World J Microbiol Biotechnol (2008) 24:209–217
DOI 10.1007/s11274-007-9458-8

ORIGINAL PAPER

Dominant chloramphenicol-resistant bacteria and resistance genes in coastal marine waters of Jiaozhou Bay, China

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Received: 15 April 2007 / Accepted: 3 June 2007 / Published online: 24 June 2007
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Abstract Studies of abundance, diversity and distribution of antibiotic-resistant bacteria and their resistance determinants are necessary for effective prevention and control of antibiotic resistance and its dissemination, critically important for public health and environment management. In order to gain an understanding of the persistence of resistance in the absence of a specific antibiotic selective pressure, microbiological surveys were carried out to investigate chloramphenicol-resistant bacteria and the chloramphenicol acetyltransferase resistance genes in Jiaozhou Bay after chloramphenicol was banned since 1999 in China. About 0.15–6.70% cultivable bacteria were chloramphenicol resistant, and the highest abundances occurred mainly in the areas near river mouths or sewage processing plants. For the dominant resistant isolates, 14 genera and 25 species were identified, mostly being indigenous estuarine or marine bacteria. Antibiotic-resistant potential human or marine animal pathogens, such as *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis* and *Shewanella algae*, were also identified. For the molecular resistance determinants, the *cat* I and *cat* III genes could be detected in some of the resistant strains, and they might have the same origins as those from clinical strains as determined via gene sequence analysis. Further investigation about the biological, environmental and anthropogenic mechanisms and their interactions that may contribute to the persistence of antibiotic-resistance in coastal marine waters in the absence of specific antibiotic selective pressure is necessary for tackling this complicated environmental issue.

Keywords Antibiotic resistance · *cat* genes · Chloramphenicol · Jiaozhou Bay · Multiplex PCR

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Introduction

Extensive use and misuse of antibiotics in medication, veterinary, agriculture and aquaculture have caused antibiotic-resistant bacteria to be widespread (Kummerer 2004). Environmental and zoonotic antibiotic-resistant bacterial pathogens may directly cause human illness and the spread of food-borne diseases (Barza 2002; Sorum and L'Abee-Lund 2002). Resistance genetic material transfer from environmental bacteria to commensal microflora may also cause bacterial pathogens to carry antibiotic resistance, complicating disease prevention and treatment (Levy and Marshall 2004). Elevated number of resistant bacteria, especially clinical strains, could be found near civic or hospital sewage discharging locations (Choi et al. 2003). High incidences of resistant bacteria in response to antibiotic usage have also been reported in coastal maricultural areas (Herwig et al. 1997). Thus, aquatic environments may

serve as reservoirs of antibiotic resistance (Biyela et al. 2004), and coastal resistant bacteria represent a serious biotic contamination and a means for the spread and evolution of resistance genes and their vectors (Young 1993).

Jiaozhou Bay, located on the western coast of the Yellow Sea (N35°38′—36°18′, E120°04′—120°23′), is a shallow semi-enclosed water with a total area of 367 km^2 and an average water depth of 7 m on the eastern part of the Shandong Peninsula of China. Environmental quality of Jiaozhou Bay has deteriorated dramatically in the past three decades due to rapid development in agriculture, industry, urbanization and mariculture in the surrounding areas (Shen 2001; Ren et al. 2006). More than ten small rivers used to form the major sources of discharged industrial and sewage wastewater. Wastewater discharge from mariculture practices makes the situation even worse. Improperly processed hospital effluent and civic wastewater could be a source of resistant bacteria. Excessive usage of antibiotics in mariculture might also stimulate the propagation of resistant bacteria in Jiaozhou Bay.

Chloramphenicol was once extensively used until it was banned in 1999 in China. The molecular determinants of chloramphenicol resistance were well studied (Schwarz et al. 2004), and the *cat* I, II, III and IV genes that encode the chloramphenicol acetyltransferases are the most common resistance mechanisms found in aquatic bacteria (Yoo et al. 2003). However, most antibiotic resistance studies focused on pathogenic bacteria (Schwarz et al. 2004). Very few studies paid attention to the environmental aspect (Mudryk 2005; Henriques et al. 2006). In order to gain an understanding of the current status of chloramphenicol resistance in a typical coastal environment of China, and to define the corresponding resistance determinants, molecular techniques were employed to determine the phylogenies of the typical culturable resistant bacteria and their resistance genes in Jiaozhou Bay.

Materials and methods

Sample collection and processing

Surface seawater from ten stations (Fig. 1) of Jiaozhou Bay was collected aseptically on the 12th and 13th of September and the 11th and 12th of October of 2004. A multifunction water quality monitoring system (model U-2001, Horiba, Japan) was used for in situ environmental factor measurements (Table 1), except for water depth and diaphaneity. Water samples were kept on ice before being transferred to the laboratory, where samples for total microbial counts were fixed with formaldehyde (2% final concentration) and stored at 4°C in the dark, and samples for bacterial cultivations were processed immediately.

Microbial number counts

For total microbial and cultivable bacteria counts, epifluorescence microscopy and 2216 marine agar plating techniques were used as described previously (Dang et al. 2006a). For chloramphenicol-resistant bacteria counts, a membrane filter incubation technique was used (Rompre et al. 2002). Water sample from each station was diluted into a ten-fold series in sterilized artificial seawater and 3 ml portions were filtered onto sterilized 0.22-μm pore-size

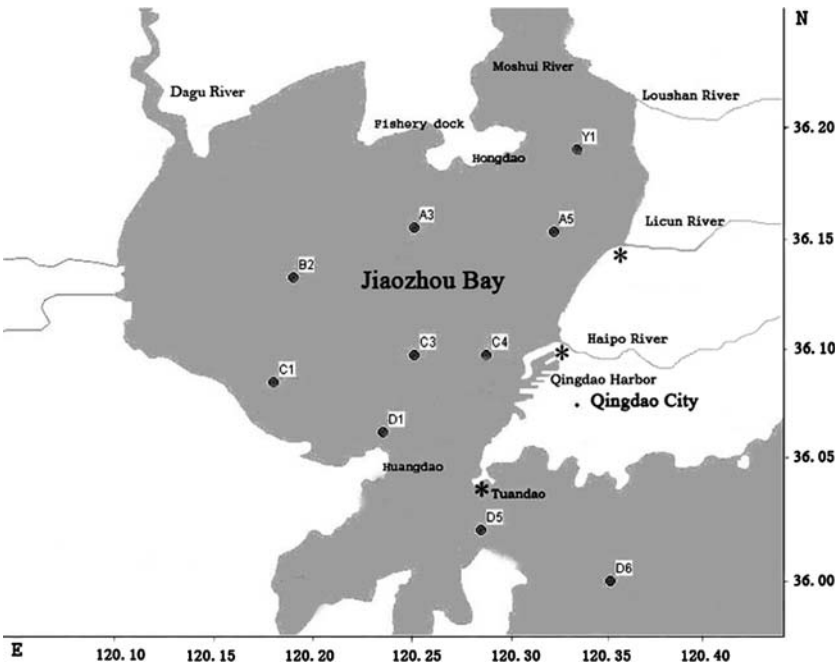

Fig. 1 Sampling stations of Jiaozhou Bay. The locations of sewage processing plants are indicated with the '*' symbols

Table 1 Measurements of in situ environmental factors of Jiaozhou Bay in September (Sep) and October (Oct) of 2004

Station	Water depth (m)		Water temperature (°C)		Salinity (‰)		pH		DO (dissolved O_2) (mg/l)		Diaphaneity (m)	
	Sep	Oct	Sep	Oct	Sep	Oct	Sep	Oct	Sep	Oct	Sep	Oct
A3	3.7	5.1	24.0	20.4	31.0	31.7	8.0	5.7	4.54	3.84	1.2	1.0
A5	9.0	8.0	24.4	21.0	31.0	31.8	7.9	5.7	4.04	3.53	1.0	1.5
B2	2.2	3.8	24.1	21.1	30.0	32.4	8.0	5.6	4.45	3.61	0.6	3.0
C1	3.7	4.6	24.5	20.5	31.0	31.7	8.0	5.8	4.40	4.55	1.1	2.8
C3	16.0	14.6	25.0	21.3	32.0	32.6	8.1	5.6	4.49	3.35	2.0	2.0
C4	6.9	4.6	24.9	20.8	32.0	32.1	8.2	5.6	4.56	3.32	1.5	1.0
D1	16.0	10.0	25.3	20.8	32.0	32.3	6.5	5.6	3.87	3.37	1.0	2.1
D5	38.0	39.0	25.0	22.1	32.0	32.7	8.1	7.0	4.24	3.49	1.8	5.0
D6	28.0	29.0	24.9	22.1	32.0	31.9	8.1	6.9	3.94	3.54	1.3	4.4
Y1	3.5	3.6	24.1	20.7	30.0	31.2	8.1	5.7	4.10	3.86	1.0	1.0

polycarbonate membrane filters. Triplicates filters were placed onto tryptic soy agar (TSA, Difco formula) plates supplemented with 3% (w/v) NaCl and 10 μg/ml chloramphenicol (CP_{10}). CP_{10}-resistant colonies on the highest or the second highest dilution (if there were too few colonies on the highest) positively growing plates were further screened with 30 μg/ml chloramphenicol (CP_{30}) based on the method described for antibiotic susceptibility tests (Yoo et al. 2003). Only CP_{30}-resistant isolates were counted as chloramphenicol-resistant bacteria, which were further assayed for higher resistance to 100 μg/ml chloramphenicol (CP_{100}).

Phylogenetic analysis of typical chloramphenicol-resistant isolates

CP_{100}-resistant bacteria were picked based on unique colony morphology and color features. Because all of these isolates were originally screened from the highest or the second highest dilution positively growing CP_{10} plates, they represented the dominant chloramphenicol-resistant bacteria in Jiaozhou Bay during our sampling period. Eventually, 60 isolates were selected for 16S rDNA sequencing and *cat* gene screening.

A simple boiling method was used for rapid bacterial genomic DNA extraction (De Medici et al. 2003). Primers 27F and 785R were used for 16S rDNA amplifications, and the standard PCR reactions and program were followed as described previously (Dang et al. 2006a). Primer 27F was also used for sequencing with purified PCR products as templates using an ABI 3770 automatic sequencer (Applied BioSystems, USA). The DNA fragments sequenced were usually about 700 bp long, covering at least the V1–V3 hypervariable regions of bacterial 16S rDNA. Bioinformatic determination of sequence affiliations followed standard methods (Dang and Lovell 2000; Dang et al. 2006a). Phylogenetic trees were constructed by using the DNADIST and NEIGHBOR programs within the PHYLIP package (version 3.6) (Felsenstein 1989).

Multiplex PCR detection of the *cat* genes

The above selected 60 CP_{100}-resistant isolates were screened for the *cat* I, II, III and IV genes via a multiplex PCR method developed by Yoo et al. (2003). The original primers (Table 2), experimental procedure and PCR amplification condition were used, only with a minor modification for total DNA (including genomic and plasmid DNA) extraction (De Medici et al. 2003). Representative PCR products were sequenced to confirm their identities as the *cat* genes.

Nucleotide sequence accession numbers

The 16S rDNA sequences determined in the current study have been deposited in the NCBI GenBank database under accession numbers DQ319006–DQ319065, and the representative *cat* gene sequences determined under accession numbers DQ319066 and DQ319067.

Results

Microbial abundance

During the sampling period, aquatic microbial density ranged from 2.7×10^6 to 3.8×10^7 cells/ml, cultivable

Table 2 Multiplex PCR primers for *cat* gene detection (from Yoo et al. 2003)

Primer	Target and position	Expected amplicon size (bp)
C-R	Common antisense primer for *cat* genes	
C-1	*cat* I, 245–264	349
C-2	*cat* II, 15–35	567
C-3	*cat* III, 307–326	275
C-4	*cat* IV, 122–141	451

bacterial abundance ranged from 1.9×10^2 to 5.2×10^5 CFUs/ml, and the density of chloramphenicol-resistant bacteria ranged from 1 to 9×10^2 CFUs/ml, which accounted for 0.15–6.70% of the total cultivable bacteria (Table 3). The highest abundance of chloramphenicol-resistant bacteria occurred at station A5, D6 and B2 in September, and A5, Y1 and D6 in October. Most of these stations are located close to a river mouth and/or sewage processing plant. Station A5 is near the river mouth of Licun and the Licun River sewage processing plant (Fig. 1), which discharged about 50,000 tons processed sewage into the bay daily. Station Y1 is close to the river mouths of Loushan and Moshui in the Hongdao mariculture area. Both stations are in the most polluted area of the Jiaozhou Bay (Li et al. 2005). Station B2 is located near the Dagu river mouth. Only station D6 is located outside of the Jiaozhou Bay.

Phylogenetic affiliations of the dominant chloramphenicol-resistant bacteria

Totally 348 CP_{30}-resistant bacteria (143 from September and 205 from October) were isolated from surface seawater samples of Jiaozhou Bay, and 100 isolates (43 from September and 57 from October) showed resistance to at least 100 μg/ml chloramphenicol. Based on colony morphology and color features, a total of 60 unique CP_{100}-resistant bacterial isolates (27 from September and 33 from October) were selected for further molecular analyses, which represent the most predominant chloramphenicol-resistant bacteria in Jiaozhou Bay during our sampling period.

All the 60 dominant chloramphenicol-resistant bacteria had more than 97% 16S rDNA sequence similarity to their best-match sequences retrieved from the GenBank database, and 85% of these isolates had 99% or higher 16S rDNA sequence similarity to their best-match sequences (data not shown). The phylogenetic tree constructed further verified their phylogenetic affiliations (Fig. 2). The chloramphenicol-resistant bacteria were quite diverse, at least 14 genera and 25 species could be identified. Most of the isolates belonged to the *γ-Proteobacteria*, and the remaining isolates belonged to the *α-Proteobacteria*, *Actinobacteria* or *Firmicutes*. Forty eight of the 60 sequenced chloramphenicol-resistant isolates were Gram-negative, and bacteria affiliated to *Pseudomonadaceae* (40%) and *Pseudoalteromonadaceae* (18.3%) formed the majority of the resistant microflora. *Pseudomonas* strains occurred in 3 and 9 of the 10 sampling stations in September and October respectively (Table 4). *Pseudoalteromonas* strains occurred in four stations in both sampling months. Gram-positive bacteria affiliated to *Micrococcaceae* and *Bacillaceae* only occurred in September, except for the *Kocuria* strain. Besides common estuarine and marine species, chloramphenicol-resistant bacteria affiliated to *Enterobacteriaceae*, such as *Escherichia coli*, *Klebsiella pneumoniae* and *Proteus mirabilis*, were also found to occur in the surface seawaters of Jiaozhou Bay during our sampling period, mainly in stations B2, C4 and Y1.

Molecular screening of the *cat* genes

Only 20% of the 60 dominant chloramphenicol-resistant bacteria isolated from Jiaozhou Bay were found to harbor the *cat* genes screened. Of the 12 *cat*-positive isolates, 10 carried *cat* I, and the remaining 2 carried *cat* III (Fig. 2). The molecular mechanisms encoding for chloramphenicol resistance of the remaining 48 strains could not be identified by the current multiplex PCR method, other mechanisms might exist.

Six of the 12 Gram-positive CP_{100}-resistant bacterial isolates carried a *cat* gene, actually *cat* I (Fig. 2). On the contrary, only 6 of the 48 Gram-negative CP_{100}-resistant bacteria carried a *cat* gene, including 3 of the 4 *Enterobacteriaceae*

Table 3 Abundance of total microbes (TM), cultivable bacteria (CB), chloramphenicol-resistant bacteria (CRB) and their percentages in the cultivable microflora (ORP) in Jiaozhou Bay of September (Sep) and October (Oct) of 2004

Station	TM (cells/ml)		CB (CFUs/ml)		CRB (CFUs/ml)		CRP (%)	
	Sep	Oct	Sep	Oct	Sep	Oct	Sep	Oct
A3	1.5×10^7	2.7×10^6	4,033	3,833	6	11	0.15	0.29
A5	3.8×10^7	8.2×10^6	516,000	24,333	900	767	0.17	3.15
B2	2.0×10^7	8.0×10^6	2,033	777	111	27	5.50	3.47
C1	1.4×10^7	5.3×10^6	360	1,433	5	11	1.40	0.77
C3	5.4×10^6	5.4×10^6	187	1,833	1	10	0.50	0.55
C4	9.5×10^6	4.4×10^6	7,133	9,233	23	22	0.32	0.24
D1	9.2×10^6	5.4×10^6	9,933	3,333	27	28	0.27	0.84
D5	2.2×10^7	3.8×10^6	4,933	5,767	32	19	0.65	0.33
D6	2.1×10^7	4.7×10^6	5,467	7,200	367	100	6.70	1.39
Y1	3.0×10^7	2.1×10^7	35,667	19,000	7	489	0.02	2.57

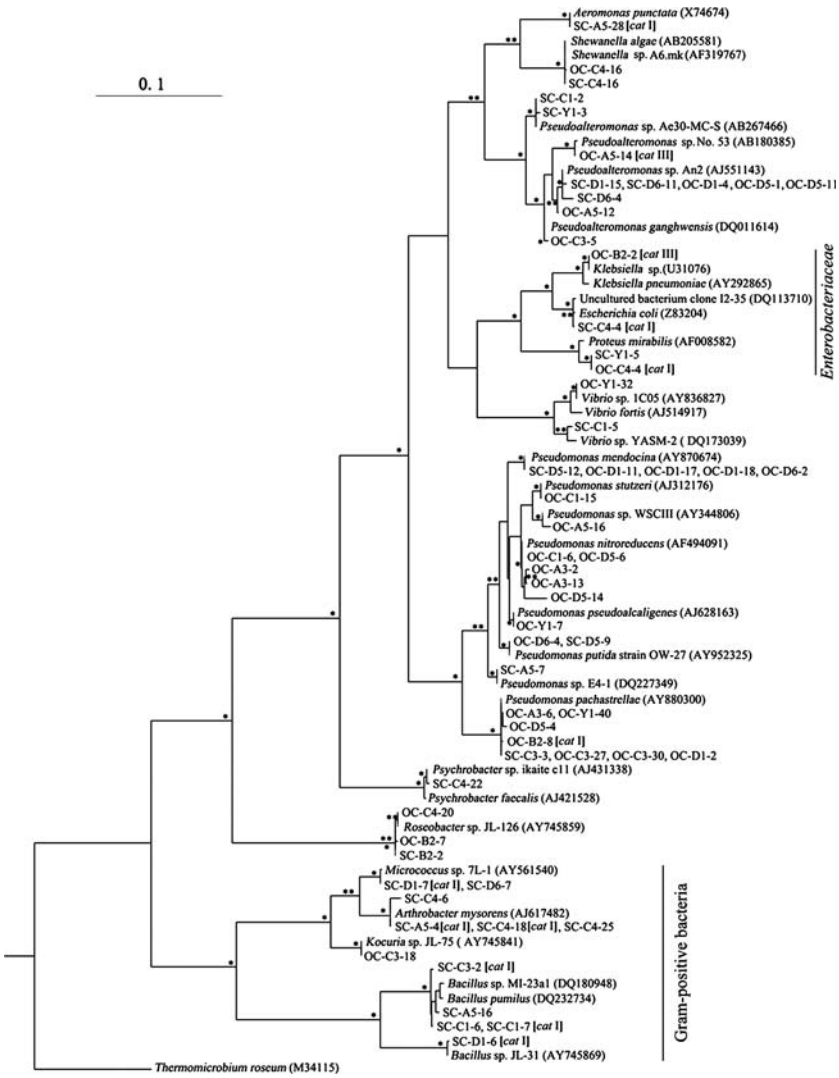

Fig. 2 Phylogenetic tree of the 60 dominant chloramphenicol-resistant bacteria isolated from Jiaozhou Bay constructed based on partial 16S rDNA sequences in a 620-bp frame length using neighbor-joining method with the Kimura 2-parameter model for nucleotide change. The tree branch distances represent nucleotide substitution rate, and the scale bar represents the expected number of changes per homologous nucleotide position. Bootstrap values greater than 70% of 100 resamplings are shown near nodes as '**', and those greater than 90% are shown as '*'. Bacterial strains are named with combinations of sampling time (SC for September and OC for October) and sampling station (such as A3, A5 and so on). The *cat* genes detected are labeled in "[ ]" besides the corresponding strains

bacteria identified. The *cat* I gene occurred in *Aeromonas*, *Arthrobacter*, *Bacillus*, *Escherichia*, *Micrococcus*, *Proteus* and *Pseudomonas*. The *cat* III gene occurred only in *Klebsiella* and *Pseudoalteromonas*, and only in October. Spatially, the *cat* genes mainly occurred in the sampling stations of A5, B2, C1, C3, C4 and D1 (Table 4), the main water body of the central Jiaozhou Bay.

The identities of the DNA fragments detected via the multiplex PCR method that might be the target *cat* genes were further verified by DNA sequencing of two representative PCR products, one for the possible *cat* I gene and the other for the possible *cat* III gene (Table 5). Both sequences had 99% or higher similarity to the best-match known *cat* genes retrieved from the GenBank database, and they also had 100% similarity to the best-match known chloramphenicol acetyltransferase sequences after they were translated into conceptual amino acid sequences. The multiplex PCR method (Yoo et al. 2003) provides a rapid and reliable approach for typical *cat* gene detection and differentiation in the marine environment studied.

Discussion

A basic understanding of the abundance, diversity and distribution of antibiotic-resistant bacteria and their resistance determinants is necessary for effective prevention and control of antibiotic resistance and its dissemination, critically important for public health and environment management. Without specific selective pressure from chloramphenicol per se, high abundance (at least 100 CFUs/ml of seawater) of resistant bacteria still could be found at some of the sampling stations in Jiaozhou Bay, such as A5, B2, D6 and Y1 (Table 3). River flow and/or wastewater discharge from the land might be the major source of antibiotic resistance in Jiaozhou Bay, as most of these stations are located near river mouths and/or sewage

Table 4 Distributions of the predominant chloramphenicol-resistant bacteria and the corresponding resistance genes in Jiaozhou Bay of September (Sep) and October (Oct) of 2004

Station	Predominant CP_{100}-resistant isolates		*cat* genes detected	
	Sep	Oct	Sep	Oct
A3[a]		*Pseudomonas*		
A5	*Aeromonas*	*Pseudoalteromonas*	*cat* I	*cat* III
	Arthrobacter	*Pseudomonas*		
	Bacillus			
	Pseudomonas			
B2	*Roseobacter*	*Klebsiella*		*cat* I
		Pseudomonas		*cat* III
		Roseobacter		
C1	*Bacillus*	*Pseudomonas*	*cat* I	
	Pseudoalteromonas			
	Vibrio			
C3	*Bacillus*	*Kocuria*	*cat* I	
	Pseudomonas	*Pseudoalteromonas*		
		Pseudomonas		
C4	*Arthrobacter*	*Proteus*	*cat* I	*cat* I
	Escherichia	*Roseobacter*		
	Psychrobacter	*Shewanella*		
	Shewanella			
D1	*Bacillus*	*Pseudoalteromonas*	*cat* I	
	Micrococcus	*Pseudomonas*		
	Pseudoalteromonas			
D5	*Pseudomonas*	*Pseudoalteromonas*		
		Pseudomonas		
D6	*Micrococcus*	*Pseudomonas*		
	Pseudoalteromonas			
Y1	*Proteus*	*Pseudomonas*		
	Pseudoalteromonas	*Vibrio*		

[a] None of the CP_{30}-resistant bacteria isolated from the seawater sample of station A3 in September was resistant to CP_{100}

Table 5 Representative *cat* gene sequences determined in the current study

Isolate	GenBank Closest sequence match	Sequence similarity	GenBank Accession number
OC-C4-4	*Photobacterium damselae* subsp. *piscicida* plasmid pSP9351, *cat* I gene	99%	D16171
OC-B2-2	*Mannheimia varigena* MVSCS1 plasmid, *cat* III gene	100%	AJ319822

processing plants. Station D6, which is located outside of the Jiaozhou Bay, also had quite high abundance of resistant bacteria. About 100,000 tons of processed sewage was discharged daily into the seawater from the Tuandao sewage processing plant, located at the mouth of the Jiaozhou Bay (Fig. 1). Tide and/or current might be the major mechanism for carrying resistance bacteria to the D6 station from the nearby contaminated areas. Thus, antibiotic resistance contamination in the coastal environment studied is complicated with a variety of factors. Comprehensive study and modeling might help understand the processes and mechanisms involved.

Two sources of chloramphenicol-resistant bacteria could be identified in Jiaozhou Bay, mainly the indigenous estuarine or marine bacteria and some of the terrestrial bacteria that might be related to anthropogenic activities. Terrestrial bacteria entering into seawater with antibiotic-resistant plasmids were proposed to be partially responsible for the prevalence of resistance genes in marine environments (Chandrasekaran et al. 1998). In Jiaozhou Bay, two sources

of *cat* genes could be identified, one from the indigenous estuarine or marine bacteria and another from the terrestrial bacteria potentially related to anthropogenic activities. The *cat*-positive *Enterobacteriaceae* bacteria were mainly detected in stations B2 and C4, close to the Dagu river mouth and the Haipo River sewage processing plant, which discharged about 80,000 tons of processed sewage into the bay daily. Although the seawater in the interior of Jiaozhou Bay is highly dynamic and water mixing happens frequently due to currents, tides and other hydrological factors, confined distribution of certain bacteria, especially the terrestrial resistant strains, still could be identified, indicating a strong terrestrial or anthropogenic impact on the distribution of certain antibiotic resistant bacteria. This finding is congruent with our previous study result about the spatial distribution of bacterial diversity in Jiaozhou Bay investigated via the microbial community 16S rDNA T-RFLP analysis method (Ren et al. 2006). The result of our current investigation indicates that sewage contamination might be a serious environmental problem of Jiaozhou Bay, at least in our sampling period. All the sewage processing plants in Qingdao had only the secondary treatment capacity. Wastewater collected from the nearby hospitals and communities might lack appropriate biological treatment, further processing of its bacteria load is needed before it can be discharged safely into the bay water.

The *cat* genes isolated from marine environments might have the same origins as those from clinical strains. *Escherichia coli* strains that harbored the *cat* I type of chloramphenicol resistance determinant have been identified from various sources around the world, including human and animal pathogens (Maynard et al. 2004; Bartoloni et al. 2006; Travis et al. 2006). A *Proteus mirabilis* strain was identified to harbor a variant of the *cat* I gene (Charles et al. 1985). Several Nigerian *Klebsiella pneumoniae* strains were identified to carry *cat* III-positive plasmids isolated from patients with community-acquired urinary tract infections (Soge et al. 2006). The isolation of similar *Enterobacteriaceae* bacteria carrying similar *cat* genes from Jiaozhou Bay indicates the wide distribution of these chloramphenicol-resistant bacteria in the world. Certain *Shewanella algae* strains were also found to be pathogenic (Holt et al. 2005). However, the molecular mechanism could not be identified for the chloramphenicol-resistant strains isolated from Jiaozhou Bay. To our knowledge, the current investigation is the first identification of the *cat* I gene in *Aeromonas punctata*, *Arthrobacter mysorens*, *Bacillus* spp., *Micrococcus* sp. and *Pseudomonas pachastrellae*, and the first identification of the *cat* III gene in *Pseudoalteromonas* sp.

While both Gram-positive and Gram-negative bacteria carried the *cat* I gene, only some Gram-negative bacteria carried the *cat* III gene in Jiaozhou Bay, at least in our sampling period. The *cat*-positive Gram-positive bacteria only occurred in September of our sampling period. What environmental and/or biological factors might influence the change of these resistance populations? Dramatic drops of temperature, pH and dissolved O_2 were observed in October (Table 1). How these changes were related to antibiotic-resistant bacteria composition in Jiaozhou Bay need to be further studied. Changes of the source of terrestrial resistance bacteria or resistance determinants via river runoff and sewage discharge might also contribute to the temporal and spatial shift of antibiotic-resistant bacteria populations in Jiaozhou Bay.

Chloramphenicol-resistant molecular determinants varied with geological locations and/or environments along the China coast of the Pacific Ocean. In our previous investigations, it was found that the detectable resistance genes were *cat* II and *cat* IV in the studied mariculture pond waters from the Dalian coast of China (Dang et al. 2006a, b), where the *cat*-positive bacteria were affiliated to marine *Vibrio* and *Pseudoalteromonas*. In the currently studied coastal environment, *cat* I and *cat* III were detected and more diverse resistant bacteria species were identified. The Dalian mariculture ponds received frequent application of various antibiotics, such as oxytetracycline, penicillin, streptomycin, enrofloxacin and furanzolidone, for bacterial disease prevention and treatment of the cultured marine animals. Persistent and high combinational antibiotic selective pressure might eliminate some of the bacterial diversity. On the contrary, it would be normal to expect a higher diversity of antibiotic-resistant bacteria in natural marine environments, though the currently studied Jiaozhou Bay was highly disturbed by anthropogenic activities and terrestrial resistant bacteria contaminations.

It has been found that chloramphenicol-resistant bacteria might persist in the environments even after the drug use has been stopped (Schwarz et al. 2004). This is probably due to the phenomenon that chloramphenicol resistant genes derived from limited sources could be transferred among aquatic microbes without a high specific selective pressure (Yoo et al. 2003). Cross-resistance or coresistance caused by cross-selection or coselection might be the mechanism involved (Alonso et al. 2001; Courvalin and Trieu-Cuot 2001; Schwarz et al. 2004). Besides chloramphenicol acetyltransferases encoded by the *cat* genes, chloramphenicol resistance could also be caused by multidrug transporters (Poole 2005), which might provide the major cross-resistance of chloramphenicol in the environment studied, as the *cat* genes were detected in only 20% of the resistant isolates. For the mechanism of coresistance, the use of one antibiotic could increase levels of resistance not only to that specific drug but also to many others, even those using very different modes of antibacterial action (Kummerer 2004). It has been found that coselection by

other antibiotics, such as oxytetracycline, could be the molecular mechanism responsible for the maintenance of the *cat* genes in some of the maricultural environments in China (Dang et al. 2006a, b). Besides antibiotics, other reagents might also contribute to the cross-selection and/or coselection mechanisms of chloramphenicol resistance. Jiaozhou Bay, especially its eastern coast, was highly polluted by heavy metals and other contaminants (Chen et al. 2005; Li et al. 2006). Exposure to heavy metals and other toxicants may confer resistance to antibiotics in the environments (Schwarz et al. 2004; Baker-Austin et al. 2006). Plasmids harboring resistance to both heavy metals and antibiotics have been reported in *Enterobacteriaceae* bacteria, such as avian pathogen *Escherichia coli* (Johnson et al. 2005) and human pathogen *Salmonella enterica* serovar Typhi CT18 (Parkhill et al. 2001), and in marine *Pseudomonas* (Rajini Rani and Mahadevan 1992). Heavy metal contamination might represent a long-standing, widespread and recalcitrant selection pressure, potentially contributing to the maintenance and spread of antibiotic resistance in Jiaozhou Bay.

Deterioration of coastal environmental quality is becoming a global issue due to various pollutions and contaminations. Oxygen-starved areas in bays and coastal waters have been expanding since the 1960s, and the number of known dead zones around the world has doubled since 1990 (UNEP 2004). The occurrence and persistence of diverse antibiotic-resistant bacteria and their resistance genes makes the situation even worse. Epidemics of pathogens in coastal marine environments may spread in large scale and at extremely rapid speed, due to their potential for long-term survival outside the host and the lack of barriers to dispersal (McCallum et al. 2003). Long-term comprehensive surveys and molecular mechanism and process studies will be needed for most of the coastal environments having similar situations to Jiaozhou Bay, in order to decode the biological, environmental and anthropogenic factors and their interactions that may contribute to the antibiotic resistance issue, for winning the battle for the health of humans and environments.

Acknowledgements This work was financially supported by the Pilot Projects of Knowledge Innovation Project of Chinese Academy of Sciences grants KZCX2-YW-211-03, KZCX3-SW-214, KZCX3-SW-223 and KZCX3-SW-233, the National Natural Science Foundation of China grants 40476058 and 40576069, and the LSC2-3-1 grant from IOCAS.

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