

Antibiotic and heavy metal resistance profile of pathogens isolated from infected fish in Tuticorin, south-east coast of India

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ABSTRACT

The antibiotic and heavy metal resistance profile of pathogens isolated from infected fishes of the Thirespuram landing centre, Tuticorin, south-east coast of India are described. The study was carried out to determine the effectiveness of antibiotic and heavy metal resistance profile to control bacterial diseases in fishes. The isolates showed multiple antibiotic resistance patterns and were tolerant to almost all the heavy metals tested. All the isolated pathogens except *Vibrio ordalli* were resistant against Ampicillin, Bacitracin, Carbenicillin and Nalidixic acid. All the pathogens were sensitive towards Gentamycin, Streptomycin and Chloramphenicol. *Vibrio harveyi* was resistant to all the antibiotics used. The results provide information on the correlation between bacterial antibiotic resistance and heavy metal tolerance. These observations indicate that the occurrence of fish pathogens with high incidence of resistance for antibiotics and heavy metals may pose risk to fish fauna and public health.

Keywords: Antibiotic resistance, Fish pathogens, Heavy metals, Seafoods

Introduction

Bacterial resistance to antibiotics and heavy metals is an increasing problem in today's society. Resistance to antibiotics and heavy metals are acquired by a change in the genetic makeup of a bacterium, which can occur either by a genetic mutation or by transfer of resistant genes from bacteria in the environment. Products such as disinfectants, sterilants, and heavy metals used in industry and in household products along with antibiotics are creating a selective pressure that leads to mutation in microorganisms that will allow them to survive better and multiply (Baquero *et al.*, 1998).

The occurrence of antibiotic-resistant pathogenic bacteria in surface waters and aquaculture environments is a wellknown phenomenon that carries a negative impact on public health and safety of fish supply (González *et al.*, 1999; Toroglu *et al.*, 2005). Recent studies showed that seafood and fish products are quite often contaminated (Samanta *et al.*, 2005; Ayas *et al.*, 2007). The presence of antibiotic-resistant bacteria in fish throughout the world has been documented (Nonaka and Suzuki, 2002; Matyar *et al.*, 2004). Heavy metals entering the fish have a possibility to get accumulated in different parts of the body and the residual amount can build upto toxic levels (Ayas *et al.*, 2007; Kumar and Achyuthan, 2007; Yoon *et al.*, 2008).

Heavy-metal resistance in a number of different bacterial genera has been shown to be plasmid mediated (Summers and Silver, 1972) and in some cases, it is present together with antibiotic resistance (Nakahara *et al.*, 1977). In instances where these genes are grouped on the same plasmid, it is reasonable to assume that either heavy metals or antibiotics could serve as selection pressure for population of bacteria hosting these plasmids.

The selective process leading to the emergence and maintenance of bacteria resistant to antibiotics are mainly brought about by the incorrect or abusive utilization of drugs (Anderson, 1968). Similarly bacterial resistance to heavy metals seems to be directly related to the presence of these elements as environmental pollutants (Khesin and Karasoya, 1984).

Studies need to be carried out to determine the distribution of antibiotic-resistant and heavy metal tolerant bacteria in freshwater basins, estuaries, municipal drinking waters, sewage waters and marine waters. There are many reports on algae, bacteria, fungi, fishes, marine animals and higher plants that remove or accumulate large amounts of heavy metals from their external environment (Samanta *et al.*, 2005; Gupta and Srivastava, 2006; Ayas *et al.*, 2007; Kumar and Achyuthan, 2007). The importance of

gram-negative bacteria has increased since the advent of broad-spectrum antibiotics because the organisms often carry multiple antibiotic resistances (Mims *et al.*, 1999).

Thirespuram Fish Landing Centre (lat. 08°48.957' and long. 078°009.795') near Tuticorin in the south-east coast of India, is highly polluted due to the rich inflow of domestic sewage highly contaminated with faecal matter. In addition, this shore is also having a rich influx of heavy metal contamination due to the presence of port. In the present work, heavy metal-resistant microorganisms were detected and resistance to antibiotics in the pathogenic bacteria isolated from the marine samples obtained from the polluted fish landing centre.

Materials and methods

Water samples and sediments were collected from sampling sites in sterile polythene bags and transported on ice to the laboratory and processed for bacteriological analysis within 6-8 h of collection. Infected finfish and shellfish samples were collected from Thirespuram Landing Centre and 10 g sample was weighed aseptically and transferred to 100 ml of sterile seawater for subsequent homogenization. The homogenized sample was serially diluted with sterile seawater and plated by spread plate method onto Zobell Marine (ZB) Agar medium (Hi Media, India) prepared with sterile seawater (Elliot *et al.*, 2001). The organisms were isolated and identified by morphological and biochemical characteristics based on Bergey's Manual of Determinative Bacteriology. Ten clinical antibiotic discs (Hi Media, Mumbai, India) with concentration of drug per disc as stated in parentheses were used in the test: ampicillin (10 µg), chloramphenicol (30 µg), bacitracin (10 µg), erythromycin (15 µg), gentamycin (10 µg), streptomycin (10 µg), oxytetracycline (30 µg), vancomycin (30 µg), penicillin (10 µg) and neomycin (30 µg). Antibiotic resistance of the selected pathogenic bacteria was determined by the disc diffusion method (Bauer *et al.*, 1966). Bacterial pathogens were multiplied in broth tubes (ZB) at 37 °C for 48 h and swabbed on Mueller-Hinton agar plates. Respective antibiotic paper discs were then placed on to the surface of the seeded medium and incubated at 37 °C for 24 h. The inhibition zone was measured and bacterial pathogens were classified

as antibiotic resistant according to the manufacturers instructions.

For testing the heavy metal tolerance of the isolated pathogens, freshly prepared nutrient agar plates were amended separately with heavy metals salts such as mercury (HgCl₂), copper (CuSO₄), zinc (ZnSO₄), iron (FeCl₃), silver (AgNO₃), nickel (NiCl₂), cobalt (CoCl₂), cadmium (CdCl₂), arsenic (AsO₂), lead (PbNO₃) and chromium (K₂Cr₂O₇) at various concentrations ranging from 25 - 400 µg ml⁻¹. The plates were inoculated with test cultures and incubated at room temperature for 48 h. The minimum concentration of metal, inhibiting complete growth was taken as Minimum Inhibitory Concentration (MIC) (Cervantes *et al.*, 1986). From the raw data, correlation coefficient was calculated at 0.05 and 0.01 levels of significance.

Results and discussion

Bacteriological analysis revealed that fish samples and water were contaminated with *Vibrio* sp. The *Vibrio* count ranged from 6.03 to 11.07 log. However, lower number of *Vibrio* were detected in sediments compared to other samples (Table 1). The prevalence of *Vibrio* sp. in each sample indicated that these were the major contaminants of seafoods in the study area. Presence of *Serratia* sp. and *Proteus* was from 1.25 to 2.10 log and 1.26 to 3.99 log respectively. The total heterotrophic bacteria ranged from 2.10 - 3.33 log. It is important to note that contamination due to *Aeromonas* was comparatively less and detected only in finfish samples. *Salmonella* was not detected in any of the sample. Marine beaches are often subjected to considerable anthropopressure (Weclawski *et al.*, 2000). To date, bacteriological studies of marine beaches are concerned mainly with their sanitary pollution and bacterial numbers (Papadakis *et al.*, 1997; Mudryk *et al.*, 2001) and to the best of our knowledge, only few studies were aimed at the problem of bacterial resistance to antibiotics, although this problem is of a great significance in the ecology of these microorganisms and in public health (Qureshi and Qureshi, 1992).

Pathogenic marine bacteria isolated from the infected fishes are characterized by large differences in the level of resistance to the studied antibiotics (Table 2). Nearly 60% of the pathogenic bacteria isolated were 100% resistant to

Table 1. Microbiology of samples collected from Thirespuram fish landing centre

Source	Bacterial count (log)					
	THB	<i>Serratia</i>	<i>Proteus</i>	<i>Vibrio</i>	<i>Salmonella</i>	<i>Aeromonas</i>
Finfish	3.33 ± 1.57	1.25 ± 0.15	1.27 ± 1.32	6.69 ± 2.35	ND	1.99 ± 0.02
Shellfish	2.10 ± 0.02	2.10 ± 0.09	3.99 ± 2.73	6.03 ± 2.45	ND	ND
Seawater	2.41 ± 0.05	1.33 ± 0.08	1.92 ± 0.90	11.07 ± 4.70	ND	ND
Sediments	2.29 ± 0.12	1.75 ± 0.07	1.26 ± 1.75	3.21 ± 2.27	ND	ND

THB- Total Viable Count; ND – Not Detected; Numbers are based on three replicates

Table 4. Inter correlation between antibiotic resistance and heavy metal tolerance

Heavy metals	Antibiotics									
	A	B	Cb	C	G	K	Na	S	T	Va
Hg	-0.398	0.245	0.583**	0.282	-0.701	0.268	-0.141	-0.605	0.141	-0.453
As	-0.357	-0.352	0.025	-0.183	-0.662	-0.412	-0.601	-0.047	-0.306	0.039
Cu	-0.595	-0.052	0.551**	0.177	-0.798	-0.398	-0.443	-0.335	0.242	-0.359
Cr	-0.549	0.224	0.427*	0.302	-0.428	-0.165	-0.191	-0.605	-0.156	-0.480
Pb	-0.576	0.052	0.508**	0.337	-0.753	-0.094	-0.362	-0.441	0.027	-0.403
Cd	-0.453	-0.013	0.409*	0.098	-0.814	-0.028	-0.403	-0.435	-0.060	-0.288
Ni	-0.496	0.114	0.442*	0.283	-0.706	0.117	-0.329	-0.452	-0.100	-0.385
Co	-0.453	-0.013	0.409*	0.098	-0.814	-0.028	-0.403	-0.435	-0.060	-0.288
Ag	-0.425	0.173	0.545**	0.234	-0.753	0.185	-0.224	-0.568	0.084	-0.414
Fe	-0.294	-0.173	0.301	-0.295	-0.807	-0.252	-0.465	-0.271	0.069	-0.097
Zn	-0.278	-0.221	0.252	-0.277	-0.793	-0.282	-0.460	-0.263	0.025	-0.055

* - $p < 0.05$; ** - $p < 0.01$; unmarked – not significant

environment of India and was irrespective of the current levels of pollution (De *et al.*, 2003).

Public health risk was further stressed by the occurrence of high frequency (94%) of strains that are typically resistant to more than one antibiotic. A routine monitoring system will allow detection of outbreaks for early control and changing drug resistance pattern (Roychowdhury *et al.*, 2008). In addition, antibiogram updates may be useful in characterizing the isolates. The multiple resistance of isolates to some antibiotic classes are of great public health concern and they underscore the need to curtail the indiscriminate use of antibiotics on humans and animals (Ashok Kumar *et al.*, 2009).

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