	Percent
Lactose Fermentation (anaerobic)	14	82.35	99	86.09
Indole	13	76.47	97	84.35
Methyl Red	11	64.71	99	86.09
Urease	11	64.71	13	11.30
Triple Sugar Iron	13	76.47	88	76.52

It was further noticed that 86.09 percent of these isolates fermented lactose. While, 84.35 and 86.09 percent of then produced indole and methyl red, respectively (Table 9).

Table 10: TSI production and other characteristics of *E.coli* in water samples.

Characteristics	TSI Producers (101)		TSI Non-Producers(31)	
	Number	Percent	Number	Percent
Lactose Fermentation (anaerobic)	90	89.11	23	74.19
Indole	89	88.12	21	67.74
Methyl Red	97	96.04	13	41.94
Urease	13	12.87	11	35.48
Citrate	9	8.91	8	25.81

Interestingly, the triple sugar iron positive *E.coli.* isolates had observed 89.11 and 88.12% fermented lactose and indole, respectively. It may be observed that 96.04, 12.87, and 8.91% of such isolates elaborated methyl red, urease and citrate, respectively. On the other hand, 25.81 and 35.48% of TSI negative isolates culture were observed to be positive for citrate and urease, respectively. A remarkable high proportion of these TSI negative isolates produced fermented lactose 74.19, indole 67.74 and methyl red 41.94 (Table 10).

It can be concluded from the table that based on these biochemical characteristics, 26 strains isolates were designated as *E.coli* as they fermented lactose and produced indole, methyl red and anaerobically fermentation of lactose, mannitol, glucose and maltose. The utensils used for storing water is not cleaned properly and frequently these are used for prolonged storage which could further deteriorate the quality of water. As availability of high microbial quality for potable water for reconstitution of infant food is strongly recommended. Use of only boiled and subsequently cooled water for preparation of infant feed is re-emphasized.

References

APHA-AWWA-WPCF. (1995). Standard methods for the Examination of Water and Waste water. 19th ed. APHA Washington, D.C.

Ayliffe, G.A.J., Collins, B.J. and Pettil, F. (1970). Contamination of infant feeds in a Milton milk kitchen. Lancet, 7646 : 559-560.

Barrell, R.A.E. and Rowland, M.G.H. (1980). Commercial milk products and indigenous weaning foods in a rural West African environment : A bacteriological perspective. J. Hyg., 84 : 191-202.

Bohra, D.L., Modasiya, V. and Bahura, C.K. (2012). The distribution of coliform bacteria in waste water microbiology research, 3 : e2. doi : 10, 4081/mr. 2012 e2.

Chan C.L., Zalifan, M.K. and Norrakiah A.S. (2007). Microbiological and physiochemical quality of Drinking water. The Malaysian Journal of analysis Sciences; 11 : (2) 414-420.

Joao, P.S. and Carbral (2010). Water Microbiology Bacterial Pathogens and water. Int. J. Environ. Res. Public Health 7 (10), 3657-3703; doi : 10. 3390/ijerph 7. 103657.

Leznik, A.I., Andrienko, A.I., Klimenko, G.N. and Gorskaya, A.F. (1973). Revision of microbiological quality indioes of infant milk formulae. Vaprosy Pitaniya, 2 : 87-89. Cited : DSA, 35 : 3455.

Miller, D.L., Geopfert J.M. and Amundson, C.H. (1972). Survival of *Salmonellae* and *Escherichia coli* during the spray drying of various food products. J. Fd. Sci., 37 : 828-831.

Rowland, M.G.M., Barrell, R.A.E. and Whitehead R.G. (1978). Bacterial contamination in traditional Gambian weaning foods. Lancet, 1 : 1368-138.

Schwab, A.H., Swartzentruber, A., Wentz, B.A. and Read, R.B. Jr. (1982). Microbiological quality of dry milk mixes and milk substitutes infant formulas. Appl. Environ. Microbiol, 43 : 389-391.

W.H.O. (1995). The treatment of diarrhea. WHO/CDR/ 95.3 WHO, Geneva.