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Prevalence and Antibiotic sensitivity of *Escherichia coli* in Extensive Brackish water Aquaculture Ponds

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Prevalence and antibiotic resistance of *Escherichia coli* in the water and sediment samples of brackish water aquaculture ponds adjacent to Cochin backwaters was analysed. More than 50% of the water samples and more than 80% of sediment samples from all the sampling stations were tested positive for *E. coli*. Risk assessment of the *E. coli* strains was carried out using multiple antibiotic resistance (MAR) indexing. Majority of the strains were found to be multiple antibiotic resistant suggesting their origin from high risk sources of contamination such as human where antibiotics are frequently used. While none of the *E. coli* strains were resistant against amikacin, chloramphenicol, streptomycin and trimethoprim, considerable levels of resistance was encountered against ampicillin, erythromycin, penicillin G and vancomycin. High prevalence of *E. coli* in the water and sediment samples of this extensive brackish water ponds indicates high degree of faecal pollution of this environment. The high risk nature of the strains warrants efficient post harvest and processing measures to avoid health risk to consumers.

Key words: ***Escherichia coli*, Aquaculture pond, Antiobiotics, MAR index**

Faecal pollution in fish growing waters poses a potential health hazard if such fish were consumed without adequate processing. The entry of pathogenic agents into the water intended for recreation, irrigation and aquaculture can become a serious human health risk or economic disaster in agriculture (Geldreich, 1996). Moreover, it is also demonstrated that the health risks associated with the consumption of fish grown under uncontrolled conditions is severe than fish grown in controlled conditions (Hejkal *et al.*, 1983). Faecal contamination entails the risk of spreading the bacterial, viral or pathogenic agents of communicable enteric diseases. It is impractical and unnecessary for routine testing for enteric pathogens, as most of them are greatly out numbered by non-pathogenic faecal flora. However, the use of marker organisms, as an index for the possible presence of enteric pathogens, provides a reliable alternative to tedious and unreliable monitoring procedures for specific pathogenic organisms. In food microbiology as well as in water microbiology, *Escherichia coli* is considered primarily as an index organism. Since *E. coli* is a typical faecal coliform having an excellent correlation with faecal contamination, it would be logical to project a positive correlation between water borne pathogens and increasing densities of faecal coliforms in the same water body (Geldriech, 1990).

It has been demonstrated repeatedly that the indiscriminate usage of antibacterial drugs to treat human/veterinary diseases or their presence in sewage and treated water has resulted in an increase in populations of antibiotic resistant bacteria as well as resistant plasmids in water. (Amstrong *et al.*, 1982). The occurrence of multiple antibiotic resistance among the enteric bacterial species in fish as well as water may make effluent from aquaculture systems of public health significance. If the resistance is plasmid

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mediated, as has been found commonly in fish pathogens, then there could be a problem associated with transfer of resistance to other organisms of human/veterinary significance (Toranzo *et al.*, 1983).

The present study was undertaken to evaluate the associated health risks before handlers and consumers of cultured fish/shellfish, in relation to the microbiological quality of the environment during extensive aquaculture practices.

Materials and Methods

Brackishwater shrimp farms located at Vypeen area adjoining the Vembanadu Lake (9°28′ & 10°10′ N Lat., 78°13′ & 76°31′ E Long.), having 6ha, 9ha and 16ha of water spread area respectively were selected for the study. Collection of samples was done on a monthly basis for one culture operation (December 2000 – May 2001). Water samples were collected in sterile conical flasks from a depth of 0.5 m from the surface and sediment samples were aseptically scooped out from the bottom and transferred into sterile polythene bags. All the samples were placed in an ice chest and transported to the laboratory within four hours for further analysis.

The microbiological quality of the samples were analyzed in terms of total heterotropic bacterial (THB) load and most probable number (MPN) of coliforms. Pour plate technique using Marine agar (Zobell 2216e, Hi Media) was employed for the estimation of THB. After incubation at room temperature for 48 – 72 hours, plates with colonies ranging from 30- 300 numbers were selected for counting and the bacterial population was expressed as number of colony forming units (cfu) per ml or g, water or sediment respectively. MPN of total coliforms was determined using lactose broth and incubation at 37°C. Tubes showing positive results after 24 - 48 h of incubation were streaked on to MacConkey agar and eosine methylene blue (EMB) agar and incubated at 37°C for 24 - 48 h. Typical *E. coli* like colonies were isolated, purified maintained on nutrient agar slants and confirmed biochemically as *E. coli* using indole, methyl red, voges-proskauer and citrate (IMViC) test.

Confirmed *E. coli* isolates were screened for resistance towards the following antibiotics by standard disc assay technique (Bauer *et al.*, 1966): Amikacin (30 mcg), Ampicillin (10 mcg), Chloramphenicol (30 mcg), Ciprofloxacin (30 mcg), Erythromycin (10 mcg), Gentamycin (10 mcg), Nalidixic acid (30 mcg), Oxytetracycline (30 mcg), Penicillin G (10 mcg), Streptomycin (10 mcg), Trimethoprim (30 mcg), and Vancomycin (10mcg). Young cultures of the isolates were swabbed on to Muellor-Hinton agar plates. After 30 min of pre-diffusion time, the antibiotic discs (Hi media) were transferred on to the plates. The plates were incubated at 37°C overnight. After incubation the inhibition zones were measured and the behavior of each strain towards individual antibiotic disc was interpreted using Kirby-Bauer interpretive chart. The strains were classified as resistant or sensitive, with the intermediate strains being included in the resistant class.

The potential risks posed by the isolates were assessed using multiple antibiotic resistance (MAR) indexing, calculated by dividing the number of antibiotics to which the isolate is resistant to the total number of antibiotics to which the isolate is exposed. Strains with MAR value higher than 0.2 were considered to have originated from high-risk sources of contamination.

Results and Discussion

The monthly variations in the bacteriological parameters analyzed in water and sediment samples collected from different sampling stations are presented in table 1. The mean THB load of the water was 7.36 x 10^4 cfu/ml, while sediment samples revealed a mean THB load one log greater than that of water. i.e. 7.15 x 10^5 cfu/g. Sugita *et al.*, (1982) reported similar levels of THB

Table 1. Monthly variations in the bacteriological parameters (THB and MPN index of coliforms) in the environmental samples from the three sampling stations.

			December	January	February	March	April	May
Sampling Station .1	Water	THB (cfu/ml)	$7.8x10^4$	$6.7x10^4$	$6.2x10^4$	$6.3x10^4$	$6.6x10^4$	$6.9x10^4$
		MPN Index/100 ml	430	2400	930	11000	4600	11000
	Sediment	THB (cfu/g)	$6.6x10^5$	$7.6x10^5$	$7.9x10^5$	$7.8x10^5$	$7.1x10^5$	$7.4x10^5$
		MPN Index/100 g	11000	11000	11000	11000	11000	11000
Sampling Station .2	Water	THB (cfu/ml)	$9.6x10^4$	$8.8x10^4$	$9.2x10^4$	$9.7x10^4$	$9.4x10^4$	$9.9x10^4$
		MPN Index/100 ml	4600	4600	430	11000	4600	11000
	Sediment	THB (cfu/g)	$7.1x10^5$	$7.9x10^5$	$7.4x10^5$	$7.9x10^5$	$7.3x10^5$	$8.1x10^5$
		MPN Index/100 g	11000	11000	11000	11000	11000	11000
Sampling Station .3	Water	THB (cfu/ml)	$5.4x10^4$	$4.8x10^4$	$5.7x10^4$	$6.2x10^4$	$5.9x10^4$	$6.4x10^4$
		MPN Index/100 ml	430	4600	4600	11000	4600	11000
	Sediment	THB (cfu/g)	$5.9x10^5$	$5.7x10^5$	$6.1x10^5$	$7.1x10^5$	$6.8x10^5$	$7.3x10^5$
		MPN Index/100 g	11000	11000	11000	11000	11000	11000

populations of around 10^3 to 10^5 in water samples collected from shellfish-growing areas. However, the obtained THB loads in sediment samples were greater than the results of the present study. The total viable heterotrophic bacteria (THB) in an environment depends on the availability of growth supporting organic matter and micronutrients. Heavy organic load and nutrient accumulation are possible in the study area since the farms are connected to the Vembanadu Lake through a net work of feeder canals and frequently affected by the tidal cycles.

As can be seen in the results, all the samples revealed relatively high levels of total coliforms both in water and sediment. The minimum value obtained was 430 MPN/100ml. The percentage of incidence of *E. coli* in water and sediment samples from different sampling stations are given in table 2. *E. coli* was detected consistently in all the samples at considerable levels. *E.coli* is primarily considered as an indicator organism. They offer highest reliability as indicators of pathogens in waters with high levels of faecal pollution. The suitability and efficiency of coliform bacteria to determine the quality of shellfish-growing waters has been examined earlier by Martinez *et al.*, (1992). The results of the study suggests that the percentage of detection of pathogens, *Salmonella, Vibrio parahaemolyticus* and *Staphylococcus aureus* are best indicated by total and faecal coliforms at concentration levels less than 700 MPN/100 ml and 100 MPN/100 ml of water respectively. According to the United States microbiological criteria to classify the shellfish-growing water quality, the total coliforms levels should not exceed 396 MPN/100ml (U.S Public Health Service., 1965). Geldreich (1996) suggested that water quality indicators should be compared with pathogens on a more realistic scale by increasing the sample volume size examined for surrogate occurrence.

Table 2. Percentage of incidence of *Escherichia coli* in water and sediment from the three sampling stations. (N* = 6).

	Percentage of incidence of *E. coli* in	
Sampling stations	Water	Sediment
Sampling station 1	50	83
Sampling station 2	83	100
Sampling station 3	67	83

* N = Number of water and sediment samples analyzed from each station

Table 3 represents the results of antimicrobial susceptibility tests and the percentage of incidence of *E. coli* strains, which revealed different MAR values. All the strains were found to have acquired multiple antibiotic resistance. Cent percent resistance was

Table 3. Percentage of antibiotic resistance of *Escherichia coli* isolates and the MAR indices encountered.

Name of the antibiotic	Percentage of resistance (N* = 47)	MAR indices encountered
Amikacin	0	0.25 (23)**
Ampicillin	55	
Chloramphenicol	0	
Ciprofloxacin	15	0.33 (40)
Erythromycin	100	
Gentamycin	9	
Nalidixic acid	19	0.42 (24)
Oxytetracycline	27	
Penicillin G	100	
Streptomycin	0	0.5 (13)
Trimethoprim	0	
Vancomycin	100	

* N = Total number of isolates.
** Figures in the parenthesis indicates percentage of isolates with given MAR index.

recorded against erythromycin, penicillin G and vancomycin. Least resistance (less than 20%) was noticed towards ciprofloxacin, gentamycin and nalidixic acid. The bacterial isolates also showed considerable levels of resistance against ampicillin (55%) and oxytetracycline (27%). However in the present study, amikacin, chloramphenicol, strepyomycin and trimethoprim proved to be the best antibiotics to treat *E. coli* infections since they were fully sensitive. The results compared favorably with those reported by Hatha *et al.*, (1999) who also obtained similar resistance patterns of *E. coli* strains isolated from river water. *E. coli* coexist with other human pathogenic strains such as salmonellae and shigellae in natural environment, where free exchange of R-plasmids can take place between them, leading to serious health risks. In the case of transmission of resistant bacteria from farmed animals to man, the rate of transmission is affected by other factors including: how the animals are produced; how they are slaughtered; how the food is manufactured; how the meal is prepared; how frequently the foodstuff is consumed; and by whom (Henrik *et al.*, 2000). In aquaculture operations, the antibiotics are presumed to enter the farming system either through drugs used for prophylactic measures or as incorporated into feed (Twiddy & Reilly, 1995).

As indicated in table 3, the MAR indices encountered varied between 0.25 and 0.5. About half of the isolates revealed a MAR value of 0.42. This is in accordance with the results obtained by Gomathinayagam *et al.*, (1994) who reported an increased prevalence of MAR *E. coli* with similar MAR value in river water and sediment samples. Strains with MAR value higher than 0.2 were considered to have originated from high-risk sources of contamination. In the present study all the isolates revealed MAR values higher than 0.2. Thus it is obvious that the study area is exposed to frequent contamination with faecal bacteria of high-risk sources. The incidence of higher number of MAR *E. coli* and its resistance to more number of antibiotics revealed the quality and unsuitability of the study area for fish farming. Moreover the present finding stresses the need for better post-harvest techniques for cultured fish/shellfish grown under extensive aquaculture operations.

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