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Effect of Antibiotics and Some Essential oils on Marine *Vibrios* Isolated from the Coastal Water of Bay of Bengal at Orissa coast

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***Vibrio* ranks very high as a human public health hazard, amongst the bacterial species as well as a contaminant in marine foods and food products. They are also considered to be one of the dreaded organisms causing a variety of diseases thereby creating havoc in both fresh water as well as in the marine environment. Seven different *Vibrio* spp. namely *V. parahaemolyticus, V. diazotrophicus, V. alginolyticus, V. nereis, V. fluvialis, V. cholerae, V. vulnificus* were isolated from the coastal waters of Bay of Bengal at Orissa coast. The antibiogram patterns of these seven different marine vibrio strains were studied. A degree of multiple antibiotic resistance was observed among the strains. Further due to mounting drug resistance by the microbes to the antibiotics these organisms were screened for the suceptibility towards six essential oils (Turmeric leaf oil, Turmeric rhizome oil, Carrot seed oil, Celery seed oil, Japanese mint leaf oil and Ginger rhizome oil). The essential oils showed vibriocidal activity at a very low concentrations and being natural in origin is suggestive for their use as natural preservatives for marine foods against costly, harmful, mutagenic/ carcinogenic chemical preservatives.**

Keywords: Marine Vibrios, Antibiogram, Essential oils.

Sea foods, which include mostly fish, prawns, crabs, molluscs etc. are consumed in many continents as a staple food and also constitutes an important part of the protein diet of the world population. Generally, the consumption of raw seafood is in high numbers throughout the globe. Though fresh flesh of newly caught healthy fish is usually considered sterile, the integument, gills and the intestines of these sea fauna carry heavy bacterial load. Besides the sea food the coastal waters of this coast were frequently used by the local peoples and the tourists for bathing purposes. Several reports are available regarding the presence of a number of pathogenic marine bacteria mostly Gram -ve rods, more specifically *Vibrio* spp. in the seafood as well as in the marine environment (Bathena *et al*., 1995).

It has been established that the marine environment contain maximum number of *Vibrio* and 9 out of 11 *Vibrios* are known to be pathogenic to humans (Lowrie *et al.* 2000). As a human public health hazard, *Vibrio* ranks very high, causing a number of different types of diseases in humans. *Vibrio* spp. are not only a human public health hazard but they are an economic hazard because every year due to *Vibrio* infection a sizeable amount of shell fish, shrimp, etc. were killed in the initial stages of their growth(Hansen *et al.,* 1991). Therefore, it is a matter of great concern

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to study the pathogenic microorganisms present in marine environment, their role in causing diseases to the marine animals as well as to the human beings. The coastal belts of Orissa along the Bay of Bengal stretches around 460 km. This part of the Bay of Bengal is usually overcrowded because of tourist importance and other activities like fishing and transportation. Therefore an attempt was made to study the presence of potential pathogenic *Vibrio* species in the coastal waters of the Bay of Bengal of Orissa coast.

Because of their known pathogenecity the marine *Vibrio* isolates were screened for their susceptibility to various antibiotics. In spite of recent advances in chemotherapy and with the mounting drug resistance to synthesised drugs, efforts are currently being made to search for new compounds of natural origin for its antimicrobial activity. Foremost amongst them are the medicinal plants and their essential oils. Thus susceptibility of these marine *Vibrio* isolates towards six common edible essential oils of plant origin was also studied.

MATERIAL AND METHODS

Isolation and identification of the isolates

The marine water samples of the Bay of Bengal at Orissa coast from four different study sites such as Chandipur, Paradip, Puri and Gopalpur were collected aseptically using sterile containers. The water samples were transported to the laboratory within 4-6 hrs.The samples were further enriched in Alkaline Peptone water medium for 18 hrs. *Vibrios* were isolated from these enriched media by streak plate and spread plate method on modified TCBS agar medium(TCBS agar medium prepared with 50% aged filter sterilized sea water and 50% distilled water). Further pure cultures of the isolated *Vibrio* strains were made in ZMA plates. The marine *Vibrio* isolates were identified by studying its morphology followed by a series of biochemical tests following the methods of Collins *et al.,* (1970). All the test results were matched with Bergy's manual of determinative bacteriology, 9thedn.1994 and one identification software PIBwin version1.9.2. (Bryant ,1986).

Antibiogram study

The marine *Vibrio* isolates were revived in ZoBell Marine broth incubated at 30°C. Subsequently, the antibiogram patterns of the isolates were studied following the disc diffusion methods of Bauer *et al.* (1966). Ten selective antibiotic discs procured from HiMedia, Mumbai were used in the study depending on their use and mode of action. Freshly grown bacterial culture from ZMB broth was sweeped onto presterilised modified MHA agar plates (50% of sea water+50% distilled water).The selected antibiotic discs were placed aseptically on the MHA plates. The plates were then incubated at 30^0 C for 24 hrs in a BOD incubator (Wadegati make). The zone of inhibition was measured with the help of a scale and was categorised as sensitive and resistant. Multiple antibiotic resistance index percentage of the isolates was determined by the method of Mohapatra et al. (2006) using the formula

MAR index % = number antibiotics to the isolate showed resistance/no. of antibiotics used in the study X100.

Susceptibility to the Essential oils

Six different types of essential oils were collected and used in this study (Turmeric leaf oil, Turmeric rhizome oil, Carrot seed oil, Celery seed oil, Japanese mint leaf oil and Ginger rhizome oil).The antibacterial activity of all these six essential oils were studied by using the tube dilution technique to find the minimum inhibitory concentration value.

RESULTS AND DISCUSSION

Isolation and identification of the isolates

From the four different study sites a total number of seven different *Vibrio* strains were isolated. The morphological and biochemical tests conducted for species level identification resulted into seven types (Table 1).

Antibiotic sensitivity pattern of the isolates

The antibiotic sensitivity test pattern indicates that all the7 species of the *Vibrios* were showing a variable degree of sensitive and resistant pattern to the antibiotics used in the study (Table 2). The multiple antibiotic resistance index percentage ranged between 20-60%. *Vibrio alginolyticus and V.vulnificus* showed 20% MAR index, whereas *Vibrio diazotrophicus* showed maximum resistance of 60% of MAR index. The increasing trend of resistance to many of these

Table 1. Different *Vibrio* species and their source of isolation.

Organisms	Strain No.	Source
Vibrio parahaemolyticus	Soc-1	Chandipur
Vibrio diazotrophicus	Soc-5	Chandipur
Vibrio alginolyticus	Sod-8	Paradip
Vibrio nereis	Sod-10	Paradip
Vibrio fluvialis	Sop-21	Puri
Vibrio cholerae	Sop-18	Puri
Vibrio vulnificus	Sog-31	Gopalpur

antibiotics by the *Vibrios* are definitely of a great concern. To a general agreement the chances of exposure of these marine *Vibrios* to antibiotics are not very frequent, still the isolates showed a rising trend of resistance towards commonly used antibiotics.This prompted us to screen these isolates for their susceptibility towards essential oils for a possible use of these oils as vibriocidal agents, more particularly in preservation of food items those are susceptible to be contaminated by vibrios, such as marine foods and food products, including foods rich in animal proteins.

Susceptibility test of the isolates against essential oils

From the minimum inhibitory concentration study of the essential oils against the seven marine *Vibrio* species, it indicates that all the isolates were highly susceptible to Japanese mint oil (MIC value <0.97-1.9 µl/ ml) and turmeric leaf oil (MIC values 0.97-1.9 µl/ ml) (Table 3). However on an average the MIC value of the oils ranged between <0.97-125 µl/ ml. Gupta *et al*. (2004) observed similar MIC values of carrot and celery seed essential oils against *Vibrio* and *Pseudomonas* .The antibacterial and antifungal activity of turmeric leaf essential oil studied by Rath *et al* (1999, 2002) corroborates with the present findings.

From the study, it is quite evident that presence of a variety of *Vibrio* species in the coastal water makes it very vulnerable for the marine animal as well as to the people inhabiting near the shore. The antibiogram pattern also made a concern regarding the trend of resistance shown by these organisms towards the common antibiotics used for treatment due to illness caused by these organisms. However, the test result of the essential oils with antibacterial activity at very low concentration gives an insight into their possible utilization as an alternative to the antibiotics. The antimicrobial activity of some essential oils and their components against food-borne pathogens, including mycotoxin-producing fungi, has also been tested (Bullerman *et al*. 1977; Kim *et al*. 1995; Ultee *et al*. 2000; Senhaji *et al*. 2007). Rath *et al.* (2007) reported the vibriocidal activity of

Table 2. Antibiogram pattern of bacterial isolates from coastal waters of Orissa

Species	Strain No	Sensitive	Resistant	Marindex
Vibrio parahaemolyticus	*SOC-1*	*Ce^{30}, Cf^{5}, Ak^{30}, G^{10}, Am^{30}*	*A^{10}, T^{30}, S^{10}, $Nx^{10}Co^{25}$*	*50%*
Vibrio cholerae	SOP-18	Cf^{5}, Nx^{10}, Ce^{30}, Ak^{30}, T^{30}	A^{10}, Am^{30}, Co^{25}, $G^{10}S^{10}$	50%
Vibrio alginolyticus	SOD-8	A^{10}, Am^{30}, Cf^{5}, Nx^{10}, Co^{25}, Ce^{30}, G^{10}, Ak^{30}	- T^{30}, S^{10}	20%
Vibrio vulnificus	SOG-31	A^{10}, Am^{30}, Cf^{5}, Nx^{10}, G^{10}, Ce^{30}, Ak^{30}, S^{10}	- Co^{25}, T^{30}	20%
Vibrio fluvialis	*SOP-21*	*Cf^{5}, Nx^{10}, Co^{25}, Ce^{30}, Ak^{30}, Am^{30},T^{30}*	*A^{10}, G^{10}, S^{10}*	*30%*
Vibrio nereis	SOD-10	Cf^{5}, Nx^{10}, Co^{25}, Ce^{30}, Ak^{30}	A^{10}, G^{10}, S^{0} Am^{30}, T^{301}	50%
Vibrio diazotrophicus	SOC-5	Cf^{5}, Nx^{10}, Ce^{30}, Ak^{30}	Am^{30}, $G^{10}A^{10}$, Co^{25}, T^{30}, S^{10}	60%

*Superscripts indicates the potency of the discs in microgram .
Antibiotics used: Ce-Cefotaxim, Nx-Norfloxacin, Cf-Ciprofloxacin, Ak-Amikacin, Am-Amoxycillin, G-Gentamycin, A-Ampicillin, Co-Co-Trimoxazole, T-Tetracycline, S-Streptomycin

Table 3. MIC value of essential oils from plant sources to Vibrios isolated from coastal waters of Orissa

Organisms	Strain No.	Essential oils (ml/ml)					
		Turmeric leaf oil	Turmeric rhizome	Carrot seed oil	Celery seed oil	Japanese mint leaf oil	Ginger rhizome oil
Vibrio parahaemolyticus	SOC-1	0.97	62.5	3.8	3.8	<0.97	7.81
Vibrio cholerae	SOP-18	0.97	125	3.8	7.81	<0.97	7.81
Vibrio alginolyticus	SOD-8	0.97	125	7.81	3.8	<0.97	15.62
Vibrio vulnificus	SOG-31	0.97	125	7.81	7.81	<0.97	15.62
Vibrio fluvialis	SOP-21	1.9	62.5	7.81	7.81	1.9	15.62
Vibrio nereis	SOD-10	0.97	125	3.8	3.8	<0.97	7.81
Vibrio diazotrophicus	SOC-5	1.9	125	15.62	7.81	1.9	62.5

coriander and aniseed essential oils against *V. cholerae*, *V. cholerae* 0139 and *V. alginolyticus*. Further, as these essential oils are edible and their odour does not make any change to the delicately prepared food items (Lis-Balchin *et al.* 1998), the possibility of their applications as a preservative agent as well as flavouring agent for preservation of sea foods is quite suggestive.

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