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Assessment of Antibiotic Resistant Pattern on Marine Bacterial Communities from Nagapattinam Coastal Zone

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Abstract

We scrutinized the impact of the effluent discharges and open defecations on the marine microorganisms in the coastal zone Nagapattinam with respect to analyses the antibiotic resistant strains. In this study, the seawater sample was collected from the Nagapattinam beach during November 2015 and isolated nearly 40 marine bacteria through pure culture techniques. The isolated 40 strains were challenged against ten different standard antibiotics on Mueller Hinton agar (MHA) by disc diffusion method for antibiotic resistance analysis. In this report, most of the strains were resistant to the tested antibiotics and the majority were resistant to Penicillin-G and Methicillin. Among a total of 40 marine isolates, 15% were susceptible to all antibiotics while 85% were resistant to at least one antibiotic. The marine bacterial isolates were highly resistant to Methicillin (72.5%) and Penicillin-G (65%) followed by Novobiocin (55%), Clindamycin (40%) and Amoxicillin (35%). All the marine isolates were sensitive to Ciprofloxacin (0%) and Erythromycin (0%). The antibiotic resistance index (ARI) was higher at Nagapattinam beach (0.08) and it indicating that the accumulation of antibiotics in coastal zone is high due to the sewage discharges and fecal matters to Nagapattinam coastal region. We concluded that determination of the number of antibiotic resistant bacteria and their occurrence in the marine beaches can possibly be treated as an alternative bioindicator of marine water and sand contamination.

Keywords: Antibiotic resistant strains, Nagapattinam Coast, Marine bacteria, Antibiotics, Seawater.

Introduction

Antibiotics are compounds produced by microorganisms and have several usages such as treatment of infections, prophylaxis, food preservation, and as growth promoters (Refsdal and Forsberg, 2000). Marine organisms that are resistant to many antibiotics are of great importance in many areas of the Indian coastal line (Vignesh et al., 2012). But nowadays, antibiotic resistance (ABR) in bacteria is a growing problem in human and veterinary medicine worldwide. The presence of antimicrobial agents at low concentration through leaching or continued usage may lead to the development of drug-resistant strains and multiple antibiotic resistance (MAR) in bacteria, which ultimately result in transfer of resistance to pathogenic bacteria and reduced efficacy of antibiotic treatment for human and animal diseases. The distribution of antibiotic-resistant bacteria in the aquatic environment is consider as an important public health issue with physicians concerned about their infectious nature (Schmidt 2002). Emergence of bacteria resistant to antibiotics is predictable in any environment where antibiotics are being used, but occurrence of antibiotic resistant bacteria is also increasing in aquatic environments (Al-Bahry et al., 2009a, 2009b; Lima-Bittercourt et al., 2007).

Being a potential public health hazard to the persons exposed to these aquatic environments through various activities, the prevalence of such antimicrobial-resistant strains in the environment is gaining worldwide concern. During the past few years, the distribution of many antimicrobial-resistant bacterial strains in aquatic environments has been observed in different regions of the globe (Vignesh et al., 2012). Studies that evaluate the antimicrobial resistance patterns in the aquatic environment are crucial for the surveillance, prevention and control of many diseases. In addition, studying antimicrobial resistance profiles is important in order to: a) detect patterns of change in resistance, b) implement control measures on the use of antimicrobial agents, and c) prevent the spread of multi-drug-resistant strains of bacteria (Duijkeren et al., 2003). The resistance is an extremely complex process and its significance for the environment is not fully understood (Alanis, 2005). Resistance in bacteria from different habitats has been increasing at an alarming rate and is becoming a serious concern throughout the world. The abuse of antibiotics not only increases resistance in pathogenic bacteria but also in the indigenous flora of animals, which in turn affects the normal microbial flora of humans (Austin and Al-Zahrani, 1988). Therefore, the present study was

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undertaken to assess the multiple antibiotic resistance in marine bacterial communities from Nagapattinam beach environment of east coast of India.

Materials and methods

Study area

Nagapattinam district is a coastal district of Tamil Nadu state in southern India. The town of Nagapattinam is the district headquarters. As of 2011, the district had a population of 1,616,450 with a sex-ratio of 1,025 females for every 1,000 males. It is the only discontiguous district in Tamil Nadu. The district of Nagapattinam lies on the shores of the Bay of Bengal between latitude 10.7906°N and Longitude 79.8428°E an area of 2,715 square kilometres (1,048 sq mi). The District capital, Nagapattinam lies on the eastern coast, 350 kilometers down south of the state capital Chennai and of Tiruchirappalli. The average literacy of the district was 75.04%, compared to the national average of 72.99%. The district had a total of 413,837 households. There were a total of 671,994 workers, comprising 54,329 cultivators, 216,353 main agricultural labourers, 7,925 in household industries, 207,721 other workers, 185,666 marginal workers, 13,153 marginal cultivators, 128,704 marginal agricultural labourers, 3,630 marginal workers in household industries and 40,179 other marginal workers (http://www.nagapattinam.tn.nic.in). The Nagapattinam coastal zone (Figure 1) consist of small distanced beach and moderate level national port. It also has huge level of fishing harbor and nearly 15000 of people/ fisher folks were reside at these regions.

Sampling

The water samples were collected from Nagapattinam beach area during November 2015 for bacteriological analysis. The seawater sample was collected from 0 to 20 cm below the surface (Vignesh et al., 2015). The 2000 mL of water samples were collected with a 2500 mL sterile container and stored in ice box at 4 °C. The samples were transported into laboratory and processed within 10 hrs.

Bacteriological analysis

In this study, the nutrient agar media was prepared with the addition of old age seawater and autoclaved properly. The bacterial populations in sea water samples were isolated by pure culture technique with (100 µL) suitable dilutions (Kumarasamy et al., 2009). All the media plates were incubated at 37°C ± 1°C for 24–48 h. After incubation, the distinguished colonies were picked and inoculated into the fresh nutrient broth which was prepared by addition of aged seawater (Vignesh et al., 2015; Muthukumar et al., 2015). A total of 40 marine bacterial strains were isolated from the seawater sample of Nagapattinam beach and stored in refrigerator.

Antibiotic resistant studies

The marine isolates (40 strains) were challenged against ten different standard antibiotics (antimicrobials) (which is purchased from Hi-media Pvt. Ltd., Mumbai, India) on Mueller Hinton agar (MHA) by disc diffusion method for antibiotic resistance analysis (Vignesh et al., 2012). The results were interpreted based on the recommendations of the National Committee for Clinical Laboratory Standards for antimicrobial susceptibility testing (NCCLS, 1998). The standard antibiotics were Ciprofloxacin (CIP) – 10 mcg, Chloramphenicol (C) – 10 mcg, Erythromycin (E) – 10 mcg, Methicillin (MET) – 10 mcg, Novobiocin (NV) – 5 mcg, Penicillin-G (P) – 10 mcg, Tetracycline (TE) – 10 mcg, Clindamycin (CD) – 10 mcg, Ampicillin (AMP) – 10 mcg and Amoxicillin (AMX) – 10 mcg. The antibacterial resistance index (ARI) of each location was calculated according to Hinton et al. (1985) and Vignesh et al. (2012), using the formula ARI = y/nx, where ‘y’ represents the actual number of resistance determinants recorded from a population of size ‘n’ and ‘x’ as the total number of antibacterial tested in the sensitivity test.

Result and discussion

In this study, the isolates were exposed to 10 different antibiotics. These antibiotics are widely used in Tamil Nadu. All of the resistant isolates were multiply resistant to the tested antibiotics and the majority were resistant to Penicillin-G and Methicillin. Interestingly, other investigators have shown that bacterial strains (*Aeromonas* spp. and *Vibrio* spp.) isolated from aquatic environment are resistant to ampicillin (Lee and Kang, 1999; Pilar et al., 1997). In Nagapattinam coastal zone, more than 14% of isolates were resistant to single antibiotics whereas double and 3 antibiotic resistances were recorded in marine bacterial isolates such as 7.5% and 2.5%, respectively. Among a total of 40 marine isolates, 15% were susceptible to all antibiotics while 85% were resistant to at least one antibiotic. The level of four and five antibiotic resistances were 7.5% and 27.5% respectively (Table 1). The nil percentage was recorded at eight, nine and ten antibiotic resistances. Undoubtedly, the improper and unnecessary use of antimicrobial drugs in humans and animals has promoted the development of resistant strains that is now affecting the environment marine habitats including the sea turtle habitats which are frequently found in Oman (Al-Bahry et al., 2009a). Antimicrobial agents and their metabolites entering the aquatic environment become highly diluted and therefore detection of these compounds becomes extremely difficult (Kümmerer, 2009). Overuse of antibiotics has led to the emergence of resistant bacteria and consequently caused an imbalance between susceptible and resistant bacteria. This eventually has sub-grouped them into susceptible and resistant variants. In addition, the potent killing and growth inhibition of bacteria have increased the number of resistant strains which have ultimately evolved into prominent populations of the microbial flora (Levy, 1994). Hence, the presence of

antibiotic resistant bacteria have been used as bio-indicators of polluted effluents since resistant bacteria can be easily isolated and detected (Al-Bahry et al., 2009b).

The studies carried out by Mudryk (2005) at the marine sand beach in Sopot showed that the bacteria from sand were highly resistant to antibiotics than water isolates. Environmental bacteria may play an important role as reservoirs of antibiotic resistance; resistance genes are exchanged by bacteria from different freshwater and marine ecosystems (Dang et al., 2008). As pointed out by Hsu et al. (1992) differences in percentage of bacterial resistance to various antibiotics may reflect the history of antibiotic application and hence there is a possibility of using bacterial drug resistance as an indicator of antibiotic application. In this study, the antibiotic resistance index (ARI) was higher at Nagapattinam beach (0.08) and it indicating that the accumulation of antibiotics in coastal zone is high due to the sewage discharges and fecal matters to Nagapattinam coastal region.

The marine bacterial isolates were highly resistant to Methicillin (72.5%) and Penicillin-G (65%) followed by Novobiocin (55%), Clindamycin (40%) and Amoxicillin (35%). All the marine isolates were sensitive to Ciprofloxacin (0%) and Erythromycin (0%). Low level of resistance were observed at Chloramphenicol (2.5%) and Ampicillin (15 %) (Table 2). Miranda and Zemelman (2001), found that bacteria from commercial marine water and pelagic fish exhibited high frequencies of resistance to ampicillin, streptomycin and tetracycline. The bacterial resistance to antibiotics is a growing medical and ecological problem worldwide, generated by the selection processes following the massive use of antibiotics (Meirelles-Pereira et al., 2002). According to Luthje and Schwarz (2007) bacterial resistance to lincosamide antibiotics can be due to target site modification, active efflux mechanisms, mutations and enzymatic inactivation on the drugs. In this study, most of the strains were resistant to at least one antibiotic and the resistance may vary depend on the type of antibiotics.

Table 1. Antimicrobial resistant strains and their susceptibilities in Chennai coastal zone

S.No	No of antimicrobial (antibiotics)	Total strains (n = 40)	
		Number	Percentage
1	One Antimicrobial	06	15%
2	Two Antimicrobial	03	7.5%
3	Three Antimicrobial	08	20%
4	Four Antimicrobial	03	7.5%
5	Five Antimicrobial	11	27.5%
6	Six Antimicrobial	02	5%
7	Seven Antimicrobial	01	2.5%
8	Eight Antimicrobial	0	0%
9	Nine Antimicrobial	0	0%
10	Ten Antimicrobial	0	0%
	Total no of resistant	35	85%
	Susceptibilities	05	15%

Table 2. The resistance of bacteria from Chennai coastal zone to specific standard antibiotics

S.No	Name of the antimicrobials (Standard antibiotics)	Total resistant strains (n = 40)	
		No of resistant strains	Percentage
1	Ciprofloxacin (CIP) – 10 mcg	0	0%
2	Chloramphenicol (C) – 10 mcg	1	2.5%
3	Erythromycin (E) – 10 mcg	0	0%
4	Methicillin (MET) – 10 mcg	29	72.5%
5	Novobiocin (NV) – 5 mcg	22	55%
6	Penicillin-G (P) – 10 mcg	26	65%
7	Tetracycline (TE) – 10 mcg	11	27.5%
8	Clindamycin (CD) – 10 mcg	16	40%
9	Ampicillin (AMP) – 10 mcg	06	15%
10	Amoxicillin (AMX) – 10 mcg	14	35%

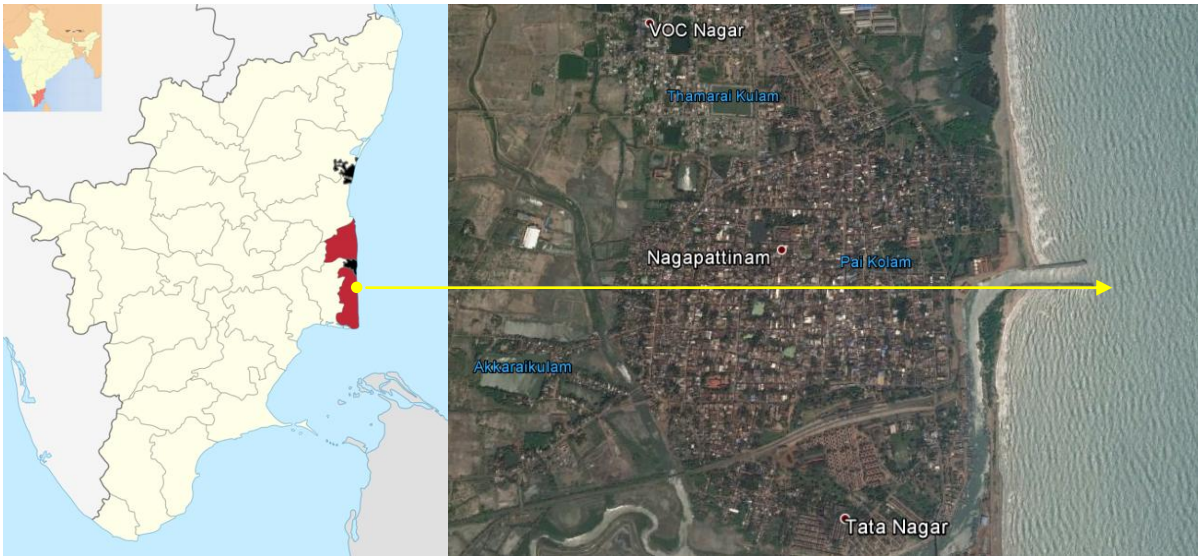

Figure 1. Study area

Conclusion

Based on the present results, the coastal area of Nagapattinam are continuously exposed to contaminated sewage discharges, regular visits and human/ animal fecal matters. The bacteria of human origin are affecting the coastal zone and antibiotic resistant determinants are being transferred to other bacteria in the area. The results presented in this paper indicated that antibiotics are a significant selection factor and probably play an important role in regulating the composition of bacterial communities of the marine beach. We concluded that determination of the number of antibiotic resistant bacteria and their occurrence in the marine beaches can possibly be treated as an alternative bioindicator of marine water and sand contamination. Hence the situation is alarming and throughout impoundment is needed.

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