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Coliphage Test: A Quick and Easy Method to Detect Faecal Pollution in Water and Fish

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Coliphages are viruses that infect *Escherichia coli*. Coliphages have been frequently proposed as rapid and inexpensive indicators of faecal pollution. This study describes a simple method termed as coliphage test for the detection of coliphages in water and fish. Coliphage test involves the assay of coliphages by the single agar layer method using nutrient agar medium. The number of plaque forming units was higher on nutrient agar (445 pfu.100ml^{-1}) than on other bacteriological media. The result of coliphage test was obtained within 6 hours. Faecal coliforms and *Escherichia coli* could survive freezing temperatures only upto 6 weeks of storage whereas coliphages resisted freezing temperature and survived even after 10 weeks of storage (maximum period tested). Frozen fish and shrimp from processing plants had low levels of coliphages (0–12 pfu.g^{-1}), faecal coliforms (0-2.5 .g^{-1}) and *E. coli* (0-0.9 .g^{-1}) whereas market samples had high levels of faecal coliforms (140+ .g^{-1}), *E. coli* (140+ .g^{-1}) and coliphages (96-158 pfu.g^{-1}). In the processing plant samples, 7% were positive for *E.coli* and 27% positive for faecal coliforms whereas 47% of the samples were positive for coliphages. The results suggested that coliphages were better indicators of faecal pollution in frozen samples than faecal coliforms and *E.coli* as they survive for a longer period at –20°C. The advantages of the coliphage test were that the test was easy to perform, result was faster (4-6 hours) and the test was less expensive.

Key words: Coliphage test, *Escherichia coli*, faecal pollution

Monitoring the sanitational quality of water and fish is necessary for predicting potential public health hazards. Water and fish are subjected to varying degrees of faecal/sewage pollution and consequently such water and fish become vectors of transmission of pathogenic bacteria (Salmonella, Shigella, Campylobacter, Enteropathogenic *Escherichia coli*, *Yersinia enterocolitica*, etc.) and viruses (Hepatitis A, Rota, Calici, Astro, Norwalk like small round structured viruses and unidentified gastroenteritis viruses).

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Monitoring for specific pathogens is not feasible because there are far too many pathogens and they are present in very small numbers. Moreover, there are still unknown and too many enteric pathogens. Hence, to protect public health it is better to monitor for indicators of faecal and sewage pollution rather than specific pathogens. An indicator is an organism whose presence indicates that pathogens may also be present.

The use of bacteriophages as indicators of enteric viruses and sanitary significant bacteria has attracted increased attention. The ubiquity of bacteriophages in the faeces of man and other animals, in sewage and sewage polluted waters has led to the suggestion that bacteriophages provide a sensitive and reliable index of contamination by entroviruses and enteric bacteria. The council directive of European communities states that water intended for humans consumption should be examined not only for coliform bacteria but also for faecal bacteriophages (EEC, 1980). Coliphages are viruses that infect *Escherichia coli.* Coliphages have been frequently proposed as rapid and inexpensive indicators of faecal pollution in water, meat and fish (Stetler, 1984; Kennedy *et al.*, 1986; Barfield & Huffsey, 1991; Morinigo *et al.*, 1992; Rao & Surendran, 2001). This study describes a simple method termed as Coliphage test for the detection of coliphages in fish and water.

Materials and Methods

Coliphage test involves the assay of coliphages by the single agar layer method using nutrient agar medium. The coliphage test was done as follows. *E. coli* host strain (NCIM 2089) was grown in nutrient broth (beef extract 0.3%, peptone 1%, sodium chloride 0.5%, pH 7.1± 0.1), incubated overnight at 37°C. Five ml of water sample or 5ml of 10^{-1} dilution of fish sample was taken in a sterile test tube. Fish samples were homogenized in Stomacher blender using nutrient broth as diluent. To this test tube, 1ml of host *E. coli* culture was added and mixed thoroughly. Approximately, 8ml of molten and cooled nutrient agar (beef extract 0.3%, peptone 1%, sodium chloride 0.5%, agar 1.5% pH 7.1±0.1) was taken in a separate sterile test tube. The contents of both the test tubes were emptied into sterile petri plate, mixed well and allowed to set. The plates were incubated at 37°C. After 4-6 h of incubation the plates were taken out and the number of plaques was counted. Plaques appear as clear areas on dense *E. coli* mat. The coliphage count was then expressed as number of plaque forming units per gram of fish or per 100 ml of water.

Faecal coliforms and *E. coli* were estimated by the Most Probable Number (MPN) method. Water used in seafood processing plants, water from fish landing centres and water from deep sea were collected in sterile bottles and analysed immediately. Frozen fish and shrimp were procured from seafood processing plants and local markets and were tested for colifoms and coliphage.

Polluted water was collected in sterile bottles and stored at three different temperatures *viz.*, 5°C (refrigeration temperature), ambient temperature (28 to 32°C) and –20°C (frozen storage temperature) in order to study survival of indicators at different temperatures. The bottles were taken out at weekly intervals and examined for coliphages by coliphage test and faecal coliforms and *E. coli* by MPN method.

Results and Discussion

The coliphage test was initially standardized using different bacteriological media. For this, polluted water sample was assayed for the level of coliphages employing coliphage test using different bacteriological media viz., Eosin Methylene Blue agar, MacConkey agar, Nutrient agar, Tergitol-7 agar, Tryptone Glucose agar, Tryptone Soy agar and Violet Red Bile Glucose agar. The number of plaque forming units was higher on nutrient agar (445 pfu.100ml^{-1}) than on other media (Table 1). Moreover, coliphages appeared as clear zones on nutrient agar whereas the zones were masked in coloured media.

Table 1. Coliphage level in water sample using different bacteriological media

Bacteriological Media	Coliphages (pfu.100ml^{-1})
Nutrient Agar	455
Tergitol 7 Agar	430
Tryptone Soy Agar	365
MacConkey Agar	350
Violet Red Bile Glucose Agar	330
Tryptone Glucose Agar	320
Eosin Methylene Blue Agar	275

Survival of coliphages and coliform bacteria at three different temperatures *viz.*, 5°C (refrigeration temperature), ambient temperature (28-32°C) and –20°C (frozen storage temperature) was as summarized in

Table 2. At ambient temperature coliphages and coliforms survived only upto 3 weeks but at 5°C coliphages and coliforms survived even after 10 weeks of storage (maximum period tested). However, marked difference was observed in their survivability at –20°C. Faecal coliforms and *E. coli* could survive frozen storage temperatures only upto 6 weeks of storage. The values of faecal coliforms and *E. coli* were greatly reduced by the 3rd week of storage and finally disappeared after 6th week of storage. On the other hand, coliphages resisted freezing temperature and survived even after 10 weeks of storage (maximum period tested). The level of coliphages in the stored sample after 10th week was 60 pfu.100ml^{-1}. The results suggested that coliphages were better indicators of faecal pollution in frozen samples than faecal coliforms and *E. coli* since coliphages survived for a longer period at –20°C than coliforms and *E. coli*.

Table 2. Survival of coliphages and faecal indicator bacteria at different temperatures

Indicator	5°C	Ambient (28 – 32°C)	-20°C
		Period of survival	
Coliphages	> 10 weeks	3 weeks	> 10 weeks
MPN Faecal Coliforms	> 10 weeks	3 weeks	6 weeks
MPN *E.coli*	> 10 weeks	3 weeks	6 weeks

Frozen fish and shrimp were analysed for coliphages using coliphage test and the results presented in Table 3. Processing plant samples had low levels of coliphages (0–12 pfu.g^{-1}), faecal coliforms (0-2.5 g^{-1}) and *E. coli* (0-0.9.g^{-1}) whereas market samples had high levels faecal coliforms (140+ g^{-1}), *E.coli* (140+ .g^{-1}) and coliphages (96-158 pfu.g^{-1}). All the processing plant samples had *E. coli* levels within acceptable limit of 20.g^{-1} (Govt. of

Table 3. Coliphages and coliforms in frozen fish and shrimp

Sample	Source	No. of samples	Indicator	No. positive	% positive	Range
IQF shrimp/ frozen fish	Processing plants	15	Coliphage	7	47	0-12 pfu.g^{-1}
			Faecal coliforms	4	27	0-2.5.g^{-1}
			E. coli	1	7	0-0.9/g
Frozen fish	Local markets	4	Coliphage	4	100	96-158 pfu.g^{-1}
			Faecal coliforms	4	100	45-140+ .g^{-1}
			E. coli	4	100	45-140+ .g^{-1}

India, 1995) while none of the frozen fish purchased from local markets met the stipulated quality requirement. The results show that coliphages survive freezing temperatures for a longer time than faecal coliforms and *E. coli*. Out of the 15 samples from processing plants, 7% were positive for *E. coli* and 27% positive for faecal coliforms whereas 47% of the samples were positive for coliphages. Coliphages were reported to be relatively more resistant to chlorination than coliforms (Muniesa, 1999). The higher incidence of coliphages may be attributed to their relatively higher resistance to freezing and chlorination.

Table 4. Coliphages and Coliforms in water samples

Sample source	Coliphages Range	Faecal coliforms	E.coli
Processing Plants	0	0	0
Landing centre	160	180+	180+
Deep Sea	0	0-4	0
Littoral zone	0-30	0-250	0-4.5

Water used for seafood processing was found to be of good quality as indicated by the absence of coliphages, faecal coliforms and *E.coli* (Table 4). Water collected from fish landing centre was of poor quality with high levels of coliphages (160pfu/100ml), faecal coliforms (180+/100ml) and *E.coli* (180+/100ml). Coliphages and *E.coli* were not detected in deep-sea waters but one sample had low level of faecal coliforms (4/100ml). In water samples coliphages were absent when *E.coli* was absent and present when *E.coli* was present. The advantage with the coliphage test was that the result was obtained on the same day within 4-6 hours whereas other indicator bacteria required longer periods for confirmation, viz., total coliforms by MPN (2 days), faecal coliforms by MPN (3days), *E.coli* by MPN (3 days), faecal streptococci by MPN (2days) and sulphite reducing clostridia (4 days). The coliphage test was a simple test that can be carried out with little technical expertise and minimum laboratory facility. Since the coliphage test requires very little inputs (requires only *E.coli* host culture, nutrient agar and nutrient broth) the cost of the test was far less than the conventional coliform test.

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