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Antibiotic sensitivity test against *Vibrio* sp. isolated from Rameswaram Island

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Abstract

Multiple drug resistant organisms are becoming common for infections in acute and long-term care units in hospitals. The emergence of the resistant bacteria has created a major concern and an urgent need for new antibacterial agents. India has an enormous and growing problem of antibiotic use and abuse in newborn care. *Vibrio* sp. are becoming increasingly important as causative agents for human diseases. The present investigation deals with antimicrobial susceptibility test using commercially available antibiotic discs against 10 different *Vibrio* species isolated from Rameswaram coastal waters. *Vibrio* species like *V. alcaligenes, V. alginolyticus, V. cholerae, V. parahaemolyticus* , and *V. vulnificus* were found to show resistant or lesser activity against one or more antibiotics.

INTRODUCTION

The treatment of infectious disease took a great leap forward with the first clinical use of penicillin in 1941. Antibiotics are considered to be "miracle drugs" that are extensively used for the treatment of infectious diseases in human, as well as for food-producing livestocks. Antibiotics greatly enhanced the human life expectancy, reduced mortality, and improved quality of life and almost won the war against many infectious diseases. But soon after antibiotics were introduced, bacteria adapted their own defenses against antibiotics to avoid possible extinction (Levy, 1992) and began demonstrating resistance to these compounds. India has an enormous and growing problem of antibiotic use and abuse in newborn care. This is resulting in the selection of increasingly resistant Gram-negative and Gram-positive bacteria (Stone *et al*., 2003). The increased use of antibiotics in medicine and agriculture over the past 60 years has been accompanied by sharp increases in the prevalence of microorganisms that are resistant to antibiotics. Antibiotic resistance is an almost inevitable result of antibiotic use. Microbes develop ways to evade the effects of antibiotics in response to exposure to these drugs (Cohen, 2006). The misuse of antibiotics led to the emergence of drug resistant microorganisms. For example, the strains of *Vibrio cholerae* in India became resistant to several antibiotics, and its multi drug resistance is increasing. Studies showed that *V. cholerae* strains isolated from patients in eastern India are resistant to several old as well as new antibiotics, including Ampicillin, Tetracycline, Furazolidone, Norfloxacin, and Ciprofloxacin (Mudur, 2000). A number of factors contribute to antibiotic resistance viz., misuse and overuse of antibiotics in human, patients' demand for and receipt of antibiotics and failure to finish an antibiotic prescription. Multiple drug resistant organisms are becoming common cause for infections in acute and long term care units in hospitals (Mudur, 2000). The emergence of the resistant bacteria has created a major concern and an urgent need for new antibacterial agents (Davies, 1997).

Microbiologists point out that, pharmaceutical companies are producing drugs containing two antimicrobial agents, such as combination of norfloxacin and metronidazole, or ciprofloxacin and tinidazole. The market is flooded with such irrational combinations that are often wrongly prescribed for diarrohea. The widespread misuse of antibiotics in India has led to the emergence of multi-drug resistant cholera, in a rerun of events that involved typhoid a decade ago (Mudur, 2000).

The Rameswaram Island, South India is nearly 88 sq. km. bounded by the Palk Bay in north and east, Gulf of Mannar in the south and connected to the mainland by Pamban Bridge. The northern side of this island has the Palk Bay coast and the Gulf of Mannar coast covers the southern side. The heterotrophic bacteria are the major components of marine as well as estuarine ecosystems. They play an important role in the degradation of organic matter and in the recycling of nutrients in the sea (Sathiyamurthy *et al*., 1990). Seasonal changes in bacterial and fungal population are likely to be more pronounced in the coastal environment than the offshore environment. Pathogenic bacteria, which can cause diseases like typhoid fever, dysentery, diarrhoea and cholera etc., are found in the coastal region (Hari Muraleedharan, 2007). Generally polluted water contains a large number of pathogens

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and their presences are indirectly detected by studying indicator organisms. The most common indicator organisms are a group of microbes called coliforms, the presence of which simply indicates that pathogens are expected to be present (Sinton *et al.,* 2002). The present investigation deals with antimicrobial susceptibility test using commercially available antibiotic discs against 10 different *Vibrio* species isolated from Rameswaram Coastal waters.

MATERIALS AND METHODS

Muller Hinton Agar plates were prepared aseptically for antibiotic sensitivity (Bauer *et al.,* 1966). *Vibrio* species isolated from water and sediment samples were identified as *V. alcaligenes, V. alginolyticus, V. cholerae, V. harveyi, V. fischeri, V. marinus, V. mimicus, V. parahaemolyticus, V. vulnificus,* and *V. proteolyticus.* Three to four similar colonies were selected and transferred to 5.0 ml of Tryptone Soya Broth (Hi-Media Ltd., India). The inoculum was incubated at 35ºC for 2-8 hours for the development of moderate turbidity.

Vibrio species were tested against Ampicillin (25µg), Chloramphenicol (25 µg), Ciprofloxacin (10 µg), Erythromycin (15 µg), Gentamycin (50µg), Kanamycin (30µg), Neomycin (30 µg), Oxytetracycline (30 µg), Penicillin-G (5units) and Streptomycin (30µg) (Hi-Media Ltd., India). The sterile non-toxic cotton swab was dipped on a wooden applicator into the standardized inoculums (turbidity so adjusted, as to obtain confluent growth on the Petri plates) and rotated the soaked swab firmly against the upper inside wall of the tube to express excess fluid. The entire agar surface of the plate was streaked with the swab three times, turning the plate at 60 º angle between each streaking and allowed them to dry for 5 to 15 minutes with lid in place. The discs were deposited at centers (24 mm) and incubated immediately at 37 ºC. The inhibition zones were measured and the diameter of the zones were recorded.

RESULTS AND DISCUSSION

In the present research, *Vibrio cholerae* was found to show high resistance against Ampicillin, Gentamycin, Neomycin and Penicillin-G (Table 1). *V. parahaemolyticus, V. alcaligenes, V. alginolyticus* and *V. vulnificus* also showed resistance to Ampicillin. Bacteria can develop resistance to antibiotics usually but not always after exposure to the antibiotics. These types of resistance might be due to changes in the bacterial genome. In bacteria, acquired immunity is a type of immunity developed by the microorganisms in response to environmental and other changes (Franklin, 1992).

Oxytetracycline and Ciprofloxacin registered good inhibition zones with all *Vibrio* species tested. Maximum (35 mm) zone of inhibition was shown by *V. mimicus*, and minimum zone of inhibition (20 mm) by *V. alginolyticus.* The maximum zone of inhibition shown by *V. cholerae* (22 mm) was for Oxytetracyclin. Garg *et al.* (2000) also reported that *V. cholerae* isolates were generally susceptible to tetracycline. Oxytetracycline has been used extensively for aquatic microbial therapy as well (Herman, 1969).When Oxytetracycline tested. In the present investigation the antibiotic Ciprofloxacin showed maximum zone of inhibition (36 mm) against *V. proteolyticus* and *V. marinus*. In India, ciprofloxacin resistant *Vibrio* sp. was reported earlier by Taneja *et al*. (2004). Chloramphenicol also reacted well with all the strains except *V. vulnificus.* Chloramphenicol has been considered as the most effective drug for controlling bacillus necrosis caused by *Vibrio* sp. in the hatcheries of *Ostraea edulis* (Loideiros *et al.,* 1987).

In the present investigation, *Vibrio* species like *V. cholerae, V. vulnificus, V. alcaligenes, V. alginolyticus,* and *V. parahaemolyticus* showed resistance or lesser activity against one or more antibiotics; these high figures are comparable to those reported elsewhere in *V. cholerae* or other Gram-negative *Vibrio* species (Kelch and Lee, 1978; Beaucage and Fox, 1979; Amaro *et al.,* 1990; Morinigo *et al*., 1990). The prevalence of antibiotic resistant microorganisms is ecologically very important and this character is plasmid borne (Isaacs, 2005). The data suggested that, antibiotic resistant *Vibrio* sp. may survive better than sensitive organisms in surface waters. This may be due to conditions similar to those described by Amaro *et al. (*1990) in *V. cholerae* and Grabow *et al.* (1973) in Gram-negative bacteria. They suggested a plasmid R-factor mediated antibiotic resistance increased survivability.

V. harveyi, V. fischeri, V. marinus V. mimicus, and V. proteolyticus were sensitive to all antibiotics tested, But strains like *V. alcaligenes, V. alginolyticus, V. cholerae, V. parahaemolyticus* and *V. vulnificus* showed resistance against Erythromycin, Ampicillin, and Penicillin-G only (Table.1). The variation in resistant pattern of different species of *Vibrio* was noticeable. In general, resistance of bacteria to antibiotics may be due to enzymatic destruction, impermeability of the cell wall by the antibiotic and addition of chemical group to an antibiotic molecule (Sathiyamurthy *et al.,* 1991). The outer membrane establishes a permeability barrier against antibiotics. An organism may lack the transport system for the antibiotics or lack the target sites or the reaction is targeted by the antibiotics (Ibezim, 2005).

Gram-negative bacteria like *Klebsiella* can produce extended spectrum of beta lactamases (ESBL) that render its resistant to almost all antibiotics (Stone *et al*., 2003). Gram-positive bacteria can carry genes conferring Vancomycin resistant enterococci (VRE) and genes coding for Methicillin resistance (Gordon and Isaacs, 2004.), such as Methicillin resistant *Staphylococcus*

Table 1. Antibiotic Sensitivity test inhibition zone (mm) against different *Vibrio* spp. Isolated from Rameswaram Island.

Antibiotics	Conc/Disc (µg)	V. cholerae non-O1	V. parahaemolyticus	V. proteolyticus	V. alginolyticus	V. mimicus	V.harveyi	V.alginolyticus	V. mimicus	V.fischeri	V. vulnificus
Ampicillin	25	0	0	6	0	16	8	0	10	12	0
Chloramphenicol	25	13	7	30	10	15	25	13	19	28	-
Ciprofloxacin	10	16	21	36	5	30	28	16	36	34	25
Erythromycin	15	11	-	12	-	9	9	-	11	13	-
Gentamycin	50	-	10	18	16	22	12	-	13	15	10
Kanamycin	30	7	-	25	10	13	14	6	24	9	16
Neomycin	30	-	-	26	20	26	22	18	10	25	9
Oxytetracyclin	30	22	21	29	24	35	32	20	33	26	24
Penicillin-G	5 units	-	-	15	-	12	23	-	16	11	-
Streptomycin	30	14	12	17	-	19	16	8	14	16	8

aureus (MRSA) and Methicillin resistant *Staphylococcus epidermidis* (MRSE). Recently a warning has been issued by WHO (2000) that unless we limit our use of antibiotics we might be entering a post antibiotic era.

Tetracycline has been so widely used and misused for decades and has become worthless for many of the infections that once designated it as a 'wonder drug' (Ibezim, 2005). All strains were found to be resistant to Tetracycline, Streptomycin, Sulfathiazole and Trimethoprim (Falbo *et al*., 1999). In India, drug resistance is of considerable importance to microbiologists and is posing a major therapeutic problem for the public and health authorities.

In the present study, it was interesting to note that, all the *Vibrio* species were sensitive to Ciprofloxacin and Oxytetracycline. Ciprofloxacin and Oxytetracycline were found to be most effective against all *Vibrio* strains tested while Chloramphenicol, Neomycin, Streptomycin, Gentamycin and Kanamycin showed moderate activity against the same. Low resistance or no sensitivity was noticed for Ampicillin, Erythromycin and Penicillin-G. Ampicillin was acid-stable but the absorption was slower and more irregular than that of some of the later developed ester analogues (Williams, 1983). This investigation proved that *V. cholerae, V. vulnificus, V. alcaligenes, V. alginolyticus,* and *V. parahaemolyticus* showed the resistant pattern against most of the antibiotics tested.

CONCLUSION

In the present investigation *V. mimicus, V. proteolyticus, V. harveyi, V. fischeri and V. marinus* were sensitive to all antibiotics tested. But strains like *V. cholerae, V. vulnificus, V. alcaligenes, V. alginolyticus,* and *V. parahaemolyticus* showed resistance against Erythromycin, Ampicillin, and Penicillin-G. The variation in resistant pattern of different species of *Vibrio* was noticeable. Antibiotic resistance is an almost inevitable result of antibiotic use. Microbes develop ways to evade the effects of antibiotics in response to exposure to these drugs. So there is an enormous chance of cholera outbreak caused by multi-drug resistant *Vibrio* sp. to the coastal community. This alarming incidence of antibiotic resistance in bacteria of medical importance indicated that there is an urgent need for new and effective therapeutic agents against them.

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