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ORIGINAL PAPER

Incidence of diverse integrons and β-lactamase genes in environmental *Enterobacteriaceae* isolates from Jiaozhou Bay, China

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Abstract Environmental microbiology investigation was performed to determine the molecular diversity of β-lactamase genes among ampicillin-resistant bacteria from Jiaozhou Bay. β-lactamase genes were detected in 93.8% of the bacterial isolates identified as *Enterobacteriaceae*. The most frequently detected gene was bla_{TEM}, followed by bla_{SHV}, $bla_{OAX\text{-}1}$, bla_{MOX} and bla_{CMY}. Most of the isolates (68.8%) were positive for the *intI1* integrase gene, and two isolates were also found for the *intI2* gene. The *dfr* and *aadA* gene cassettes were predominant. Anthropogenic contamination from onshore sewage processing plants might contribute predominantly to the β-lactamase gene reservoir in the studied coastal waters. Environmental antibiotic-resistant bacteria and resistance genes may serve as bioindicators of coastal environmental quality or biotracers of the potential contamination sources. This is the first report of the prevalence and characterization of β-lactamase genes and integrons in coastal *Enterobacteriaceae* from China.

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Introduction

Extensive and intensive use and misuse of antibiotics in medication, veterinary, agriculture and aquaculture have resulted in new selective pressures on environmental antibiotic-resistant bacteria (Costanzo et al. 2005; Cabello 2006). The emergence of antibiotic resistance among bacterial pathogens has become a serious global health and economical problem (French 2005). Resistance genetic determinants have also been detected in a large number of microbial communities from natural environments (Alonso et al. 2001). Antibiotic resistance in *Enterobacteriaceae*, partially bacterial opportunist pathogens, has increased dramatically in aquatic environments (Schwartz et al. 2003; Henriques et al. 2006; Mukherjee and Chakraborty 2006). The prevalence of diverse antibiotic-resistant bacteria and resistance genes makes coastal ecosystems a reservoir of antibiotic resistance (Young 1993; Biyela et al. 2004).

Bacterial resistance to β-lactam antibiotics caused by the spread of the extended-spectrum β-lactamases (ESBLs) and plasmid-mediated AmpC β-lactamases is becoming more and more important globally for clinical and environmental concerns (Bradford 2001; Bonfiglio et al. 2002; Philippon et al. 2002; Walther-Rasmussen and Hoiby 2002; Lee et al. 2006). Most ESBLs genes are usually derivatives of the common TEM, SHV or OXA genes (Bradford 2001). The transfer of ESBL genes in the *Enterobacteriaceae* are usually plasmid-mediated, too (Rupp and Fey 2003; Shah et al. 2004; Pfaller and Segreti 2006), facilitating the spread of resistance within and

between species. These genes might also be strongly correlated with multidrug resistance in *Enterobacteriaceae* (Nathisuwan et al. 2001; Philippon et al. 2002). Moreover, the coexistence of ESBLs and AmpC β-lactamases in certain *Enterobacteriaceae* strains might provide a broader spectrum of resistance (Alvarez et al. 2004). The β-lactamase gene analyses may provide an understanding of the complexity of the environmental resistance determinants and their origins.

Transfer of antibiotic resistance can be facilitated by mobile genetic elements, such as plasmids, transposons and integrons (Olsen 1999; White and McDermott 2001). Recent studies have indicated that integrons play a particularly important role in the spread of multidrug resistance in Gram-negative bacteria due to their ability to capture and exchange resistance genes via site-specific recombination (Ploy et al. 2000; White et al. 2001; Weldhagen 2004). The detection and characterization of integrons and gene cassettes may be critical in understanding the resistance dissemination pathways and in evaluating the potential of an environment to act as an antibiotic resistance reservoir (Henriques et al. 2006).

Although the studies of resistance genetic determinants in aquatic environments are important, there are few reports from China. Jiaozhou Bay, located on the western coast of the Yellow Sea, is a semi-enclosed waterbody with significant anthropogenic perturbations. Surrounding small rivers used to form the major sources of discharged sewage. Improperly processed hospital effluent and civic wastewater could be a source of antibiotics and antibiotic-resistant bacteria (Kim and Aga 2007). Excessive usage of antibiotics in mariculture may also stimulate the propagation of resistant bacteria (Cabello 2006). Our previous studies indicated that the Jiaozhou Bay was contaminated with tetracycline- and chloramphenicol-resistant *Enterobacteriaceae*, especially at the eastern coast stations close to the onshore sewage processing plants of the Qingdao city (Dang et al. 2008a, b). In the current work, we studied the molecular diversity of the β-lactamase genes (especially those of the plasmid-mediated AmpC or other *bla* genes), integrons and gene cassettes of the environmental multidrug-resistant bacteria isolates, in order to gain a basic understanding of the potential influence of anthropogenic activities, such as wastewater discharging, on the clinically and environmentally important *Enterobacteriaceae* population in Jiaozhou Bay.

Materials and methods

Bacterial isolates and antimicrobial susceptibility tests

A total of 99 isolates resistant to OTC_{100} (100 μg/ml oxytetracycline), including 42 from A5 station, 42 from C4 station, 11 from Y1 station and 4 from D6 station (Fig. 1), were selected from a previous study (Dang et al. 2008a). Resistance to ampicillin might be a predictor of the presence of integrons (Leverstein-van Hall et al. 2003), thus this antibiotic was used as the starting reagent for multidrug-resistance screening. Isolates were inoculated onto tryptic soy agar (TSA, Difco formula) plates supplemented with 3% (w/v) NaCl and 100 μg/ml ampicillin (AMP_{100}). The positively growing resistant isolates were selected for further study.

Antibiotic susceptibility tests were determined based on the method described by Dang et al. (2007). The TSA plates were supplemented with 3% (w/v) NaCl and one of the following antimicrobials: streptomycin (30 μg/ml), chloramphenicol (30 μg/ml), kanamycin (30 μg/ml), trimethoprime-sulfamethoxazole (30 μg/ml), erythromycin (15 μg/ml) and nalidixic acid (30 μg/ml), for multidrug resistance assay.

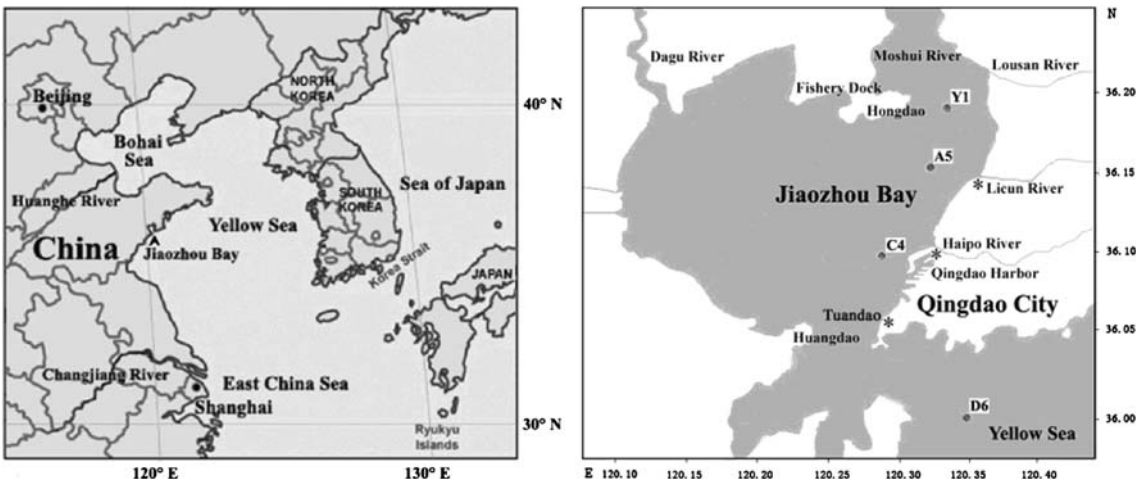

Fig. 1 Sampling stations of Jiaozhou Bay. The locations of sewage processing plants are indicated with the '*' symbols

β-lactamase-specific PCRs

Primers used to amplify the plasmid-mediated AmpC (Hong et al. 2005) and other major members of the β-lactamase genes (Colom et al. 2003) are shown in Table 1. A boiling method was used for extraction of the total DNA from AMP_{100}-resistant strains, including genomic and plasmid DNA (Dang et al. 2007). PCR products were analyzed by electrophoresis on 1% agarose gels stained with ethidium bromide.

Molecular determination of phylogenetic affiliations

The isolates harboring the β-lactamase genes determined in the above step were analyzed of 16S rRNA gene (rDNA) sequences of the isolates to determine their phylogenetic affiliations. The primers 27F and 1387R, and the PCR conditions were described previously (Dang and Lovell 2000). When an isolate' phylogenetic affiliation could not be unambiguously determined with 16S rDNA sequence analysis, phenotypic characterization, including colony morphology, Gram stain, lactose fermentation, glucose oxidation and acetate reduction, was determined.

PCR amplification for integron detection

PCR amplification of specific integrase gene fragments was used for integron identifications of bacteria carrying β-lactamase genes (Table 1). PCR conditions for the detection of the class 1 and class 2 integrons and also the gene cassettes have been described elsewhere (Kraft et al. 1986; Levesque et al. 1995; Goldstein et al. 2001; White et al. 2001). Amplicons of gene cassettes were digested with *MspI* and *HhaI*, respectively. Restriction fragments were resolved by electrophoresis on 4% agarose gels in 0.5× TBE, and digitally photographed with an Image-Master VDS imaging system (Pharmacia Biotech, USA). The band patterns of the RFLP analysis were compared to identify potential identical sequences. PCR products of typical RFLP profiles were purified and ligated into pMD19-T simple vectors (Takara, Japan), transformed into *E. coli* TOP10 competent cells. The transformants were

Table 1 PCR primers used in this study

Primer	Target	Sequence (5′–3′)	Amplicon size (bp)	Reference
SHV-F	bla_{SHV}	AGGATTGACTGCCTTTTTG	392	Colom et al. (2003)
SHV-R		ATTTGCTGATTTCGCTCG		
TEM-C	bla_{TEM}	ATCAGCAATAAACCAGC	516	Colom et al. (2003)
TEM-H		CCCCGAAGAACGTTTTC		
OXA-F	bla_{OXA}	ATATCTCTACTGTTGCATCTCC	619	Colom et al. (2003)
OXA-R		AAACCCTTCAAACCATCC		
MOXMF	MOX-1, MOX-2, CMY-1	GCT GCT CAA GGA GCA CAG GAT	520	Hong et al. (2005)
MOXMR	CMY-8 to CMY-11	CAC ATT GAC ATA GGT GTG GTG C		
CITMF	LAT-1 to LAT-4, CMY-2	TGG CCA GAA CTG ACA GGC AAA	462	Hong et al. (2005)
CITMF	CMY-7, BIL-1	TTT CTC CTG AAC GTG GCT GGC		
DHAMF	DHA-1, DHA-2	AAC TTT CAC AGG TGT GCT GGG T	405	Hong et al. (2005)
DHAMR		CCG TAC GCA TAC TGG CTT TGC		
ACCMF	ACC	AAC AGC CTC AGC AGC CGG TTA	346	Hong et al. (2005)
ACCMR	MIR-1T ACT-1	TTC GCC GCA ATC ATC CCT AGC		
EBCMF		TCG GTA AAG CCG ATG TTG CGG	320	Hong et al. (2005)
EBCMR	FOX-1 to FOX-5b	CTT CCA CTG CGG CTG CCA GTT		
FOXMF		AAC ATG GGG TAT CAG GGA GAT G	190	Hong et al. (2005)
FOXMR		CAA AGC GCG TAA CCG GAT TGG		
IntI1-F	Class 1 integrase gene	CCTCCCGCACGATGATC	280	Kraft et al. (1986)
IntI1-R		TCCACGCATCGTCAGGC		
IntI2-F	Class 2 integrase gene	TTATTGCTGGGATTAGGC	233	Goldstein et al. (2001)
IntI2-R		ACGGCTACCCTCTGTTATC		
5′_CS	Variable region of class 1	GGCATCCAAGCAGCAAG	Variable	Levesque et al. (1995)
3′_CS	Integron	AAGCAGACTTGACCTGA		
Hep-F	Variable region of class 2	CGGGATCCCGGACGGCATGCACGATTTGTA	Variable	White et al. (2001)
Hep-R	Integron	GATGCCATCGCAAGTACGAG		

selected by using X-Gal-IPTG Luria-Bertani (LB) indicator plates supplemented with 100 μg/ml ampicillin.

Sequencing and sequence analyses

Primers for amplification of each gene fragment in the above steps were used for sequencing, except for the gene cassette DNA fragments, which were sequenced using the clone vector primers M13-D (5′-AGGGTTTTCCCAGTCACGACG-3′) and RV-M (5′-GAGCGGATAACAATTTCACACAGG-3′). Bioinformatic determination of the sequence phylogenetic affiliations followed standard methods (Dang and Lovell 2000). NCBI GenBank database was queried for initial determinations of the nearest neighbor sequences via the BLAST program (Altschul et al. 1997). 16S rDNA sequences were aligned using the ClustalX program (version 1.8) (Thompson et al. 1994). Phylogenetic trees were constructed using the DNADIST and NEIGHBOR programs within the PHYLIP package (version 3.67) (Felsenstein 1989).

Nucleotide sequence accession numbers

The β-lactamase gene and the 16S rDNA sequences determined in the current study have been deposited in the GenBank database under accession nos. EU420893 to EU420956, and the representative class 1 and class 2 integrons and the gene cassettes sequences determined under accession nos. EU523051 to EU523064.

Results

Antibiotic resistance profiles

A total of 66 isolates (30 from C4 station, 28 from A5 station, 7 from Y1 station and 1 from D6 station) showed resistance to 100 μg/ml ampicillin. All these isolates were also multidrug-resistant (resistant to more than two classes of antibiotics). Most frequently those additional resistances were to trimethoprime-sulfamethoxazole (86.4%) and nalidixic acid (83.3%). Particularly among the resistant strains, 7 isolates from C4 station, 1 isolate from A5 station and 1 isolate from Y1 station were resistant to all the antibiotics tested (Table 2).

Occurrence and diversity of β-lactamase genes

A total of 66 AMP_{100}-resistant isolates were selected to screen the β-lactamase genes by multiplex PCR methods. About 32 isolates were found to carry at least one of the β-lactamase genes (Table 2). Based on 16S rDNA analyses (Fig. 2), these isolates included 30 *Enterobacteriaceae* strains, 1 *Acinetobacter* strain and 1 *Aeromonas* strain. The predominant resistance gene was bla_{TEM}, occurring in 17 isolates (53.1%), affiliated with *Escherichia coli* (10 isolates), *Klebsiella oxytoca* (5 isolates), *Enterobacter sp.* (1 isolates) and *Kluyvera intermedia* (1 isolate). The resistance gene bla_{SHV} was detected in 9 isolates, affiliated with *Klebsiella pneumoniae* (8 isolates) and *Citrobacter freundii* (1 isolate). The resistance gene bla_{OXA-1} was detected in 1 *Citrobacter freundii* isolate and 1 *Acinetobacter seohaensis* isolate. For the plasmid-mediated AmpC β-lactamase genes, bla_{CMY} was observed in 2 *Citrobacter freundii* isolates, and bla_{MOX-2} was observed in 1 *Acinetobacter* isolate and 1 *Citrobacter freundii* isolate. All the *E. coli* and *K. oxytoca* isolates carried the bla_{TEM} gene, while all the *K. pneumoniae* isolates carried the bla_{SHV} gene. Nucleotide sequence analysis revealed that all the sequences of the screened genes had 98% or higher similarity to their best-match known genes retrieved from the GenBank database, but it is difficult to distinguish from these fragments sequenced whether the bla_{TEM} and bla_{SHV} correspond to ESBLs or not, except that bla_{OXA-1} gene can be identified as ESBLs. Because the amplification fragments of bla_{TEM} and bla_{SHV} with the primers are too short to contain the point mutations. More specific techniques should be used to discriminate wild-type bla_{TEM} and bla_{SHV} genes.

Occurrence and diversity of integrons and gene cassettes

A total of 22 out of the 32 β-lactamase gene-carrying isolates (68.8%) had the class 1 integrase gene *intI1*. Two strains, SA-C4-59 and SA-A5-61, were also found to possess the class 2 integrase gene *intI2*. The class 1 and class 2 regions of the gene cassettes of the integrase positive isolates were analysed. About 12 isolates were found to carry the gene cassette fragments amplified with 5′_CS/3′_CS (Table 2). Sequence analysis of the cassette arrays revealed a predominance of *dfr* and *aadA* cassettes that conferred resistance to trimethoprim, and streptomycin and spectinomycin, respectively. Additional gene cassette arrays were also detected, including *catB8* and *cmlA* encoding resistance to chloramphenicol, *aacA4* encoding resistance to aminoglycosides, *arr-3* encoding resistance to rifampicin, and *orfF* with unknown function. Seven isolates (3 *Klebsiella oxytoca*, 3 *Escherichia coli* and 1 *Kluyvera intermedia*) carried the same class 1 integron gene cassette arrays (*dfr17*, *aadA5*).

Discussion

Enterobacteriaceae remain important causes of both nosocomial and community-acquired infections. The emergence

Table 2 Characteristics of ampicillin-resistant isolates with β-lactamase genes including resistance phenotypes, *bla* genotypes and integrons content

Isolate	Closest sequence match (% similarity)	Phenotypic identification	Resistance phenotypes[b]	*bla* Genotypes	Integrase	Putative gene cassettes[c]
SA-A5-17	*Escherichia coli/Shigella sonnei* (99%)[a]	*Escherichia coli*	OTC, AMP, SM, NA, SXT	TEM	ND	ND[d]
SA-A5-32	*Escherichia coli/Shigella dysenteriae* (98%)[a]	*Escherichia coli*	OTC, AMP, SM, NA, EM, SXT	TEM	I	dfr17, aadA5
SA-A5-78	*Escherichia coli/Shigella dysenteriae* (98%)[a]	*Escherichia coli*	OTC, AMP, SM, KM, NA, SXT	TEM	I	dfr17, aadA5
SA-A5-100	*Escherichia coli/Shigella boydii* (99%)[a]	*Escherichia coli*	OTC, AMP, SM, SXT	TEM	ND	ND
SA-A5-125	*Escherichia coli/Shigella dysenteriae*(99%)[a]	*Escherichia coli*	OTC, AMP, SM, KM, NA, EM, SXT	TEM	I	ND
SA-A5-24	*Escherichia coli/Shigella boydii* (99%)[a]	*Escherichia coli*	OTC, AMP, SM, NA, SXT	TEM	ND	ND
SA-Y1-43	*Escherichia coli/Shigella flexneri* (98%)[a]	*Escherichia coli*	OTC, AMP, SM, NA, SXT	TEM	I	ND
SA-Y1-39	*Escherichia coli/Shigella dysenteriae* (98%)[a]	*Escherichia coli*	OTC, AMP, SM, KM, NA, SXT	TEM	I	ND
SA-C4-50	*Escherichia coli/Shigella dysenteriae*(99%)[a]	*Escherichia coli*	OTC, AMP, SM, NA, EM, SXT	TEM	I	ND
SA-C4-59	*Escherichia coli/Shigella sonnei* (99%)[a]	*Escherichia coli*	OTC, AMP, CP, SM, KM, NA, EM, SXT	TEM	I, II	aacA4, cmlA1
SA-C4-27	*Klebsiella pneumoniae* (99%)		OTC, AMP, CP, SM, NA, SXT	SHV	I	ND
SA-C4-51	*Klebsiella pneumoniae* (99%)		OTC, AMP, SM, KM, NA, SXT	SHV	I	ND
SA-C4-53	*Klebsiella pneumoniae* (99%)		OTC, AMP, SM, NA, SXT	SHV	ND	ND
SA-C4-60	*Klebsiella pneumoniae* (99%)		OTC, AMP, NA, SXT	SHV	ND	ND
SA-C4-61	*Klebsiella pneumoniae* (99%)		OTC, AMP, NA, SXT	SHV	ND	ND
SA-Y1-7	*Klebsiella pneumoniae* (99%)		OTC, AMP, CP, SM, KM, NA, EM, SXT	SHV	I	Arr-3 dhfrv
SA-Y1-23	*Klebsiella pneumoniae* (99%)		OTC, AMP, SM, KM, NA, EM, SXT	SHV	I	ND
SA-D6-7	*Klebsiella pneumoniae* (99%)		OTC, AMP, NA, EM, SXT	SHV	ND	ND
SA-C4-8	*Klebsiella oxytoca* (99%)		OTC, AMP, CP, SM, KM, NA, EM, SXT	TEM	I	dfr17, aadA5
SA-C4-13	*Klebsiella oxytoca* (98%)		OTC, AMP, CP, SM, KM, NA, EM, SXT	TEM	I	ND
SA-C4-21	*Klebsiella oxytoca* (99%)		OTC, AMP, CP, SM, KM, NA, EM, SXT	TEM	I	dfr17, aadA5
SA-C4-35	*Klebsiella oxytoca* (99%)		OTC, AMP, CP, SM, KM, NA, EM, SXT	TEM	I	aadA1, catB8 dhfrv
SA-C4-38	*Klebsiella oxytoca* (99%)		OTC, AMP, CP, SM, KM, NA, EM, SXT	TEM	I	dfr17, aadA5
SA-A5-89	*Citrobacter freundii* (99%)		OTC, AMP, SM, KM, NA, EM, SXT	OXA-1	I	ND
SA-A5-98	*Citrobacter freundii* (99%)		OTC, AMP, SM, KM, NA, EM, SXT	CMY	I	ND
SA-A5-118	*Citrobacter freundii* (99%)		OTC, AMP, SM, NA, EM, SXT	SHV	ND	ND
SA-C4-58	*Citrobacter freundii* (98%)		OTC, AMP, SM, NA, SXT	CMY	ND	ND
SA-Y1-11	*Citrobacter freundii* (98%)		OTC, AMP, SM, KM, NA, SXT	MOX-2	I	dfrA12, orfF, aadA
SA-A5-114	*Enterobacter sp.* (99%)		OTC, AMP, CP, SM, KM, NA, EM, SXT	TEM	I	dfr17, aadA5
SA-C4-45	*Kluyvera intermedia* (98%)		OTC, AMP, CP, SM, KM, NA, EM, SXT	TEM	I	dfr17, aadA5
SA-A5-86	*Aeromonas* sp. (99%)		OTC, AMP, CP, SM, KM, NA, SXT	MOX-2	ND	ND
SA-A5-61	*Acinetobacter seohaensis* (99%)		OTC, AMP, CP, SM, KM, NA, SXT	OXA-1	I, II	ND

[a] 16S rRNA genes sequencing cannot affiliate the isolate to only one genus unambiguously

[b] OTC, oxytetracycline; AMP, ampicillin; CP, chloramphenicol; SM, streptomycin; NA, nalidixic acid; EM, erythromycin; KM, kanamycin; SXT, trimethoprime-sulfamethoxazole

[c] Putative gene cassettes were identified by nucleotide sequencing and comparison with the sequences in the GenBank database by use of the BLAST service

[d] ND, not detected

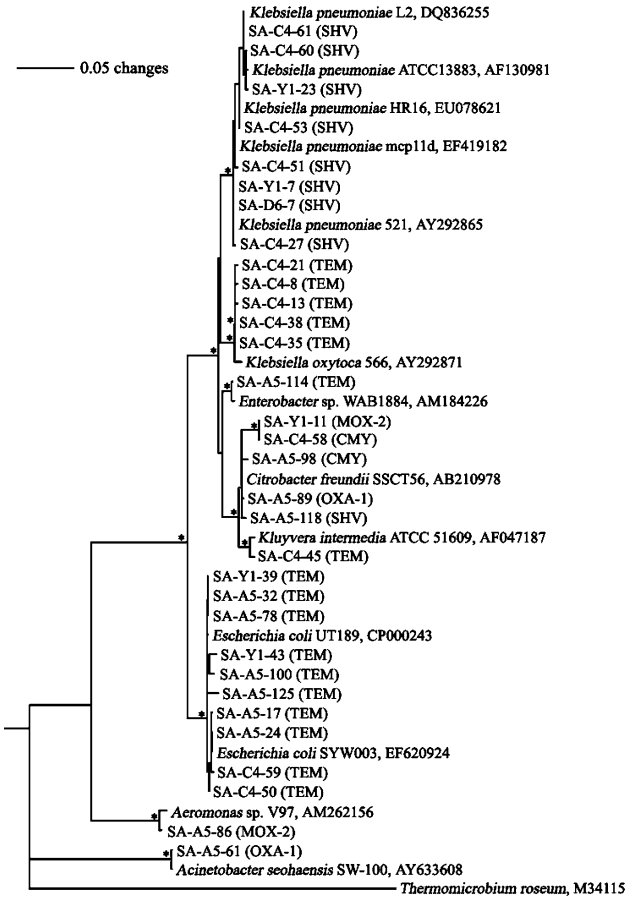

Fig. 2 Phylogenetic tree constructed based on partial 16S rRNA genes sequences using neighbor-joining method for the 32 β-lactamase gene-harboring bacterial isolates. Bootstrap values greater than 70% were indicated at the branch points as '*', generated by 100 replicates of neighbor-joining and parsimony analysis. The β-lactamase genes detected are labeled in parentheses for the corresponding isolates

and wide dissemination of multidrug resistant *Enterobacteriaceae* environmental strains represent a potential risk for human health. Our previous studies showed that antibiotic-resistant *Enterobacteriaceae* strains might be a serious contamination in Jiaozhou Bay (Dang et al. 2008a, b). In the current study, 30 out of 32 (93.8%) bacterial isolates with β-lactamase genes were identified as *Enterobacteriaceae* strains, with *Klebsiella* sp. and *Escherichia coli* being the most predominant strains. Our data indicate that the Jiaozhou Bay provides a good model for studying the molecular diversity and potential sources of antibiotic-resistant bacteria and resistance genes. Similar situations from other areas of the world coastal environments about antibiotic-resistance contamination from potential commensal bacteria worth being further studied.

The distribution of lactam-resistant bacteria was quite heterogeneous and area-specific in Jiaozhou Bay. More than 60% of the OTC_{100}-resistant bacteria isolated from the eastern coast stations, such as A5, C4 and Y1, were found to be AMP_{100} resistant, while only 25% were found from station D6. Station A5 is located near the river mouth of Licun and station C4 is located near the river mouth of Haipo (Fig. 1), where the nearby sewage processing plants discharge a huge amount of processed wastewater into the bay (Liu et al. 2005). Station Y1 is located at the river mouths of Loushan and Moshui near the Hongdao mariculture area. Station D6 is located at the outside of Jiaozhou Bay, possibly with less impact from terrestrial or mariculture contamination. Although the seawater of Jiaozhou Bay is dynamic and water mixing happens frequently due to currents, tides and other hydrological factors (Liu et al. 2004), confined distribution of resistant bacteria, especially the *Enterobacteriaceae* strains, still could be identified along the eastern coast, indicating a strong terrestrial or anthropogenic impact. Our current environmental microbiology study indicates that microbial spatial distribution might be closely related to geographical characteristics and anthropogenic activities, and sewage contamination might be a serious environmental problem of Jiaozhou Bay. The prevalence and dissemination of antibiotic-resistant commensal bacteria may serve as a bioindicator or biotracer, potentially useful in revealing the coastal environmental quality or contamination source (Dang et al. 2008a, b).

To date, although a variety of β-lactamase gene types have been described, bla_{TEM} and bla_{SHV} are the major types found in members of the family *Enterobacteriaceae* (Cantón et al. 2008; Villegas et al. 2008). In coastal waters of Jiaozhou Bay, the most frequently detected gene was bla_{TEM}, followed by bla_{SHV}, bla_{OXA-1}, bla_{MOX} and bla_{CMY}. Our result is in agreement with other reports on the diversity and dominance of the lactam-resistant bacteria and β-lactamase genes in aquatic environments (Henriques et al. 2006; Alpay-Karaoglu et al. 2007), indicating that it might be a global phenomenon for the prevalence and dominance of *Enterobacteriaceae* bacteria and their lactam-resistant genes in aquatic environments with significant anthropogenic contamination impact.

In the current study, class 1 integrons were detected in 68.8% of the β-lactamase gene-carrying bacterial isolates from Jiaozhou Bay. This proportion is slightly higher than what was found in some aquatic environments (Lin and Biyela 2005), but much higher than other aquatic environments (Rosser and Young 1999; Roe et al. 2003; Henriques et al. 2006; Ozgumus et al. 2007). It has been found that there is a strong correlation between the presence of integrons and multidrug resistance (White et al. 2001). According to our susceptibility test result (Table 2), integron-positive isolates were more likely to be multidrug resistant than integron-negative isolates. These data indicated that integrons were prevalent and might play an important role in multidrug resistance in the environmental β-lactamase-producing *Enterobacteriaceae* strains.

Gene cassettes were found to be associated with resistance to several major classes of antibiotics. The most frequently found resistance gene cassettes *dfr*17 and *aad*A5 in the current study might be widespread in *Enterobacteriaceae* strains of aquatic environments (Ozgumus et al. 2007), similar to clinical settings (White et al. 2001). In our study, only aminoglycoside, trimethoprim and chloramphenicol could be directly related to the presence of the gene cassettes (Table 2). The association of the other antibiotics, such as ampicillin, erythromycin and tetracycline, with the presence of an integron is likely to be due to genetic linkage between integrons and conjugative plasmids or transposons (White et al. 2001). Our result showed that 11 isolates containing the *intI1* gene were not detected with gene cassette regions. This was probably due to the lack of a 3′ conserved region or the variable region was too long to be amplified in these isolates (Barlow et al. 2004; Yao et al. 2007).

Coastal environment deterioration is becoming more complicated due to contaminations of antimicrobials and antibiotic-resistant bacteria. Discharged civic and hospital wastewater, even after processing, might contribute to the resistance bacteria and gene reservoir in aquatic ecosystems. Our study indicates the possibility that antibiotic-resistant bacteria and resistance genes may serve as bioindicators of coastal environmental quality or biotracers of major contamination sources. Efficient and timely surveillance is crucial for high-quality environmental management and for prevention and control of microbial diseases and infections caused by environmental antibiotic-resistant bacteria.

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