Antibiotic resistance of bacteria from Krishna Godavari Basin, Bay of Bengal, India

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Abstract

The present study investigates the responses exhibited by the bacterial flora from the Krishna Godavari basin sediment from Bay of Bengal, India to antibiotics. The levels of resistance and sensitivity of the strains were examined using standard methods. A total of 53 isolates were screened, of which 67.92% strains were resistant to tetracycline, streptomycin, and chloramphenicol. The study revealed that 30.18, 24.52, 13.20, 0.0% of the isolates were resistant to ampicillin, chloramphenicol, streptomycin and tetracycline, respectively. The inhibitory sequence of the antibiotics was ampicillin > chloramphenicol > streptomycin > tetracycline.

Key words: antibiotics, bacteria, resistance, marine sediment, Krishna Godavari basin.

Introduction

Many bacteria isolated from natural environments possess an important ecological quality, namely that of resistance to antibiotics, which can be gained in the course of selective processes (Nair et al. 1992; Silva, Hofer 1995). Over recent years many studies have been carried out to investigate the occurrence and distribution of antibiotic-resistant bacteria in water basins (Baya et al. 1986; Herwig et al. 1997; Lemos et al. 1991; Mudryk et al. 1994). Antibiotic resistant bacteria in the aquatic environment, which have been demonstrated in many studies (Andersen et al. 1994), develops as a consequence of uncontrolled discharges of urban and animal wastewater (Guardabassi et al. 1998; Goni-Urriza et al. 2000). During recent decades, antibiotics have been widely used as therapy for bacterial infections in humans and animals, and as growth promoters in agriculture and aquaculture, increasing proportion of antibiotic resistant bacteria in various environments (Pathak et al. 1993; Young, Hilary-Kay 1993).

There is a great diversity among bacteria, and they do not share all of the same biochemical and physiological pathways. Therefore, not all antibiotics are active against all bacteria and and bacterial species can have intrinsic resistance to one or more antibiotics. Intrinsic resistance refers to resistant microorganisms without any chromosomal mutation or acquisition of plasmid carrying resistance factors. Inherent features of the bacterial cell prevent antimicrobial action, and these properties are typically characteristics of species. The extent of antibiotic use is a measure of the selection pressure exerted on bacteria (Schwartz et al. 1993). Antibiotic resistance in microorganisms may be associated with reduced penetration of the antibiotic into the cell, or in a result from active processes such as changes in the transport of these compounds into or from bacterial cells (Hermansson et al. 1987). Bacterial resistance to antibiotics is located in plasmids of 1 to 30 megadaltons molecular weight (Kobori et al. 1984). Genes assembled in plasmids protect bacterial populations against antibiotics.

The aim of the present study was to screen the bacteria from Krishna Godavari Basin sediments, Bay of Bengal for antibiotic resistance.

Materials and methods

Sediment samples were collected from Krishna-Godavari basin, Bay of Bengal (located between 13° 07' N and 19° 20' N, and 73° 22' E) (Gunaseelan, Ruban 2011). The total area of basin is 14 000 km^3. Reliance Industries Ltd., various hotels and hospitals are present in the surrounding area. The back water of the industries and hotels has been discharged in to the basin. Sediment samples were collected from different depths ranging from 1 to 13 m by using gravity and pressure corers. All samples were transported to the laboratory in a cool container (10 ± 2 °C).

Sediment samples (5 g) were resuspended in 50 mL of sterile sea water, forming a slurry. Individual samples of 25 µL of the slurry were inoculated on nutrient agar plates under sterile conditions by the spread plate technique. The plates were incubated in room temperature for 24 to 36 h until colonies clearly developed (Gunaseelan, Ruban

Table 1. Morphological characterization, growth and identification of isolates of marine sediment at different depths and their response to antibiotics. Letters indicate different morphological colonies at specific depths of sediment sample. +, resistance; –, sensitivty

No.	Depth (m)	CFU (g^{-1})	Morphology	Bacteria	Antibiotics			
					Ampicillin	Chloramphenicol	Streptomycin	Tetracycline
1	13 (a)	1.8×10^5	Rods	*Bacillus* spp.	+	–	–	–
2	13 (b)		Cocci	*Actinobacter* spp.	–	+	–	–
3	13 (c)		Rods	*E. coli*	–	–	–	–
4	12 (a)	1.9×10^5	Rods	*Enterobacter* spp.	–	–	–	–
5	12(b)		Rods	*Legionella* spp.	–	–	–	–
6	12 (c)		Rods	*Pseudomonas* spp.	–	–	–	–
7	12 (d)		Rods	*Klebsiella* spp.	+	–	+	–
8	12 (e)		Rods	*Microbacterium* spp.	+	–	–	–
9	11 (a)	2.0×10^4	Cocci	*Lactobacillus* spp.	–	–	+	–
10	11 (b)		Rods	*Bacillus* spp.	–	–	+	–
11	11 (a)		Rods	*Clostridium* spp.	+	+	–	–
12	10 (b)	2.4×10^4	Rods	*Rhizobium* spp.	–	+	–	–
13	10 (c)		Rods	*Yersinia* spp.	+	+	–	–
14	10 (d)		Rods	*Carnybacterium* spp.	+	+	–	–
15	9 (a)	3.2×10^4	Rods	*Serratia* spp.	+	+	–	–
16	9 (b)		Rods	*Klebsiella* spp.	–	–	–	–
17	9 (c)		Rods	*Proteus* spp.	–	+	–	–
18	9 (d)		Rods	*Morganella* spp.	+	–	+	–
19	8 (a)	4.0×10^4	Rods	*Enterobacter* spp.	+	+	–	–
20	8 (b)		Rods	*Actinobacter* spp.	–	–	–	–
21	8 (c)		Rods	*Bacillus* spp.	+	+	–	–
22	8 (d)		Cocci	*Enterobacter* spp.	+	–	–	–
23	7 (a)	7.9×10^4	Cocci	*Carnybacterium* spp.	–	–	–	–
24	7 (b)		Rods	*Vibrio* spp.	–	–	–	–
25	7 (c)		Cocci	*Enterobacter* spp.	+	–	–	–
26	7 (d)		Rods	*Microbacterium* spp.	–	–	+	–
27	6 (a)	8.6×10^4	Rods	*Pseudomonas* spp.	+	+	–	–
28	6 (b)		Rods	*E. coli*	–	–	–	–
29	6 (c)		Rods	*Staphylococcs* spp.	+	+	–	–
30	6 (d)		Rods	*Vibrio* spp.	–	–	–	–
31	6 (e)		Rods	*Moraxella* spp.	+	+	–	–
32	6 (f)		Cocci	*Actinobacter* spp.	–	+	–	–
33	6 (g)		Rods	*Actinobacter* spp.	–	–	–	–
34	5 (a)	8.78×10^4	Rods	*Aeromonas* spp.	+	–	–	–
35	5 (b)		Rods	*E. coli*	–	–	–	–
36	5 (c)		Cocci	*Pseudomonas* spp.	–	–	–	–
37	5 (d)		Cocci	*Nesaria* spp.	–	–	+	–
38	5 (e)		Rods	*Serratia* spp.	–	–	–	–
39	4 (a)	10×10^4	Rods	*Klebsiella* spp.	–	–	–	–
40	4 (b)		Rods	*E. coli*	–	–	–	–
41	4 (c)		Rods	*Vibrio* spp.	–	–	–	–
42	3 (a)	10.3×10^4	Rods	*Serratia* spp.	–	–	–	–
43	3 (b)		Rods	*Pseudomonas* spp.	–	–	–	–
44	3 (c)		Rods	*Serratia* spp.	–	–	–	–
45	3 (d)		Rods	*E. coli*	–	–	–	–
46	2 (a)	14×10^4	Cocci	*Enterococcus* spp.	–	–	+	–
47	2 (b)		Rods	*Moraxella* spp.	–	–	–	–
48	2 (c)		Rods	*Serratia* spp.	–	–	–	–
49	2 (d)		Rods	*Klebsiella* spp.	–	–	+	–
50	2 (e)		Rods	*Clostridium* spp.	–	–	–	–
51	2 (f)		Rods	*Proteus* spp.	–	–	–	–
52	1 (a)	14.8×10^4	Cocci	*Enterococcus* spp.	–	–	–	–
53	1 (b)		Cocci	*Micrococcus* spp.	–	–	–	–

2011). The colonies were counted by colony forming unit and identified based on morphological and phenotypic characters (Holt et al. 1997; Mahalakshmi et al. 2011)

Four different antibiotics ampicillin, chloramphenicol, streptomycin, and tetracycline were used for analysis in 50, 60, 70.75, 80 μg mL^{-1} concentrations, respectively, incorporated in Yeast Peptone medium. The representative organisms were inoculated on this prepared medium. The minimum inhibitory concentrations of the antibiotics were determined.

Results

A total of 53 isolates were isolated from different depths of sediment sea floor (1 to 13 m) in Krishna Godavari Basin. Based on the colony morphology and phenotypic characteristics, the isolates were identified (Table 1).

In primary antibiotic resistance screening 17, 13 and 8 isolates were resistant to ampicillin, chloramphenicol and streptomycin, respectively. All these isolates were observed to be sensitive to tetracycline (Table 1).

Discussion

Presence of antibiotic resistance bacteria in a given environment may be an indication that the area is contaminated with antibiotics. Such an area may foster adaptation and selection leading to antibiotic resistant organisms. This type of study has been carried out on clinical material, but little is known of bacterial resistance to antibiotics in the natural environment (Klech, Lee 1978; Jones et al. 1986; Mudryk 2002). Hence, the role of antibiotic substances secreted into the natural environment has not been recognized in a comprehensive way and has been one of the most controversial issues of microbial ecology (Williams et al. 1986). Antibiotic substances may be an important factor in forming the species composition of bacteriocenoses in water ecosystems and may also play a substantial role in food competition systems (Barja et al. 1989; Lemos et al. 1991; Dan et al. 2008). The sensitivity to antibiotics has also been used as a character to identify bacteria on a taxonomic level (Klech, Lee 1978).

The results from this study of resistance of bacterial isolates to antibiotics from the marine sediments of various depths reflects the extent and character of pollution, and the level of adaptation of natural bacteria to their surrounding environment. It was found that resistant isolates were found at all sediment depths. According to Chandrasekarn et al. (1998) and Tendencia, De la Pena (2001) such a high level of antibiotic resistance in marine bacteria might result from entry of terrestrial bacteria with antibiotic resistant plasmids to seawater. This might explain the observed prevalence of resistance genes in the marine environment. Also about 50% of marine bacteria have plasmids in which antibiotic-fighting genes are assembled, which protect the microorganisms from being affected by antibiotics (Kobori et al. 1984). In Krishna Godavari basin sediment samples, the highest bacterial resistance was noted in the cases of ampicillin, chloramphenicol, while bacteria were most sensitive to streptomycin, tetracycline.

Bacteria inhabiting many water basins are characterized by multiple antibiotic resistance. Multiple resistance may be coded on plasmids, mutational events or on even smaller and mobile genetic elements called transposons (Herwig et al. 1997; Chapman, 2003; Baker-Austin et al. 2006). Transposons are able to move between plasmids and bacterial chromosomes. The present study shows that bacterial strains inhabiting the sediments of Krishna Godavari basin have multiple antibiotic resistance indicating that bacteria are able to detoxicate these antibacterial substances.

According to Foster (1983) and Nair et al. (1992) bacterial resistance to antibiotics depends on their chemical structure, which is well confirmed by the results of the present investigation. Bacteria isolated from the sediments were most susceptible to aminoglycosides, β-lactams and tetracyclins. These antibiotics interfere with bacterial ribosomal translation and consequently inhibit protein synthesis.

Mudryk and Skorczewski (1992) obtained identical results during their investigation of bacterial resistance to antibiotics in Gdansk Deep. A high proportion of strains inhabiting sediments were resistant to β-lactam antibiotics, which inhibit enzyme activity in the biosynthesis process of cell walls (Foster 1983). Those results show that bacteria are capable of detoxifying these antimicrobial agents. The resistance of bacteria to β-lactam antibiotics lies in their ability to synthesis three extracellular enzymes; β-actamase, acylase and penicillinase. These biocatalysts limit the permeability of the cytoplasmatic membrane to those antibiotics or transform them, by hydrolysis of the β-lactam bond, into antibiotically inactive penicilloic acid (Herwig et al. 1997; Hermansson et al. 1983). The results presented in this paper show that antibiotics are a significant selection factor and probably are important in regulating the composition of bacterial communities in marine environments.

In conclusion, the present study outlines the possibility of public health risk through food chains, as this site harbours a high frequency of bacteria resistant to antibiotics. The study indicates that antibiotics are a significant selection factor and probably play an important role in regulating the composition of bacterial communities in the Krishna Godavari basin. Hence, further studies are necessary to establish the role of antibiotic substances in control of marine bacterial populations.

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