MUSSEL POLLUTION AT KORAPUZHA ESTUARY (MALABAR), WITH AN ACCOUNT OF CERTAIN COLIFORM TYPES*

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Introduction

Oysters, mussels and other shell-fish are highly nutritious foods, which, apart from their usual proximate principles, contain fairly high amounts of minerals like iodine, copper, iron, etc., and vitamins. In the shallow rocky areas of Malabar Coast there are numerous green mussel (*Mytilus edulis*) beds from which large quantities of mussels are taken out almost throughout the year, which form an important item of food for people of low incomes. As the mussels grow in shallow areas subject to the influence of land drainage, sewage and river systems, these beds constitute a potential hazard to public health on account of possible epidemic infections being carried by the shell-fish.

Outbreaks of innumerable cases of enteric fever and other epidemics traceable to polluted shell-fish, have been cited and reviewed in great detail by Tanner (1944) and Shraeder (1952). It is known that pathogens, especially when present in large numbers, survive freezing and cooking. Cooking, unless it is vigorous, may not always kill *B. coli* and it is impossible to remove *B. coli* from the body of the polluted oysters by washing (Hunter and Harrison, 1928). Albuquerque and Bhat (1953) have shown that the Bombay-duck, infested with any of the intestinal pathogens, would constitute a grave danger to human health since the latter survive certain culinary processes. When the mussel beds are located in shallow areas and are grossly polluted by fæcal organisms, the latter constitute a danger to the consumers.

In many villages and small towns there are no organised systems of sanitation and hence the refuse is not always discharged into the sea. During the rainy days the stormwater carries with it the town refuse and during the South-West monsoon this is the main source of pollution.

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Hydrography of the Area

The mussel beds studied are in Elathur, 7 miles north of Calicut. The coast is shallow with rocky projections on which the green mussels grow in abundance. The depth of water above the bed is about 6 to 8 feet at low tide, when they are fished for. The beds are located about 2 to 4 furlongs from the shore and the river Korapuzha empties into the sea at Elathur.

Material and Methods

" Overlaying water " was collected in sterile bottles and simultaneously other samples were collected for estimating dissolved gases, salinity, pH, etc., which were determined by standard methods. Bacteriological examination of the water and mussels was made according to the methods of the American Public Health Association (1943) with the modification that for dilutions 50% sea-water was used. The most probable numbers (M.P.N.) of coliforms was calculated by Hoskin's method (Presscott *et al.*, 1947). Tests for cholera vibrios was done as described by Thresh, Beale and Suckling (1949) and by direct plating on desoxycholate citrate agar of pH 8·6. For the isolation of enteric pathogens the water or shell-fish were enriched in tetrathionate broth for 18 to 24 hours at 37° C. and a loopful streaked on Bismuth sulfite agar, S.S. agar, and MacConkey's agar. Suspicious non-lactose fermenting colonies were subcultured and studied in detail. The coliform types isolated on E.M.B. agar and V.R.B. agar were also studied in detail (Wilson, 1935).

Four periods were chosen to represent the different seasons. January represented the colder month with very low rainfall (December, January, February), April represented the period of summer (March, April, May) with increasing temperature and occasional pre-monsoon showers but rainfall more than during the previous period, July represented the peak South-West monsoon period (June, July, August) when more than two-thirds of the rainfall of the year occurred, and October represented the post South-West monsoon period (September, October, November) of lull and advent of North-East monsoon.

Results and Discussion

The results are summarized in Table I. There is a parallelism in the coliform numbers of 'overlaying water', mussels from bed, and mussels from the market. The numbers are low in January, but increase slightly in April, reach phenomenal figures in July immediately after the South-West monsoon, and decrease to the lowest level in October. The total bacterial counts are the lowest in October, but highest in July. Normally counts on

TABLE I

Showing the Hydrological Conditions and Bacterial Quality of Shell-Fish Waters and Shell-Fish at Elathur

Period	Temperature °C.		Salinity S‰	pH	Overlaying water				Mussels from bed				Mussels from market			
					Total counts per ml. on		MPN of coli-forms per 100 ml.	Ratio of coliforms to total counts	Total counts per ml. on		MPN of coliforms per 100 ml.	Ratio of coliforms to total counts	Total counts per ml. on		MPN of coliforms per 100 ml.	Ratio of coliforms to total counts
	Air	Water			Sea-water agar	Fresh-water agar			Sea-water agar	Fresh-water agar			Sea-water agar	Fresh-water agar		
January 1953	28·5	29·0	33·50	8·5	310	230	33	1 : 940	8200	1020	540	1 : 1517	6200	3300	1700	1 : 365
April 1953	30·0	30·5	34·98	8·4	390	50	49	1 : 790	18800	13800	79	1 : 23770	12900	5400	2400	1 : 538
July 1953	25·5	27·0	9·09	8·1	3800	7700	1100	1 : 345	150000	Spreaders	160000	1 : 94	3900	3100	160000	1 : 24
October 1953	27·0	29·5	31·93	8·2	190	35	12	1 : 1583	1880	1350	280	1 : 671	8600	960	540	1 : 592

sea-water agar are greater than on fresh-water agar because of the preponderance of 'marine' forms.

Effect of seasons on the bacterial quality of shell-fish and water.—A comparison of the data on rainfall given in Table II with the bacterial numbers in Table I indicates clearly the effect of various seasons on the quality of

TABLE II

Showing the Rainfall and Temperature

(Data kindly supplied by the Marine Biological Station of the Madras Fisheries Department, West Hill)

Months	Rainfall	Temperature ° F.	
	Inches	Maximum	Minimum
1952			
October	12·12	85·0	71·0
November	0·48	90	83
December	1·48	96	82
1953			
January	0·00	86	67
February	1·69	85	67
March	0·24	90	73
April	4·64	88	70
May	2·03	87	75
June	12·61	86	72
July	40·42	83	77
August	7·63	82	72
September	2·29	84	72
October	9·52	85	73
November	0·63	86	67
December	nil	86	62

shell-fish and waters. In the case of water and market mussels a slight increase in total counts and coliform counts was noticed from January through April, with the advent of pre-monsoon showers and with higher day temperatures. During the rainy South-West monsoon period there was a conspicuous increase in total counts as well as in coliform counts. This is clearly due to the influx of a large quantity of river water bringing down with it a lot of organic matter and town refuse, as is evidenced by the marked fall in salinity from 34·98‰ in April to just 9·09‰ in July in the overlaying water. Gray (1951) has also strongly suggested that the increase in bacterial counts in a stream was due to higher rainfall which washed down soil organisms (Topley and Wilson, 1948, p. 2018).

Significance of coliform counts.—The U.S. Public Health Service lays down that samples of water from 'approved' areas shall not show an M.P.N. of coliforms in excess of 70 per 100 ml., while 'moderately' polluted areas (restricted areas) shall not contain more than 700 coliforms per 100 ml. In excess of this number, the area was classified as 'grossly polluted'. On the basis of this the shell-fish waters may be considered to be in the approved area in all months except during the South-West monsoon, when it is 'grossly' polluted. The same is true of mussel beds. But with market mussels an entirely different picture is obtained. The U.S. Public Health Service considers that for oysters an M.P.N. of 230 or more of coliforms per 100 ml. and for other shell-fish 2,400 or more, should be considered unfavourable. On this basis the market mussels collected from the various centres in and around Calicut during the period, April to July, should be considered as grossly polluted. More detailed investigation is needed, however, to say whether any legislation is to be recommended. Hunter and Harrison (1928) prescribed that the *B. coli* 'score' should be less than 50 before marketing, while Shraeder (1952) stated that a 'score' of above 50 was considered to be indicative of dangerous pollution, warranting condemnation of the product. In market mussels the 'score' was 50 and above at all periods except in October, thus indicating undesirable contamination.

Significance of coliform types.—*E. coli* type I (IMViC $+ + - -$) was present throughout the year in the 'overlaying' water and at most of the periods in the mussels. Hunter (1939) emphasized that the *E. coli*, which are IMViC $+ + - -$, are of direct or indirect fæcal origin and their presence in a food may be accepted as a substantive evidence of excremental pollution. Sherwood (1952) also considered *B. coli* type I to be a criterion of pollution. Perry (1939) considers *E. coli* to be the logical indicator of fæcal pollution in shell-fish and shell-fish waters. The unfavourable condition in the mussels is thus self-evident.

Epidemiological significance.—A number of suspicious colonies were isolated by methods enumerated earlier. These consisted of non-lactose fermenting organisms, some of which produced acid and gas from dextrose and other sugars, and the others produced only acid. The ærogenic organisms belonged to the genera *Paracolobactrum* and *Proteus*. There has been indisputable evidence about the pathogenic nature of paracolon strains

TABLE III

Showing the Coliform Types isolated from Shell-Fish Water and Mussels

Period	Water	Bed mussels	Market mussels
1953			
January ..	*E. coli* type I	*E. coli* type I *A. cloacæ*	*E. coli* type I *A. cloacæ*
	E. coli type II	*A. ærogenes* types I and II	*A. ærogenes* types I and II
April ..	*E. coli* type I *A. cloacæ* *A. ærogenes*	*E. coli* types I and II *A. cloacæ*	*E. coli* type II *A. cloacæ* *A. ærogenes* type I
July ..	*E. coli* types I and II *A. ærogenes* type I	*E. coli* type I *A. cloacæ* *A. ærogenes* type II	*E. coli* types I and II *A. ærogenes* type II
October ..	*E. coli* type I *A. cloacæ*	*A. ærogenes*	*E. coli* types I and II *A. ærogenes* *E. freundii*

(Christensen, 1947; Griffin and Sniezsko, 1950; Hinshaw and McNeil, 1946; Stuart *et al.*, 1938; Stuart *et al.*, 1940). We have isolated a few of these slow and non-lactose-fermenting paracolons from mussels. Parr (1939) has adduced enough authoritative evidence to show that coliform organisms are responsible for causing infectious diarrhœa, enteritis, etc., and that they are secondary invaders complicating infections. Dack (1953) has recently reviewed that *E. coli* are capable of causing food poisoning and that they cause infantile diarrhœa. Recently a great deal of evidence has come forth about the presence in paracolons, of antigens that agglutinate *Salmonella* and *Shigella* (Young, 1945; Ferguson and Wheeler, 1945). Agglutination tests done at the King Institute, Guindy, did not, however, reveal any organism belonging to the *Salmonella-Shigella* group. *Proteus*, Paracoli and certain *E. coli* strains are suspected ætiological agents of infectious diarrhœa and enteritis (Rubbo, 1952) and hence the presence of these in mussels indicates possible adverse conditions in the taking and marketing of mussels.

Presence of cholera vibrios was not demonstrated at any time during the period of investigation, but this coincides with a period when there was no epidemic outbreak of cholera in this part of the State.

TABLE IV

Fermentative Types of Coliform Bacteria Isolated from Marine Environment

Number of cultures	IMViC characters	Sucrose	Salicin	Dulcitol	Adonitol	Glycerol	Gelatin lique-faction	Motility	
6	++−−	AG	AG	AG	−		−	−	*Escherichia neapolitana* (Emmerich)*
5		AG	AG	AG	− (1 positive)		−	+	*E. Communior* (Durham)*
2		AG	AG	−	−		−	−	*Bacterium neapolitanum* (Winslow *et al.*)†
2		−	AG	−	−		+ (slow)	−	*E. colitropicalis* (Castellani)*
2		AG	AG	−	AG		+	+	*E. Pseudocoloides* (Castellani)*
3		AG	−	AG	AG		−	+	
1		−	AG	−	−		−	+	
6		AG	−	AG	−		−	−	*Bact. coscoroba* (Winslow *et al.*)†
2		−	−	−	AG		−	−	*Bact. Acidi-laetici* (Winslow *et al.*)†
2		AG	−	−	−		−	±	*E. Pseudocoscoroba* ((Castellani and Chalmers)*
2	−+−−	AG	AG	−	−		±	±	

1		AG	A	AG	AG		–	–	
4		AG	–	AG	–		– (1 positive slow)	+ (1 non-motile)	*Bact. colicommunius* (Winslow *et al.*)†
2		–	AG	–	–		–	+	
13	––++			–	–	–	+	+	Aerobacter
2				–	–	AG	+	+	
5				–	AG	AG	–	– (1 positive)	
3				–	AG	AG	+	+ (1 negative)	
1				–	AG	–	+	+	
3				AG	–	AG	–	–	
4				AG	–	– (1 positive)	+	+	
1				AG	AG	–	+	+	
7				AG	AG	–	– (1 positive)	– (1 positive)	
4	+–++			AG	AG	AG	+	+	Aerobacter
1				AG	AG	AG	–	–	
1				–	–	–	+	+	

* = Nicholls and Saltz (1936).
† = Topley and Wilson (1948), p. 666.
AG = Acid and gas.
A = Acid alone.
– = Not fermented (sugars).
+ = Not liquefied (gelatin).

Fermentative reactions of coliform types.—In Table IV are given the fermentative reactions of the coliform organisms. The following salient features may be noted: (1) In the genus *Escherichia*, (IMViC + + — — and — + — —) gelatin liquefaction is not pronounced, but is either negative or slow, while motility is fairly universal, though the two are not always correlated. (2) A very large number of *Escherichia* ferment sucrose (33 out of 40) the inverse correlation of sucrose and salicin fermentation was irregular, many of them fermenting both. (3) Very few fermented adonitol, though dulcitol was fermented by greater number of cultures. (4) In regard to the *Aerobacter*, there is a very high degree of correlation between gelatin liquefaction and motility. (5) The inverse correlation of gelatin liquefaction and glycerol fermentation is not a rule, but is fairly common in *Aerobacter*. (6) Dulicitol and adonitol fermentation was variable and not correlated with other characters. (7) Sucrose, salicin, sorbitol, dextrin and riffinose were fermented by all *Aerobacter*. (8) All *Escherichia* fermented glycerol and dextrin.

Fermentation of sucrose by such a large number of Escherichi is interesting, as members of this genus rarely ferment sucrose. Clemesha (1912) showed that the proportion of saccharose fermenters to non-saccharose fermenters was 1 : 2½ and considered that " this increase in the non-saccharose fermenters in India. . . .emphasizes the difference between the two parts of the World. . . . ." Parr (1939) considers the sucrose fermenting *E. coli* to be *E. coli communior*. Though Parr (1938) suggests that *A. cloacæ* demand taxonomic difference with *A. ærogenes*, Griffin and Stuart (1940) feel that the separation of *Aerobacter* into *ærogenes* and *cloacæ* is of questionable value, since there is lack of correlation between glycerol fermentation, gelatin liquefaction and motility. As recorded above we found a high degree of correlation between gelatin liquefaction and motility, but equally good correlation between gelatin liquefaction and glycerol fermentation was lacking. Stuart, Griffin and Baker (1938) suggest that coliforms could be divided only into three sections, *viz.*, *Aerobacter*, Intermediate and *Escherichia*, and this appears to be reasonable unless sufficient evidence is let in on the basis of permanent non-variable characteristics to further split up *Aerobacter* and *Escherichia*. Fermentation of sugars is not of much taxonomical value in the separation of the coliform group into different species due to absence of a set pattern as could be seen from the table.

SUMMARY AND CONCLUSIONS

The mussel beds in shallow coastal areas are continually polluted and show the presence of *E. coli* type I, throughout the year. The peak of pollution is attained immediately following the advent of South-West monsoon. After South-West monsoon abates and till summer, there appears to be a 'recovery' and self-purification and the coliform numbers, as well as the total counts, are low. *Salmonella-Shigella* group as well as cholera vibrios were absent, but *Paracoli, Proteus* and *E. coli* were present, which themselves have epidemiological significance as causative agents of infectious gastro-enteritis.

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