Current Research in Bacteriology 3 (4): 227-237, 2010
ISSN 1994-5426

Plasmid Profiling and Multiple Antibiotic Resistance of Heterotrophic Bacteria Isolated from Muthupettai Mangrove Environment, Southeast Coast of India

S. Ramesh, P. Manivasagan, S. Ashokkumar, G. Rajaram and P. Mayavu
CAS in Marine Biology, Annamalai University, Parangipettai-608 502, Tamilnadu, India

Abstract: A study was carried out multiple antibiotic resistance of total heterotrophic bacteria isolated from sediment samples in mangrove environment of Muthupettai, Southeast coast of India was during the period of April 2006-March 07. Six stations at different area of the mangrove sites were selected for sampling. The results of the present study revealed that resistance of heterotrophic bacteria strains (six hundred and eighty) were isolated and identified from mangrove environment. The levels of resistance of bacteria to various antibiotics differed considerably. Among these 6 species (22 strains) multiple antibiotic resistance bacteria were identified from all isolates such as *Escherichia coli* (6 strains), *Vibrio parahaemolyticus* (5 strains), *Vibrio vulnificus* (3 strains), *Pseudomonas fluotescens* (4 strains), *Pseudomonas cepacia* (2 strains) and *Proteus vulgaris* (2 strains). The all strains were also able to resistance concentration of antibiotics up to 150 μg mL^{-1}. The isolated strains were screened for plasmid DNA by agarose gel electrophoresis and tested for susceptibility to 10 antibiotics by the agar dilution method. Twenty two strains belonging to 6 species have been found to Muthupettai mangroves 1-5 plasmids, with sizes ranging from 8-137 kb.

Key words: Muthupettai, mangroves, multiple antibiotic resistance, heterotrophic bacteria, plasmid profiling

INTRODUCTION

Mangrove wetlands along the coastal zone act as barrier against cyclones, protect coastal erosion and provide good nursery ground for number of commercially important aquatic organisms (Janaki-Raman *et al*., 2007). The study on physiological and genetic diversity of natural microflora is among the most intriguing problems of microbial ecology. In particular, the impact of biotic and abiotic factors on heterotrophic bacteria has been investigated (Carlsson *et al*., 1998; Hadas and Berman, 1998; Vrede, 1999; Adrian *et al*., 2001; Pomeroy and Wiebe, 2001). Bacteria are the most sensitive organisms in natural ecosystems. They possess unique adaptive features, which help them acquire high resistance to environmental factors (Lobova *et al*., 2002). Dissemination of antibiotic resistance genes among bacteria is one of the most obvious example (Chandrasekaran *et al*., 1998; Gomes-Lus, 1998; Ho *et al*., 1998; Van Elsas *et al*., 1998; Radu *et al*., 2001; Shoemaker *et al*., 2001).

Corresponding Author: P. Manivasagan, CAS in Marine Biology, Annamalai University, Parangipettai-608 502, Tamilnadu, India

Extensive use and misuse of antibiotics in medication, veterinary, agriculture and aquaculture have caused antibiotic-resistant bacteria to be widespread (Kummerer, 2004). Resistance genetic material transfer from environmental bacteria to commensal microflora may also cause bacterial pathogens to carry antibiotic resistance, complicating disease prevention and treatment (Levy and Marshall, 2004). High incidences of resistant bacteria in response to antibiotic usage have also been reported in coastal maricultural areas (Herwig *et al*., 1997). A correlation between environmental stress, e.g., pollution, resistance to antibiotics and pollutants and increased plasmid incidence in marine bacterial populations has been observed (Glassman and McNicol, 1981; Hada and Sizemore, 1981; Burton *et al*., 1982; Baya *et al*., 1986).

Plasmids have been found in heterotrophic bacteria (Lobova *et al*., 2002) and in some cases, their involvement in resistance to many antibiotics has been proven (Toranzo *et al*., 1983; Zhao *et al*., 1992). To our knowledge, plasmid presence, profiling, or their relationship with antibiotic resistance, have not been reported from bacterial strains so far the present study has been made to investigate the presence of plasmids and their relationship with multiple antibiotic resistances of heterotrophic bacteria strains isolated from Muthupettai mangroves environment in the Southeast Coast of India.

MATERIALS AND METHODS

Study Area

Muthupettai mangroves (Lat. 10° 25'N; Long. 79° 39'E) situated 400 km South of Chennai lies along the South east coast of India. It has total area of 6800 ha in which the water spread area covers approximately 2720 ha. It has two specialized habitats were noted viz., mangroves and lagoon (Fig. 1). Many tributaries of the river Cauvery delta viz., Paminiyar, Koraiyar, Kilaithangiyar, Kandankurichanar and Marakkakoraiyar flows through Muthupettai and nearby villages and form a lagoon before they enter in to the sea, Bay of Bengal. *Avicennia marina* is the dominant mangrove species in Muthupettai and accounts

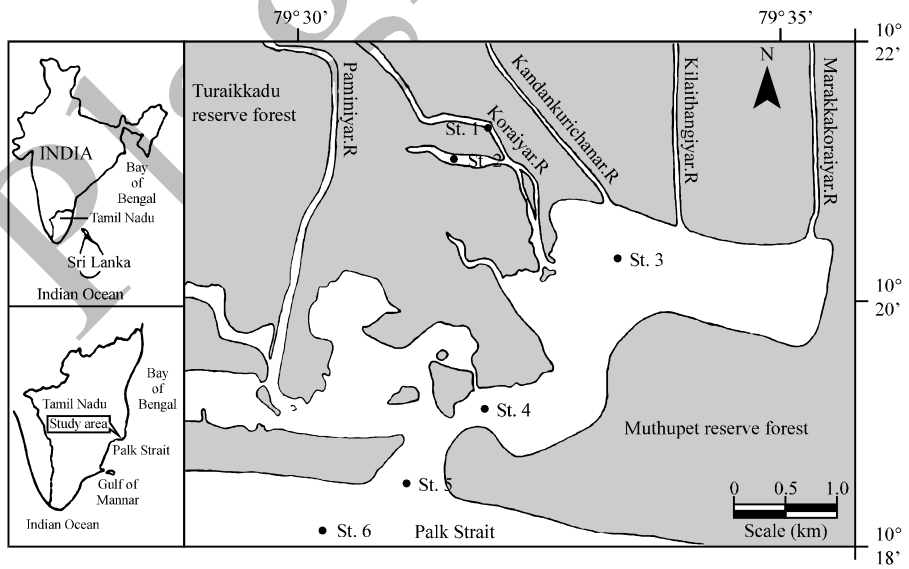

Fig. 1: Map of Muthupettai mangrove environment showing different locations

for nearly 95% of the vegetative cover. The sampling areas for present study viz., (1) Aquaculture discharge area, (2) Sethuguda, (3) Lagoon, (4) Sellimunai, (5) sea mouth region and (6) open sea.

A sediment samples at six stations were collected during April (2006) to March (2007), kept on ice box and transferred to the laboratory and analyzed within 18-24 h.

Isolation of Bacteria

One gram of sediment samples were weighed, dissolved in 99 mL of sterile seawater and serially diluted up to the dilution 10^5. Serially diluted samples were plated on ZoBell 2216 marine agar (ZB) (Rheinheimer, 1977) prepared with 50% aged sea water. Triplicate plates from each dilution were incubated for 14 days at 27°C. Afterwards, 45 bacterial colonies from each sampling site were collected randomly and transferred to same medium to purify the culture. After purity control, bacteria were stored at 4°C, with inoculation on fresh medium carried out every 3 months and used for further studies in order to determine their antibiotic resistance (Mudryk, 2005).

Identification of Heterotrophic Bacteria

Morphological and biochemical properties of the bacteria were investigated according to Bergey's manual of determinative bacteriology (Holt *et al*., 1997).

Determination of Antibiotic Resistance

Antibiotic resistance of bacteria was determined by the single disc diffusion method with the use of Mueller-Hinton agar, according to the Bauer-Kirby method (Arvanitodou *et al*., 1997). Bacteria were multiplied on agar slants (ZB) at 20°C. After 72 h they were washed off the slants with 5 cm^3 of sterile buffered water and adjusted to a turbidity of 4 on the Mac Farland scale, which corresponds to 10^9 bacterial cells per 1 cm^3. Subsequently, 0.2 cm^3 of bacterial suspension prepared and introduced into steriled Mueller-Hinton medium cooled to 40°C. After mixing, the sample was poured onto Petri dishes and dried in a drier at 37°C for 1 h. Paper discs impregnated with an antibiotic were than applied to the surface of the seeded medium. The blotting paper discs (13 mm) were manufactured by HIMEDIA. The dishes were kept at 4°C for 1h in order to allow antibiotic diffusion from the discs into the agar medium. The dishes were then incubated at 27°C for 24 h. Bacteria were classified as antibiotic resistant according to the manufacturer instructions. The following ten clinical antibiotics, with their concentrations given in parentheses were used in antibiograms: Ampicillin (AM, 10 µg), Chloramphenicol (CP, 30 µg), Gentamycin (GE, 10 µg), Kanamycin (KM, 30 µg), Nalidixic acid (NA, 30 µg), Novobiocin (NB, 30 µg), Penicillin (PL, 10 µg), Rifampicin (RF, 10 µg), Streptomycin (SM, 30 µg) and Tetracycline (TE, 30 µg). The results were used to calculate the Antibiotic Resistance Index (ARI) for bacteria (Jones *et al*., 1986).

Time Course for Growth of the Bacterial Isolates

Exponentially grown cultures of the test organisms were inoculated into treated (30, 60, 90, 120 and 150 µg mLG^1 of antibiotics) and untreated liquid culture medium and incubated at 28°C for different time intervals. A control was also run simultaneously. The growth was determined turbidometrically at different time intervals by measuring the Optical Density (OD) at 540 nm in a Spectronic-20 spectrophotometer (Shafiani and Malik, 2003).

Extraction of Plasmid

Plasmid DNA of bacterial isolates was extracted using alkaline lysis method as described by Sambrook *et al*. (1989). QIAprep spin miniprep kit (Valencia, CA, USA) was also applied

to confirm the plasmid extraction result by alkaline lysis method. The plasmid DNAs were loaded onto 0.7% horizontal agarose gels for separation and viewing. Gels were run at 5 V cm^{-1}, strained in ethidium bromide, destained in water and photographed on a UV transilluminator (Wang *et al*., 2006; Zhang *et al*., 2006).

RESULTS

Heterotrophic bacteria isolated from different stations of the Muthupettai mangroves environment were subjected to analysis for resistance to ten widely used antibiotics.

The results of present study has been showed (Fig. 2) over 50% of the bacterial microflora was resistant to clinically used antibiotics such as kanamycin, nalidixic acid, novobiocin and pencillin; less than 20% of the isolates were resistant to gentamycin and streptomycin.

Differences in the level of antibiotics resistance between the bacteria and stations of the mangroves environment was determined (Table 1), most of the antibiotic resistant bacteria were found to be in the aquaculture pond discharge area of the mangrove environment (Station 1) (ARI 0.48) and the most sensitive bacteria was isolated from open sea (station 6) (ARI 0.33). At all stations, most of the bacteria were resistant to nalidixic acid, novobiocin and pencillin and most sensitive to gentamycin and streptomycin.

Figure 3 shows the results of the present study of antibiotic resistance in pigmented and non-pigmented bacteria isolated from the Muthupettai mangroves environment. Generally

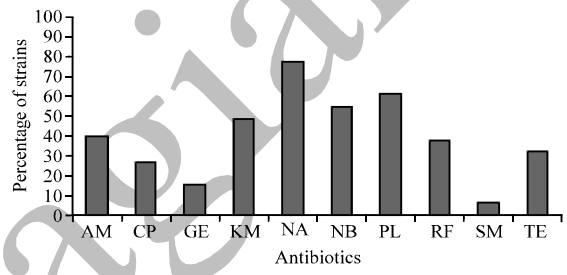

Fig. 2: Resistance to different antibiotic among bacteria isolated from Muthupettai mangroves environment (percentages derived from the pooled date of all stations)

Table 1: Resistance to the antibiotics of bacteria isolated from different stations in (%)

	Stations					
Antibiotics	1	2	3	4	5	6
Ampicillin (AM)	49.60	43.70	40.20	38.50	35.40	31.80
Chloramphenicol (CP)	34.40	31.70	28.60	25.40	21.50	20.20
Gentamycin (GE)	22.40	17.50	12.50	10.70	14.70	16.90
Kanamycin (KM)	54.60	52.70	48.60	45.60	49.50	42.40
Nalidixicacid (NA)	87.60	85.10	74.40	80.30	72.60	63.50
Novobiocin (NB)	65.10	60.50	56.40	52.80	48.60	45.70
Penicillin (PL)	71.30	65.70	60.60	64.90	53.60	50.60
Rifampicin (RF)	44.50	40.90	39.50	33.80	35.60	30.20
Streptomycin (SM)	11.30	8.60	5.60	3.20	7.60	3.10
Tetracycline (TE)	39.50	35.70	30.60	28.50	30.40	26.60
ARI	0.48	0.44	0.40	0.38	0.37	0.33

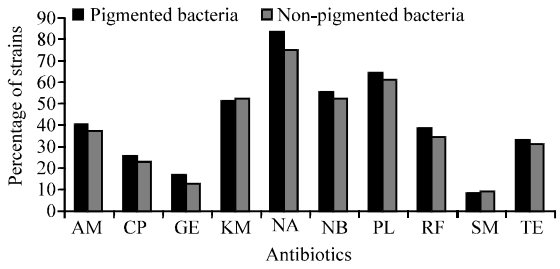

Fig. 3: Differential resistance of pigmented and non-pigmented bacteria to studied antibiotics

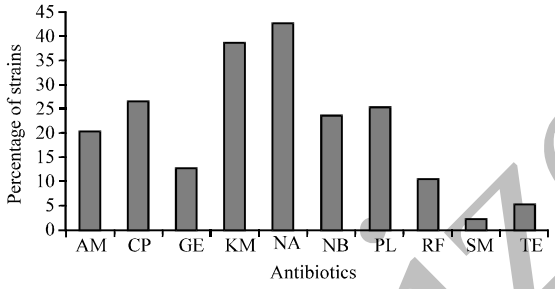

Fig. 4: Multiple antibiotic resistance bacterial strains inhabiting Muthupettai mangrove environment

no difference between pigmented and non-pigmented bacteria was noted, with the exception of pigmented bacteria being more resistant nalidixic acid, novobiocin and pencillin and non-pigmented bacteria to gentamycin and streptomycin.

Chosen strains were analyzed for multiple antibiotic resistance (MAR) (Fig. 4). About 30-45% of the studied bacteria were resistant to KM and NA. 10- 25% of the studied bacteria showed an AM, CP, NB and PL MAR pattern (i.e., resistance to KM and NA of the 10 antibiotics tested). Only small percentages of studied bacteria showed a SM and TE MAR. A total of 680 strains were isolated based on colony morphology, pigmentation and non-pigmentation from antibiotic resistance of heterotrophic bacteria for identification (Table 2). The majority of the strains isolated were from station 1 and the lowest number of strains was isolates from station 6. Present study also observed that highly resistance of the 22 (MAR) strains isolates were identified and classified as 6 species using MIC of resistance bacteria and plasmid profile, (Table. 3) such as *Escherichia coli* (6 strains), *Vibrio parahaemolyticus* (5 strains), *Vibrio vulnificus* (3 strains), *Pseudomonas fluotescens* (4 strains), *Pseudomonas cepacia* (2 strains) and *Proteus vulgaris* (2 strains). These bacterial strains were tested for their tolerance to the antibiotics ampicillin, chloramphenicol, gentamycin, kanamycin, nalidixic acid, novobiocin, penicillin, rifampicin, streptomycin and tetracycline. The *E. coli* strain was resistance up to the concentration of 150 µg mLG^{1} of CP, KM and NA and remaining antibiotics 120 µg mLG^{1}. The *V. parahaemolyticus* strain was resistance to up to concentration of 150 µg mLG^{1} of 9 antibiotics and remaining the concentration of 120 µg mLG^{1} of SM. The *V. vulnificus* resistance to the concentration up to 150 µg mLG^{1} of CP, KM, NA, NB and PL and remaining antibiotic resistance concentration 120 µg mLG^{1} AM, GE, RF, SM and TE. The *P. fluotescens* antibiotic resistance to the concentration up to 150 µg mLG^{1} of CP, KM and NA and 120 µg mLG^{1} of remaining

Table 2: Antibiotic resistance of heterotrophic bacteria identified from mangrove environment

	Stations						
Bacterial species	1	2	3	4	5	6	Total
Enterobacter sp.	18	15	13	11	13	10	80
Aeromonas sp.	12	8	10	7	5	2	44
Micrococcus sp.	10	6	8	5	3	5	37
Serratia plymuthica	8	-	2	-	1	1	12
Citrobacter freundii	4	2	5	3	4	2	20
Escherichia coli	18	16	12	10	10	12	78
E. hermanii	8	7	6	3	4	4	32
E. tarda	5	4	9	7	-	6	31
Pseudomonas fluotescens	10	11	10	8	8	5	52
P. cepacia	8	6	4	2	6	-	26
P. pseudomallei	10	11	8	10	10	6	55
Vibrio cholerae	13	11	15	12	11	13	75
V. harveyi	10	8	4	3	6	3	34
V. mimicus	5	3	7	-	1	-	16
V. splendidus	5	-	-	-	-	-	5
V. aestuarianus	3	-	3	-	-	1	7
V. vulnificus	6	2	-	-	2	2	12
V. parahaemolyticus	10	16	11	5	6	7	55
V. metschnikovii	4	-	-	-	1	-	5
Proteus vulgaris	2	-	1	-	-	1	4
Total	169	126	128	86	91	80	680

Table 3: Multiple antibiotic resistance of 6 species isolates

		Antibiotics									
Resistance of bacteria	MIC (µg mL^{-1})	AM	CP	GE	KM	NA	NB	PL	RF	SM	TE
E. coli	30	-	-		-	-	-	-	-	-	-
	60	1(3.3)	-	1(3.3)	-	-	2(6.6)	1(3.3)	2(6.6)	2(6.6)	1(3.3)
	90	3(10)	2(6.6)	3(10)	2(6.6)	1(3.3)	2(6.6)	3(10)	3(10)	3(10)	3(10)
	120	2(6.6)	2(6.6)	2(6.6)	3(10)	2(6.6)	2(6.6)	2(6.6)	1(3.3)	1(3.3)	2(6.6)
	150	-	2(6.6)	-	1(3.3)	3(10)	-	-	-	-	-
V. parahaemolyticus	30	-	-	-	-	-	-	-	-	1(2.5)	-
	60	1(2.5)	1(2.5)	2(5.0)	1(2.5)	1(2.5)	1(2.5)	1(2.5)	1(2.5)	1(2.5)	1(2.5)
	90	1(2.5)	2(5.0)	1(2.5)	1(2.5)	1(2.5)	2(5.0)	2(5.0)	1(2.5)	2(5.0)	1(2.5)
	120	1(2.5)	1(2.5)	1(2.5)	2(5.0)	1(2.5)	1(2.5)	1(2.5)	2(5.0)	1(2.5)	2(5.0)
	150	2(5.0)	1(2.5)	1(2.5)	1(2.5)	2(5.0)	1(2.5)	1(2.5)	1(2.5)	-	1(2.5)
V. vulnificus	30	-	-	-	-	-	-	-	-	-	-
	60	-	-	-	-	-	-	-	-	-	-
	90	1(5.0)	-	2(10)	-	-	-	-	1(5.0)	2(10)	2(10)
	120	2(10)	2(10)	1(5.0)	1(5.0)	1(5.0)	2(10)	2(10)	2(10)	1(5.0)	1(5.0)
	150	-	1(5.0)	-	2(10)	2(10)	1(5.0)	1(5.0)	-	-	-
P. fluotescens	30	-	-	-	-	-	-	-	-	-	-
	60	1(3.3)	-	1(3.3)	-	-	1(3.3)	1(3.3)	1(3.3)	2(6.6)	1(3.3)
	90	1(3.3)	2(6.6)	1(3.3)	1(3.3)	1(3.3)	2(6.6)	1(3.3)	2(6.6)	1(3.3)	2(6.6)
	120	2(6.6)	1(3.3)	2(6.6)	2(6.6)	1(3.3)	1(3.3)	2(6.6)	1(3.3)	1(3.3)	1(3.3)
	150	-	1(3.3)	-	1(3.3)	2(6.6)	-	-	-	-	-
P. cepacia	30	-	-	-	-	-	-	-	-	-	-
	60	-	-	-	-	-	-	-	-	1(5.0)	1(5.0)
	90	-	-	-	-	-	-	-	1(5.0)	1(5.0)	1(5.0)
	120	1(5.0)	1(5.0)	2(20)	-	1(5.0)	2(20)	1(5.0)	1(5.0)	-	-
	150	1(5.0)	1(5.0)	-	2(20)	1(5.0)	-	1(5.0)	-	-	-
P. vulgaris	30	-	-	-	-	-	-	-	-	-	-
	60	-	-	-	-	-	-	-	-	1(5.0)	1(5.0)
	90	1(5.0)	1(5.0)	2(20)	1(5.0)	-	1(5.0)	1(5.0)	2(20)	1(5.0)	1(5.0)
	120	1(5.0)	1(5.0)	-	1(5.0)	1(5.0)	1(5.0)	1(5.0)	-	-	-
	150	-	-	-	-	1(5.0)	-	-	-	-	-

Values in parentheses indicates the percentage of the total isolates. Total No. of *E. coli* isolates = 6, Total No. of *V. parahaemolyticus* isolates = 5, Total No. of *V. vulnificus* isolates = 3, Total No. of *P. fluotescens* isolates = 4, Total No. of *P. cepacia* isolates = 2, Total No. of *Proteus. vulgaris* isolates = 2, Not detected = -

antibiotics. Regarding *P. cepacia* was antibiotic resistance to the concentration up to 150 µg mL^{-1} of AM, CP, KM, NA and PL and other antibiotic resistance concentration of 120 µg mL^{-1}. However, *P. vulgaris* strain was able to resistance the concentration of 150 µg mL^{-1} of NA and other antibiotic resistance concentration of 120 µg mL^{-1} of AM, CP, KM, NB and PL, concentration up to 90 µg mL^{-1}.

Table 4: Plasmid profiles of the 22 heterotrophic bacteria isolates

Species	Strain No.	No. of plasmids	Plasmid size (kb)
E. coli	18	1	34
	93	1	48
	154	2	85,106
	297	2	85,106
	464	5	22, 53, 77, 93, 137
	531	1	34
V. parahaemolyticus	6	1	46
	186	1	46
	458	1	58
	591	2	39, 62
	642	1	44
V. vulnificus	26	1	11
	193	1	11
	442	1	23
P. fluotescens	57	2	56, 62
	229	1	39
	495	1	39
	656	1	64
P. cepacia	462	1	28
	650	1	36
P. vulgaris	95	1	8
	405	1	15

Twenty two strains were belonging to *E. coli*, *V. parahaemolyticus*, *V.vulnificus*, *P. fluotescens*, *P. cepacia* and *P. vulgaris* have been found to be 1-5 plasmids, with sizes ranging from 8-137 kb (Table 4). Seventeen strains were found to contain 1 plasmid, 4 strains contained 2 plasmids and 1 strain (strain no 464) contained 5 plasmids of different molecular weights and also observed that the antimicrobial resistant patterns of these strains one or more plasmids were very similar, almost all of them were resistant to all antibiotics.

DISCUSSION

Bacteria inhabiting mangroves environment are dominant microorganisms, fairly well adapted to the extreme conditions of mangrove ecosystem. To date, bacteriological studies of mangrove environment concerned mainly their sanitary pollution and bacterial number (Papadakis *et al*., 1997) and only limited studies were aimed at the problem of bacterial resistance to antibiotics, although this problem is of a great significance in the ecology of those microorganisms and in the public health risk (Qureshi and Qureshi, 1992).

In the present study, cultivable antibiotic- resistant bacteria were widespread in the Muthupettai mangrove environment. They were most resistant to nalidixic acid, novobiocin and penicillin and most sensitive to gentamycin and streptomycin. Similar results were obtained in southern Baltic Sea by Mudryk (2005). According to Chandrasekaran *et al*. (1998) and Tendencia and Pena (2001), observed that high level of antibiotic resistance in marine bacteria might result from terrestrial bacteria with antibiotic resistant plasmids entering the seawater; this fact may be responsible for the observed prevalence of resistance genes in the marine environment.

In the present study, generally no such peculiar characterization between antibiotic resistance and pigmentation of bacteria; the similar result was obtained by Mudryk (2005) in marine beach, Mudryk (2002) in estuarine Lake Gardno. The pigmented bacteria were more resistant to antibiotics than non-pigmented ones in different regions of Arabian Sea, (Nair *et al*., 1992) and Antarctic marine waters (De Souza *et al*., 2006).

The present study, the majority of the MAR bacteria were resistant to KM and NA of Muthupettai mangroves. That means that they are perfectly capable of detoxicating those

antibacterial substances. The percentage of MAR was higher than those reported by Tendencia and Pena (2001), Mudryk (2002, 2005) and Lobova *et al*. (2002, 2008).

In the present study, all the 680 strains of bacteria featuring antibiotic resistance in the sample collected at different stations, which are affected by monsoon season heavy fresh water inflow, agricultural discharges, shrimp effluent pollution with indicators of sewage pollution and this result suggests that perhaps other anthropogenic sources of pollution are present and influencing the microbial communities at all sites. The majority of the isolated strains from station 1 were identified, which are affected by aquaculture pond discharge water in mangrove environment. Similar results were observed by Tendencia and Pena (2001). The lowest number of isolates was identified at station 6. In the station, fresh water inflow, pollution sources and aquaculture effluent were low in marine environment. Highly resistance of the 22 (MAR) strains isolates were classified and identified as 6 species using MIC of resistance bacteria and plasmid profile such as *E. coli* (6 strains), *V. parahaemolyticus* (5 strains), *V. vulnificus* (3 strains), *P. fluotescens (*4 strains), *P. cepacia* (2 strains) and *Proteus vulgaris* (2 strains) were tested for their resistance to the all antibiotics. Growth pattern of 6 species of isolates in broth at different time intervals has been revealed that the lowest concentration (30 µg mL^{-1}) was comparable to that of control. However, the growth was declined at (120 µg mL^{-1}) and dropped more sharply at 150 µg mL^{-1}. *Vibrio parahaemolyticus* resistance of high concentration up to 150 µg mL^{-1} of 9 antibiotics.

It is well known that plasmid is one of the most important mediators facilitating the fast spreading of antibiotic resistance among bacteria (Dale and Park, 2004). In order to examine if there is any plasmid involved in antibiotic resistance profile mentioned above, plasmid extraction with alkaline lysis and QIAgen miniprep kit were also applied in this study. From the results of the plasmid extraction experiment, bacteria gave large plasmids (1-5 plasmids per strain) with molecular weights ranging from 8-137 kb. The plasmids were higher than those reported by Li *et al*. (1999), Aja *et al*. (2002), Shafiani and Malik (2003) and Wang *et al*. (2006). However, in the present study, a large number of strains were devoid of plasmids but were resistant to all antibiotics an observations which indicates that resistance to these antibiotics is chromosomal. However, the presence of plasmids in these isolates seemed to increase their antibiotic resistance.

According to Qureshi and Qureshi (1992), adaptive responses of bacterial communities to several antibiotics observed in the present investigation may have possible implications for the public health. Public health risk is further stressed by the occurrence of a high frequency (77%) of strains that are typically resistant to more than one antibiotic. Result obtained from this study indicates that antibiotics are a significant selection factor and probably play an important role in regulating the composition of bacterial communities in mangroves environments. Hence, further studies on establishing the role of antibiotic substances in controlling mangroves sediment bacterial populations are needed.

In view of these studies, it is evident that the bacterial strains isolated from Muthupettai mangroves sediment were able to grow in the presence of antibiotics. This property of antibiotic resistance in these bacteria may be important in the decontamination of mangrove sediment polluted by the antibiotics. This is the few report where a comprehensive study on the plasmids present in heterotrophic bacteria isolated. Resistance to antibiotics is widespread in heterotrophic bacteria and their relationship with transferable plasmids should be further studied.

REFERENCES

Adrian, R., S.A. Wickham and N.M. Bulter, 2001. Trophic interactions between zooplankton and the Microbial community in contrasting food webs: the epilimnion and deep chlorophyll maximum of a mesotrophic lake. Aquat. Microbial. Ecol., 24: 83-97.

Aja, A.M., A.G. Gasca, A.A. Grobois, C.B. Mejia, A. Roque and B.G. Gil, 2002. Plasmid profiling and antibiotic resistance of *Vibrio* strains isolated from cultured penaeid shrimp. FEMS Microbiol. Lett., 213: 7-12.

Arvanitodou, M., A. Tsakris, T.C. Constantindis and V.C. Katsouyannopulus, 1997. Transferable antibiotic resistance among Salmonella strains isolated from surface water. Water Res., 37: 1112-1116.

Baya, A.M., P.R. Brayton, V.L. Brown, D.J. Grimes, E. Russek-Cohen and R.R. Colwell, 1986. Coincident plasmids and antimicrobial resistance in marine bacteria isolated from polluted and unpolluted Atlantic Ocean samples. Applied Environ. Microbiol., 51: 1285-1292.

Burton, N.F., J.D. Marin and A.T. Bull, 1982. Distribution of bacterial plasmids in clean and polluted sites in a South Wales river. Applied Environ. Microbiol., 44: 1026-1029.

Carlsson, P., H. Edling and C. Bechemin, 1998. Interaction between a marine dinoflagellate (*Alexandrium catenella*) and a bacterial community utilizing riverine humic substances. Aquat. Microbial. Ecol., 16: 65-80.

Chandrasekaran, S., B. Venkatesh and D. Laithakumari, 1998. Transfer and expression of a multiple antibiotic resistance plasmid in marine bacteria. Curr. Microbiol., 37: 347-351.

Dale, J.W. and S. Park, 2004. Molecular Genetics of Bacteria. 4th Edn., John Wiley and Sons Inc., Chichester, UK.

De Souza, M.J., S. Nair, P.A. Loka-Bharathi and D. Chandramohan, 2006. Metal and antibiotic-resistance in psychrotrophic bacteria from Antarctic marine waters. Ecotoxicology, 15: 379-384.

Glassman, D.L. and L.A. McNicol, 1981. Plasmid frequency in natural population estuarine microorganisms. Plasmid, 5: 231-232.

Gomes-Lus, R., 1998. Evolution of bacterial resistance to antibiotics during the last three decades. Int. Microbiol., 1: 279-284.

Hada, H.S. and R.K. Sizemore, 1981. Incidence of plasmids in marine *vibrio* sp. isolated from an oil field in the northwestern Gulf of Mexico. Applied Environ. Microbiol., 41: 199-202.

Hadas, O. and T. Berman, 1998. Seasonal abundance and vertical distribution of protozoa (flagellates, ciliates) and bacteria in lake Kinneret, Israel. Aquat. Microbial. Ecol., 14: 161-170.

Herwig, R.P., J.P. Gray and D.P. Weston, 1997. Antibacterial resistant bacteria in surficial sediments near salmon net-cage farms in Puget Sound, Washington. Aquaculture, 149: 263-283.

Ho, M.W., T. Traavik, T. Olsvik, B. Tappeser, C.V. Howard, C. von Weizsacker and G.C. McGavin, 1998. Gene technology and gene ecology of infections disease. Microbial. Ecol. Health Dis., 10: 33-59.

Holt, G.H., N.R. Kreig, P.H.A. Sneath, J.T. Staley and S.T. Williams, 1997. Bergey's Manual of Determinative Bacteriology. 9th Edn., Williams and Wilkins, New York.

Janaki-Raman, D., M.P. Jonathan, S. Srinivasalu, J.S. Armstrong-Altrin, S.P. Mohan and V. Ram-Mohan, 2007. Trace metal enrichments in core sediments in Muthupet mangroves, SE coast of India: Application of acid leachable technique. Environ. Pollut., 145: 245-257.

Jones, J.G., S. Gardner, B.M. Simon and R.W. Pickup, 1986. Factors affecting the measurement of antibiotic resistance in bacteria isolated from lake water. J. Applied Bacteriol., 60: 455-462.

Kummerer, K., 2004. Resistance in the environment. J. Antimicrob Chemother., 54: 311-320.

Levy, S.B. and B. Marshall, 2004. Antibacterial resistance worldwide: Causes, challenges and responses. Nat. Med., 10: S122-S129.

Li, J., J. Yie, R.W.T. Foo, J.M.L. Ling, H. Xu and N.Y.S. Woo, 1999. Antibiotic resistance and plasmid profiles of *Vibrio* isolates from cultured silver sea bream, *Sparus sarba*. Marine Pollut. Bull., 39: 245-249.

Lobova, T.I., E.Y. Maksimova, L.Y. Popova and N.S. Pechurkin, 2002. Geographical and seasonal distribution of multiple antibiotic resistance of heterotrophic bacteria of Lake Shira. Aquat. Ecol., 36: 299-307.

Lobova, T.I., Y.V. Bakhatov, O.V. Salamatina and L.Y. Popova, 2008. Multiple antibiotic resistance of heterotrophic bacteria in the littoral zone of Lake Shira as an indicator of human impact on the ecosystem. Microbiol. Res., 163: 152-160.

Mudryk, Z.J., 2002. Antibiotic resistance among bacteria inhabiting surface and subsurface water layers in estuarine lake Gardno. Polish J. Environ. Stud., 11: 401-406.

Mudryk, Z.J., 2005. Occurrence and distribution antibiotic resistance of hetrotrophic bacteria isolated from a marine beach. Marine Pollut. Bull., 50: 80-86.

Nair, S., D. Chandramohan and P.A. Loka-Bharathi, 1992. Differential sensitivity of pigmented and non-pigmented marine bacteria to metals and antibiotics. Water Res., 26: 431-434.

Papadakis, J.A., A. Mavridou, S.C. Richardson, M. Lampiri and U. Marcelou, 1997. Bather-related microbial and yeast populations in sand and seawater. Water Res., 31: 799-804.

Pomeroy, L.R. and W.J. Wiebe, 2001. Temperature and substrates as interactive limiting factors for marine heterotrophic bacteria. Aquat. Microbial. Ecol., 23: 187-204.

Qureshi, A.A. and M.A. Qureshi, 1992. Multiple antibiotic resistant fecal coliforms in raw sewage. Water Air Soil Pollut., 61: 47-56.

Radu, S., S.A. Mutalib, G. Rusul, Z. Hassan and L.K. Yeang, 2001. Molecular characterization of *Salmonella weltevreden* isolated from poultry: evidence of conjugal transfer of plasmid and antibiotic resistance. Microbios, 407: 39-47.

Rheinheimer, G., 1977. Microbial ecology of a brackish water environment. Ecol. Stud., 25: 39-60.

Sambrook, J., E.F. Fritsch and T. Maniatis, 1989. Small Scale Preparations of Plasmid DNA, in Molecular Cloning : A Laboratory Manual. 2nd Edn., Cold Spring Harbor Laboratory Press, New York, pp: 1.25-1.28.

Shafiani, S. and A. Malik, 2003. Tolerance of pesticides and antibiotic resistance in bacteria isolated from wastewater irrigated soil. World J. Microbiol. Biotechnol., 19: 897-901.

Shoemaker, N.B., H. Vlamkis, K. Hayes and A.A. Sayers, 2001. Evidence for extensive resistance gene transfer among *Bacteroides* sp. and among other genera in the colon. Applied Environ. Microbiol., 67: 561-568.

Tendencia, E.A. and L.D. Pena, 2001. Antibiotic resistance of bacteria from shrimp ponds. Aquaculture, 195: 193-204.

Toranzo, A.E., J.L. Barja, R.R. Colwell and F.M. Hetrick, 1983. Characterization of plamids in bacterial fish pathogen. Infect. Immun., 39: 184-192.

Van Elsas, J.D., B.B. McSpadden-Gardener, A.C. Wolters and E. Smit, 1998. Isolation, characterization and transfer of cryptic gene-mobilizing plasmid in the wheat rhizospere. Applied Environ. Microbiol., 64: 880-889.

Vrede, K., 1999. Effects of inorganic nutrients and zooplankton on the growth of heterotrophic Bacterioplanton enclosure experiments in an oligotrophic clear water lake. Aquat. Microbial. Ecol. 18: 133-144.

Wang, Y., P.C. Leung, P.Y. Qian and J.D. Gu, 2006. Antibiotic resistance and plasmid profile of environmental isolates of *Vibrio* species from Mai Po Nature Reserve, Hong Kong. Ecotoxicology, 15: 371-378.

Zhang, R., J. Wang and J. Dong, 2006. Identification of environmental plasmid-bearing *Vibrio* species isolated from polluted and pristine marine reserves of Hong Kong and resistance to antibiotics and mercury. Antonie Van Leeuwenhoek, 89: 307-315.

Zhao, J., E.H. Kim, T. Kobayashi and T. Aoki, 1992. Drug resistance of *Vibrio anguillarum* isolated from ayu between 1989 and 1991. Nippon Suisan Gakkaishi, 58: 1523-1527.