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STUDIES ON BACTERIAL FLORA OF TRIVANDRUM COASTAL WATER*

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ABSTRACT

The bacterial flora of coastal water from Trivandrum, was investigated to understand the rate of pollution by human pathogens. A total of 43 bacterial strains were isolated from 13 samples of water. Out of total isolates, 22 were obtained from Shankumughom Beach, 11 from Kovalam and 3 from Vizhinjam. The morphological and biochemical features of all strains were studied. Most of the isolates belong to *Pseudomonas* spp. (37.2%), Coliforms (37.2%) and *Aeromoas* spp. (20.9%). All the isolates were nonpigment producers except *Pseudomonas* sp. *Streptococcus faecalis* and *Vibrio* strains (4.6%) were encountered in two samples. Maximum isolates, including members of Enterobacteriaceae were isolated from Shankumughom Beach water. 67.4% of the isolates were motile and 51.2% were highly fermentative. The antibiotic sensitivity pattern against 10 different antibiotics showed 34% average resistant rate for marine bacteria. The danger of the emergence of resistant strains due to repeated use of antibiotics, use of sublethal doses of antibiotics and the potential role of transfer factor (R+ factor) and its significance in fishery resources are discussed.

INTRODUCTION

BACTERIA in the marine environment at the sediment-water interface have been studied amply. Most of these studies were on the generic composition and percentage occurrence, the nature of organic substances, their origin and degradation by bacteria, seasonal variations in quantity and quality (Murchelano and Brown, 1970; Altschuter and Riley, 1967; Zo Bell, 1946; Wood, 1953; Simidu *et al.*, 1980; Venkataraman and Sreenivasan, 1957; Velankar, 1960; Gore, 1971) and methods for estimating phosphatase-producing bacteria in sediment-water interface, biochemical micromethods based on direct enzymatic reactions (Ayyakkannu and Chandramohan, 1970). Isolation of pure cultures from active sediment and water and distribution of bacteria has been shown to depend on the nutrients of sediment-water interface (Karthiayani and Mahadeva Iyer, 1975; Chandrika, 1983).

This paper describes the biochemical characters and characterisation of marine bacteria isolated from surf waters in the sediment-water interface. Apart from characterisation of marine bacteria isolated from surf water, the distribution of antibiotic-resistant bacteria in the coastal area of Shankumughom, Vizhinjam and Kovalam has been found out as the preliminary study of antibiotic pattern will be of help in future research for detection of R^+-factors and their transfer in Enterobacteriaceae.

MATERIAL AND METHODS

The bacterial flora of 13 surf-water samples from the three beaches *viz.*, Vizhinjam, Kovalam and Shankumughom were analysed according to standard methods. 27 strains were isolated from 6 water samples from Shanku-

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mughom. 12 strains from 5 water samples from the Kovalam Coast and 4 isolates from 2 water samples collected from Vizhinjam Beach. The results of bio-chemical investigations indicated the existence of 5 genera of marine flora according to the Scheme of Shewan (1960).

Marine bacteria isolated from littoral waters (43 nos.) were emulsified in nutrient broth. Inoculation of plates was done within 15 minutes of emulsifying broth culture so that standardisation remains correct. The emulsified suspension is streaked evenly in 3 directions on the surface of the medium. The petri-dish was kept aside for drying for at least 5 minutes, but not longer than 30 minutes. Appropriate antibiotic disc was dispensed manually on the inoculated plate and gently pressed it down with sterile forceps flamed and cooled between each disc. Discs were placed 10-15 mm from each other to avoid overlapping of zones.

Results and Discussion

All the strains were catalase positive except 2 *Aeromonas* and one *Enterobactor* strains. Almost all the results were clearly positive or negative. Table 1 gives the types of bacterial genera isolated from the beach water samples. All the five bacterial genera namely *Aeromonas, Pseudomonas, Vibrio, Paracolons/ E. coli* and *Alcaligenes* were present in Shankumughom Beach water. *Vibrio, Aeromonas* and *Pseudomonas* were not encountered in Vizhinjam Beach whereas *E. coli/Paracolons* were comparatively more than the other two beaches. *Vibrio* spp. were not encountered in Kovalam Beach and only 2 strains were isolated from Shankumughom waters. This was supported by the investigation by Karthiayani and Mahadeva Iyer (1975) who reported the paucity of *Vibrio* spp. in surface water and bottom mud whereas percentage of this genera on fresh sardines and prawns seems to be fairly high. *Streptococcus faecalis* was isolated in Kovalam and Shankumughom Beach waters. Absence of Gram positive *Micrococcus* in all the 13 sea water samples was a striking observation. Gram-positive organisms were prevalent in the bottom mud and their paucity was reported by Karthiayani and Mahadeva Iyer (1975) in surface waters. Altogether 43 bacterial strains were isolated from 13 random samples of beach water and maximum isolates were from Shankumughom Beach water. Table 3 shows the generic composition of bacteria. Table 3

Table 1. *Bacterial genera isolated from the beach water near Trivandrum*

Place of collection	No. of samples	*Aeromonas*	*Pseudomonas* (No. 4%)	*Vibrio* (No. 4%)	*Paracolons/ E. coli* (No. 6)	*Alcaligenes*	Total isolates
Shankumughom Beach	6	10	6	2	2	7	27
Vizhinjam Coast ..	2	0	0	0	3	1	4
Kovalam Beach ..	5	5	2	0	2	3	12
Total ..	13	15	8	2	7	11	43

Plates were incubated immediately or within 30 minutes for overnight at 35° - 37° C. Best results were obtained after 18 hours of incubation. Zone of inhibition equal to size of disc or even slightly greater *e.g.* 6.5 - 7·0 mm was taken as negative and zone of inhibition more than 12 - 15 mm was taken as positive.

A total of 43 strains of bacteria isolated from marine environment were screened for resistance to selected antibiotics.

gives the biochemical characters of selected isolates.

TABLE 2. *Generic composition of marine bacteria isolated from littoral waters of Trivandrum*

Identified strains	No. of strains	Percentage
Pseudomonas	8	19
Alcaligenes	10	23
Vibrio	2	5
Aeromonas	15	38
E. coli	7	16
Unidentified	1	2

Table 4 shows reports of marine bacterial genera isolated by various investigators in different parts of the world. A perusal of the Table shows that *Pseudomonas* predominates North Sea and is completely absent in N. Cape Norway and occurs in all the other parts in lesser quantities. *Aeromonas* was encountered only by Simidu *et al.* (1971) in inshore waters of Japan. *Vibrio* dominated in Chesapeake Bay, but absent in Australian waters. Enterobacteriaceae (*E. coli*) were found in inshore waters of Japan. *Alcaligenes* was encountered in all parts of the area, but encountered in moderate numbers.

Table 4 incorporates also reports of bacterial genera from Calicut, Cochin and Trivandrum Coastal waters. In the present study *Pseudomonas* occurred in all the 3 places whereas *Aeromonas* was recorded only in Trivandrum coastal waters. *Vibrio* and *E. coli* were dominant in the coastal waters of Cochin. *Alcaligenes* occurred in moderate numbers in Calicut, Cochin and Trivandrum surf waters.

From the results, it was noted that there is not much differentiation in the genera of bacteria in different parts of the sea though they differ much in biochemical characters. All the 5 genera isolated from Trivandrum coastal water occurred only in moderate numbers in other parts of the world. Much differentiation was not observed regarding biochemical characters of marine bacteria except that marine bacteria were more sensitive to antibiotics than human bacteria. The abundance of *Aeromonas*, *Pseudomonas*, *Para-*

TABLE 3. *Results of 30 biochemical tests with 43 marine bacteria isolated from the surf water near Trivandrum*

Name of the Test	Positive strains	%
Motility test	29	67.4
Gas production	20	46.5
Glucose fermentation	20	46.5
Lactose fermentation	15	34.9
Sucrose fermentation	19	44.2
Mannose fermentation	20	46.5
Mannitol fermentation	21	48.8
Dulcitol fermentation	6	13.9
Adonitol fermentation	7	17.3
Inositol fermentation	12	27.9
Salicin fermentation	17	39.5
Arabinose fermentation	14	32.6
Xylose fermentation	16	37.2
Indole production	7	17.3
M-R test	9	20.9
V.P. test	18	41.9
Citrate utilization	32	74.4
Urease test	24	55.8
Oxidase test	25	58.1
H_2S production	2	4.6
Nitrate reduction	26	60.4
O-F reaction	20	46.5
Ejkmann test	14	32.2
Aesculin hydrolysis	29	67.4
Gelatin hydrolysis	19	44.1
Starch hydrolysis	18	41.8
Pigment production	2	4.6
Catalase test	40	92.0
Sensi. to 2.5 I.U. Penicillin	4	9.2
Sensi. to 10 mg Terramycin	29	67.4

colons/E. coli in beach sea water is an indication that the three beaches are intensely faecally polluted and there is contamination of sand within human faeces. Breyenski and Russonauno (1969) and Grunnet and Nielsen (1969) also isolated *E. coli* and other enteric-pathogens from seventeen beaches of New York, but ZoBell (1941) failed to find coliform

bacteria in any of 961 samples of sea water collected at stations remote from possibilities of terrigenous contamination although large numbers of *E. coli* were found in polluted bays and estuaries. The factor contributing to high occurrence of pathogens in beach water may be due to (*i*) Temperature of the water, (*ii*) presence of organic matter, easily decomposed, and (*iii*) continuous supply of bacteria to the recipient water, through sewage, rainfall, freshwater run-off and drainage.

Penicillin (22), Ampicillin (16), Erythromycin (16), Gentamycin (18), Septran (11) and Cephoroon (19).

Details of sensitivity pattern of 43 marine strains is given in Table 6. In Gram-negative bacteria alteration of the cell envelope resulting in decreased penetration of the antibiotic to the targets responsible for lethality in cytoplasmic membrane has been proposed. Several investigators have used selective media con-

TABLE 4. *Marine bacterial genera expressed as percentage isolated by various authors in different coastal waters of the world*

Place of study	Author	*Pseudomonas*	*Aeromonas*	*Vibrio*	*Paracolon/ E. coli*	*Alcaigene*
Australia	Wood	10.0	0.0	0.0	0.0	26.0
Australia	Wood	0.9	0.0	0.0	0.0	0.0
North Sea	Shewan and Hodgkiss	94.0	0.0	0.0	0.0	6.0
N. Cape, Norway	Shewan and Hodgkiss	0.0	0.0	0.0	0.0	14.0
Narrangansett Bay, R.I. U.S.A.	Murchellano	28.3	0.0	13.3	0.0	12.2
Kamogawa Bay, Japan	Simidu and Aiso	29.8	0.0	37.3	0.0	21.3
Long Island, Sound Connecticut, USA	Murchellano and Brown	40.6	0.0	4.9	0.0	28.6
Inshore water of Japan	U. Simidu Ashino and Kaneko	28.1	9.4	34.4	3.1	15.6
Chesapeake Bay, Mi.	Lovelace, Tubiash and Colwell	18.0	0.0	56.0	0.0	13.0
Calicut, India	Venkataraman and Sreenivasan	18.0	0.0	1.4	0.0	11.6
Cochin, India	Gore	18.5	0.0	24.1	31.4	9.2
Trivandrum, India	Present Report	19.0	38.0	5.0	16.0	21.0

The pattern of drug sensitivity of marine bacteria observed during the investigation is given in Table 5. Multiple drug resistance was more prevalent than resistance to one or two drugs among marine bacteria. None of the strains was resistant to gentamycin and only one strain was resistant to septran. Tetracycline was found to be resistant to only 2 strains and 3 strains were resistant to streptomycin. 5 strains were resistant to Kanamycin and 7 strains to chloramphenicol. A higher rate of resistance was found in taining antibiotics to isolate antibiotic resistant bacteria (Koditscheck and Guyre, 1974; Sturtevant *et al.*, 1971) from raw or treated sewage, but in the present investigation this approach yielded poor results when plated with sea water. It is also observed that certain bacteria especially those naturally present in the intestine of man, when introduced into an aqueous environment become stressed and debilated. Bissonnette *et al.*, (1975), Maxey (1970) and Stuart *et al.* (1977) demonstrated that selective media used to enumerate stressed or debilated

coliforms could cause significant loss of these indicator bacteria which are resistant to antibiotics. In the present investigation it was found that approximately 25% of the coliforms appearing in MacConky agar were resistant to penicillin, ampicillin and cephoron.

Based on *E. coli* distribution the Vizhinjam Beach was considered to be most polluted of all the three beaches studied. The intensity of *E. coli* in Shankumughom and Kovalam Beaches was found to be the same.

numbers of resistant organisms than rural communities in the areas of Kovalam and Vizhinjam which are less exposed to the administration of antibiotics for their ailments.

Since the use of new and old antibiotics is constantly increasing both for clinical, agricultural and maricultural purposes, it is necessary to carry out an area wise survey to assess the incidence and extent of the multiple drug-resistance.

TABLE 5. *Sensitivity pattern of* 43 *marine bacterial strains* isolated from littoral water near Trivandrum (Values in parenthesis are %)*

Antibiotics	No. of sensitive strains	No. of moderately sensitive strains	No. of resistant strains
Penicillin ..	15 (34.8)	4 (9.7)	22 (51.1)
Ampicillin ..	22 (51.1)	3 (6.9)	16 (36.8)
Chloramphenicol ..	32 (74.4)	2 (4.6)	7 (16.1)
Kanamycin ..	33 (75.9)	3 (6.9)	5 (11.5)
Erythromycin ..	23 (52.9)	2 (4.6)	16 (36.8)
Streptomycin ..	38 (87.4)	— —	3 (6.9)
Tetracycline ..	36 (82.8)	3 (6.9)	2 (4.6)
Gentamycin ..	22 (50.6)	1 (2.3)	18 (41.4)
Septran ..	24 (55.2)	— —	17 (39.1)
Cepheron ..	21 (48.3)	1 (2.3)	19 (43.7)

* 15-*Aeromonas* ; 8-*Pseudomonas* ; 2-*Vibrio* ; 7-*Paracolons* and *E. coli* ; 11-*Alcaligenes*

Eventhough Vizhinjam Beach possessed more faecal coliforms than the beaches of Shankumughom and Kovalam, the Shankumughom Beach possessed antibiotic resistant microflora distinct from that of other sites and had an average of 16 *E. coli*/ml in spread plate count technique. These data support the view that the three beaches were considerably polluted by human waste during the study period and the quantity of antibiotic resistant microflora support the view that urban communities living in and around Trivandrum may harbour significantly greater

Selection of R^+ - factors in culture system

Regarding usage of polluted surf water for maricultural studies it is believed that situations such as those mentioned above contribute measurably as causes responsible for the recovery of R^+ - factor carrying organisms. In natural ecosystem the initial introduction of an antibiotic resistant bacterial population in fish is of very little or no consequence since it may occur at a low frequency in the total bacterial population even in the absence of antibiotic pressure (Smith, 1971).

TABLE 6. *Sensitivity pattern of* 43 *marine bacterial*

Antibiotics	Bacterial strain																	
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Penicillin ..	R	R	R	R	Dead	R	R	R	R	18	R	R	R	R	30	20	30
Ampicillin ..	R	15	R	R	,,	R	R	R	R	R	20	20	R	30	15	30	20
Chloramphenicol ..	R	R	20	30	,,	R	R	20	25	18	30	30	30	30	40	30	40
Kanamycin ..	20	10	20	20	,,	R	20	20	20	20	22	30	40	30	36	20	20
Erythromycin ..	24	R	15	R	,,	R	20	R	R	R	10	R	15	25	40	R	50
Streptomycin ..	20	15	30	20	,,	R	23	25	25	25	25	40	15	50	20	30	40
Tetracycline ..	20	20	20	20	,,	R	22	24	20	20	22	30	30	30	30	30	30
Gentamycin ..	22	24	20	20	,,	20	20	20	30	30	30	40	40	15	50	15	40
Septran ..	28	30	30	30	,,	20	25	30	25	30	24	50	40	40	40	30	40
Cephoron ..	R	R	R	R	,,	R	R	R	15	R	16	50	20	40	40	40	40

R—Resistant. Above 12-15 mm zone of inhibition is treated as sensitive.

The potential danger is due to the indiscriminate use of antibiotics particularly tetracycline as 'cure all' in maricultural practices. The misuse of antibiotics in culture system also may result in the selection of R^+-factor carrying clones of pathogenic bacteria. Ultimately an antibiotic resistant bacterial population may result in a fish disease that would not respond to usual antibiotic therapy. Resistance to different aminoglycoside antibiotics like Streptomycin, Kanamycin, etc. is due to several enzymes produced by different R^+-factors (Panse, 1981). Several enzymes have been characterized which modify different groups of aminoglycoside antibiotics. Cautious and responsible use of antibiotics will aid in minimising the development and spread of potential R^+-factors carrying organims that may confer antibiotic resistance to otherwise antibiotic sensitive bacterial species.

Such a transfer would create a pathogen with formidable capabilities, possessing enhanced infectivity and virulence.

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strains isolated from littoral waters of Trivandrum

Bacterial strain																							
19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43
R	R	R	R	30	12	30	15	20	30	R	15	16	R	30	8	10	12	20	R	30	R	R	R	40
15	R	15	R	30	R	30	13	22	30	15	10	14	10	30	15	R	8	22	R	25	R	20	R	30
20	25	40	40	30	20	30	R	20	30	25	10	15	15	20	28	20	8	20	24	24	R	20	R	16
30	30	30	32	30	40	30	R	ND	30	20	R	10	R	16	20	20	20	20	20	20	20	25	R	20
30	15	12	20	30	R	30	26	15	30	R	24	R	R	24	25	R	20	20	14	25	R	12	R	20
R	36	R	40	30	30	30	30	30	30	25	20	30	26	22	30	30	22	24	20	22	20	24	18	18
36	30	30	40	30	24	30	22	20	20	20	24	R	20	25	25	20	12	22	22	30	20	20	12	20
30	35	30	40	30	24	30	20	36	30	15	20	10	22	18	20	16	20	24	18	22	15	20	15	18
40	50	20	50	30	42	30	24	40	40	25	24	30	30	30	28	30	30	30	30	40	15	30	R	20
40	44	R	30	30	28	30	R	30	30	ND	14	14	R	36	20	20	R	R	18	44	R	R	R	24

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