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Characterization and quantification of class 1 integrons and associated gene cassettes in sewage treatment plants

Xu-Xiang Zhang · Tong Zhang · Ming Zhang · Herbert H. P. Fang · Shu-Pei Cheng

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Abstract Class 1 integrons and gene cassettes containing antibiotic resistance genes (ARGs) in five different sewage treatment plants (STPs) were characterized and quantified using polymerase chain reaction (PCR), sequencing, and quantitative real-time PCR (qRT-PCR) in this study. Class 1 integronase gene (*int*I1) was found commonly occurring in all of activated sludge samples from the five STPs, as well as in influent and effluent of two STPs at Hong Kong. One hundred and nine lactose-fermenting *Enterobacteriaceae* (LFE) strains were isolated from activated sludge of Shatin STP. Among them, 36 strains (33.0%) were found to carry class 1 integrons. PCR assays showed that 11 of the 36 *int*I1-carrying isolates harbored a common type of gene cassette array of about 1,600 bps, as well as the static genes (*sul*I and *qacE*Δ1) on class 1 integrons. This gene cassette array was found phylogenetically close to antibiotic resistance genes *dfr*17 and *aad*A5, encoding dihydrofolate reductase conferring resistance to trimethoprim and adenylyltransferase conferring resistance to spectinomycin/streptomycin, respectively. Antimicrobial susceptibility analysis demonstrated that all the 11 LFEs carrying gene cassette were multi-resistant, especially having common resistance to trimethoprim and streptomycin. qRT-PCR assay showed that genes copies of both class 1 integron and the gene cassette varied significantly among the activated sludge sampled from different STPs, at different time points or different treatment steps. More than 90% of class 1 integrons and the gene cassette were removed by activated sludge processes in two STPs, while the disinfection process removed 94% integron and 77% gene cassette in one STP.

Keywords Antibiotic resistance gene · Gene cassette · Integron · qRT-PCR · Sewage treatment plant

X.-X. Zhang · T. Zhang (✉) · H. H. P. Fang
Environmental Biotechnology Lab, Department of Civil Engineering, The University of Hong Kong,
Pokfulam Road,
Hong Kong, SAR, China
e-mail: zhangt@hkucc.hku.hk

X.-X. Zhang · S.-P. Cheng
State Key Laboratory of Pollution Control and Resource Reuse, Department of Environmental Science, Nanjing University,
Nanjing, China

M. Zhang
Key Laboratory of Urbanization and Ecological Restoration of Shanghai, Department of Environmental Science, East China Normal University,
Shanghai, China

Introduction

Antibiotics have wide applications in protecting the health of human beings and animals or to increase growth rate of animals as food additive. The most of antibiotics are excreted unchanged into the environment. Concerns about the potential impact of antibiotic residues in the aquatic environment keep growing recently (Sarmah et al. 2006; Wright 2007; Kemper 2008). In addition to chemical pollution caused by antibiotics themselves, the use of antibiotics may also accelerate the development of antibiotic resistance genes (ARGs) and bacteria which shade health risks to humans and animals (Kemper 2008). These bacteria may transmit from environment to human via direct or indirect contact (Iversen et al. 2004; Kim et al. 2005; Rodríguez et al. 2006). Considering the growing evidences that clinical resistance is intimately associated with environmental ARGs and bacteria (Tatavarthy et al. 2006; Prabhu et al. 2007; Abriouel et al. 2008), it is clear that the research

activities need to be expended to include nonpathogenic or environmental microorganisms.

ARG may be carried in many forms in antibiotic resistant bacteria, including insertion sequences, transposons, conjugative plasmids, and integrons. So far, at least five classes of mobile integrons have been observed to have a role in the dissemination of ARGs, among which class 1 integrons are the most common one (Mazel 2006). All integrons known today contain three key elements: (1) an *int*I1 gene encoding a site-specific integrase responsible for integration and excision of gene cassettes, which occurs at (2) the *att*I site, and (3) a promoter (P_{int}) that drives expression of inserted gene cassettes. A gene cassette generally consists of at least one gene and an imperfect inverted repeat of 57 to 141 bp, i.e., *att*C which is the recognition site for the integrase (Mazel 2006). Class 1 integron possesses a conserved segment at downstream of the gene cassette, which is composed of the genes *qacE*Δ1, *sul*1, and *orf*5 (Fig. 1), encoding a small exporter protein that confers resistance to quaternary ammonium compounds, a dihydropteroate synthetase for sulphonamide resistance, and a hypothetical protein of unknown function, respectively (Paulsen et al. 1993).

Class 1 integrons are frequently reported to carry gene cassettes associated with clinical antibiotic resistance (Li et al. 2006; Mendes et al. 2007; Labuschagne et al. 2008). Recently, integrons have been detected in some microbial species from various environments, including wastewater (Tennstedt et al. 2003; Taviani et al. 2008), river water (Mukherjee and Chakraborty 2006), and soil (Agersø and Sandvang 2005), suggesting that the dissemination of integrons may be a noteworthy problem of environmental pollution and public health (Stokes et al. 2006). Different from direct detection of environmental ARGs, investigation on integrons and associated gene cassettes can provide evidence for multiple antibiotic resistances of environmental bacteria and horizontal transfer of ARGs among the species since integrons are able to incorporate and disseminate gene cassettes containing antibiotic resistance genes (Alekshun and Levy 2007).

Previous studies were mainly focused on characterization of integrons and antibiotic-resistance gene cassettes recovered from environmental bacterial species. However, few researches have been conducted to investigate elimination and fate of integrons and gene cassettes in sewage treatment plants (STPs). The objectives of this study are (1) to detect class 1 integrons and associated gene cassettes in isolated bacteria from activated sludge of STP and (2) to quantitatively investigate elimination of the integrons and gene cassettes in STPs.

Materials and methods

Sampling and DNA isolation

Activated sludge samples were sampled from aeration tank of Shatin STP and Stanley STP (Hong Kong, China), Minhang STP (Shanghai, China), San Jose STP (CA, USA), and Palo Alto STP (CA, USA). The sampling in the two STPs of Hong Kong was conducted every month from March 2007 to March 2008. Water samples were collected from influent of primary settler, effluent of secondary sedimentation tank in both Shatin and Stanley STPs, and effluent of disinfection tank in Stanley STP. Biosolids (digestion sludge) were also sampled from the two STPs of Hong Kong. Water and sludge samples were kept on ice and transported to the lab for immediate processing (within 2–4 h). Wastewater (50–200 ml) and sludge samples (10 ml) were centrifuged at 4,000 rpm for 10 min at 4°C. Supernatant was discarded, and the pellet was used for DNA extraction using the FastDNA soil kit (Zymo Research, USA).

Isolation and identification of bacterial isolates

Lactose-fermenting *Enterobacteriaceae* (LFE) strains were isolated from STPs on MacConkey media, and polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was used to group the isolates. In detail,

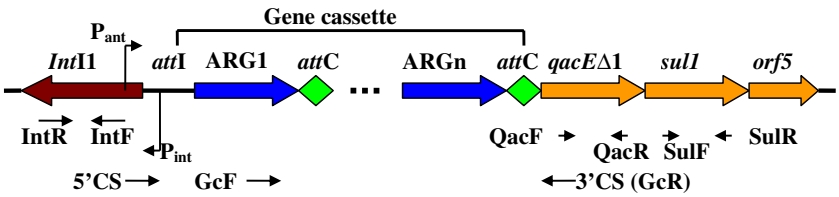

Fig. 1 Components of class 1 integron and target positions of PCR primers. Class 1 integron consists of the integrase gene *int*I1, the promoters P_{int} and P_{ant}, the integron-specific attachment site (*att*I), quaternary ammonium compound resistance gene *qacE*Δ1, sulphonamide resistance gene *sul*I, and *orf*5 of unknown function. Integron acquires gene cassette via site-specific recombination at *att*I and *att*C. IntF and IntR, QacF and QacR, SulF and SulR, 5′CS and 3′CR, and GcF and GcR represent forward and reverse primers for PCRs of *int*I1, *qacE*Δ1, *sul*I, gene cassette, and partial fragment of the common detectable gene cassette, respectively

activated sludge samples (10 ml) collected from Shatin STP were mixed with 90 ml PBS buffer (pH7.2) and 5 ml 5-mm glass bead and shaken at 200 rpm for 2 h. The suspension was used to isolate LFE on Macconkey agar (Difco) using the spread plate method after serial dilution (Kim et al. 2007). Colonies with pink color were defined as LFE. Almost full sequence of 16S rDNA of the LFE strains was amplified using the primer set EUB8F/Univ1392R and digested using *Rsp*I. Strains were typed based on RFLP profile on 1% agarose gel. One to three representative sequences of each RFLP type were sequenced to identify the taxonomy affiliation.

Specific PCRs of class 1 integron and gene cassettes

With the primers listed in Table 1, PCR was used to detect *int*I1, gene cassette, *qacE*Δ1, and *sul*I in the environmental samples and bacterial isolates. Primers specific for the 5′ conserved segment (5′CS) and 3′ conserved segment (3′CS) were used to amplify the variable region of class 1 integrons. Based on DNA sequence of the common gene cassette detected with 5′CS and 3′CS, the primer set of GcF and GcR was designed and used to target a partial fragment (895 bp) on the gene cassette commonly present in the environmental samples and bacterial isolates. PCR amplifications of *int*I1, *sul*I, and *qacE*Δ1 were performed in 30 µl volumes solution with 1× PCR buffer, 1.5 mM $MgCl_2$, 100 µM dNTP, 3 pmol of each primer, 100 ng of template DNA, and 1 U of *Taq* polymerase. Conditions of these PCRs were initial denaturation at 94°C for 5 min, followed by 30 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 60 s, with a final extension at 72°C for 8 min. PCRs of gene cassettes were performed in 30 µl reaction system including 1× PCR buffer, 1.5 mM $MgCl_2$, 200 µM dNTP, 6 pmol of each primer, 200 ng of template DNA, and 2 U of *Taq* polymerase. Conditions of gene cassette PCRs were initial denaturation at 94°C for 7 min, followed by 30 cycles of 94°C for 40 s, 55°C for 40 s, and 72°C for 80 s, with a final extension at 72°C for 10 min. Duplicate PCR reactions were performed for each combination of sample and primer set. Sterile water was used as the negative control for each assay.

PCR products were analyzed by electrophoresis using 1% agarose gel with ethidium bromide in 1× TAE buffer at 120 V for 15 min. After agarose gel electrophoresis, PCR products of the target genes (1 µl each) present in each sample were mixed up in a centrifuge tube. The mixture (1 µl) was added in each sample well of DNA chips to analyze the size of the DNA fragments using Agilent 2100 BioAnalyzer (USA). The analysis was conducted with Agilent DNA100 Kit (USA) according to the manufacturer instructions. Amplification product of each target gene was purified with PCRquick spin™ PCR Product Purification Kit (iNtRon Biotechnology, South Korea) and sent out for DNA sequencing (TechDragon, Hong Kong, China) to check if the target DNA fragments were amplified.

qRT-PCR

Class 1 integronase gene *int*I1 and the gene cassette detected in specific PCR assay was quantified with quantitative real-time PCR (qRT-PCR) for the wastewater and sludge samples from the five STPs wastewater treatment plants. The qRT-PCR primers of *int*I1 were the same with those used in specific PCRs (Table 1). The specific primer set (GcF and GcR) was used for qRT-PCR of the gene cassette detected in specific PCRs. Reactions were conducted in 96-well plates with a final volume of 20 µl using 10 µl iQ™ SYBR® Green Supper Mix (BioRad, Hercules, CA), plus 1 µl each primer of 2 mM and 8 µl template DNA. Thermal cycling and fluorescence detection were conducted on a BioRad iCycler with the software iCycler iQ version 3.0 (BioRad), using the following protocol: 94°C for 3 min, followed by 45 cycles of 94°C for 30 s, 55°C for 30 s, 72 °C for 60 s. Each reaction was run with duplicate.

PCR products of *int*I1 and the gene cassette were cloned using the TA Cloning Kit (Invitrogen, Carlsbad, CA, USA),

Table 1 Primers of *int*I1, *qacE*Δ1, *sul*I, and gene cassette located on class 1 integron

Target gene	Primer	Sequence (5′–3′)	Product size	References
*int*I1	IntF	GGGTCAAGGATCTGGATTTCG	484 bp	Mazel et al. 2000
	IntR	ACATGCGTGTAAATCATCGTCG		
*qacE*Δ1	QacF	ATCGCAATAGTTGGCGAAGT	230 bp	Sandvang et al. 1998
	QacR	CAAGCTTTTGCCCATGAAGC		
*sul*I	SulF	CGGCGTGGGCTACCTGAACG	433 bp	Kerrn et al. 2002
	SulR	GCCGATCGCGTGAAGTTCCG		
Gene cassette	5′CS	GGCATCCAAGCAGCAAG	Variable	Lévesque et al. 1995
	3′CS	AAGCAGACTTGACCTGA		
	GcF	AAAAGCTGCCGCTGAACTTA	895 bp	This study
	GcR	AAGCAGACTTGACCTGA		

and the plasmid-carrying target gene was extracted and purified using PureLink™ Quick Plasmid Miniprep Kit (Invitrogen). The copy number of *int*I1 and gene cassette per microliter plasmid DNA solution was calculated according to Liang et al. (2008). Calibration curves (C_t value versus Log value of initial target gene copy number per reaction) with at least five points for qRT-PCR were generated using tenfold serial dilution of the plasmid-carrying target gene (Zhang and Fang 2006; Liang et al. 2008). Calibration curve was run together with each measurement. The PCR efficiencies ranged from 97.6% to 102.2%, and R^2 values were over 0.992 for all calibration curves. Based on the calibration curves, C_t value of a test sample with an unknown concentration was used to calculate copy number of target gene, and then the latter was normalized against mass (nanogram) of the extracted DNA samples and the volume (milliliter) of original water or sludge samples.

Antibiotic susceptibility analysis

LFE strains carrying gene cassettes were sent to the Department of Microbiology, Queens Mary Hospital, The University of Hong Kong for antibiotic susceptibility analysis using disc diffusion method according to Clinical and Laboratory Standards Institute (2006).

Nucleotide sequence accession numbers

DNA sequences of the test gene cassettes were submitted to GenBank, and the accession numbers were provided for the gene cassettes isolated from *Escherichia coli* (FJ463742) and *Klebsiella pneumoniae* (FJ463743).

Results

Detection of integrons in activated sludge and sewage samples

PCR amplifications suggested that class 1 integronase gene *int*I1 was present in all activated sludge samples of the five STPs, influent and effluent water of both STPs in Hong Kong. The PCR products of *int*I1 were verified by DNA sequencing.

PCRs using the primer set of 5′CS and 3′CS showed that several types of gene cassettes with different sizes occurred in the activated sludge samples (data not shown).

Detection of integrons and gene cassettes in isolated bacteria

Among the 109 LFE strains isolated from activated sludge of Shatin STP, 36 isolates (33.0%) were found to carry *int*I1 (Table 2). PCR assays showed 11 of the 36 *int*I1-carrying isolates harbored a common type of gene cassette, as well as *sul*I and *qacE*Δ1, the static genes on class 1 integrons (Table 2 and Fig. 2). The full length of the common gene cassette detected with 5′CS and 3′CS as PCR primers was about 1,600 bp. Based on DNA sequencing and Blast results, the sequence obtained demonstrated the detectable gene cassette array with the highest similarity (87.7%) to class 1 integron *dfr*17-*aadA*5 for dihydrofolate reductase and aminoglycoside-3′-adenylyltransferase, which are associated with resistance to trimethoprim and streptomycin, respectively. Figure 3 showed that the gene cassette array identified in *E. coli* or *K. pneumoniae* strains obtained in this study were phylogenetically close to those found in the isolates of

Table 2 *sul*I, *qacE*Δ1, and gene cassette in the LFE isolates carrying *int*I1

Strain no.	*sul*I	*qacE*Δ1	Gene cassette
M4	+	+	−
M8	−	−	−
M16	+	+	−
M36	+	+	+
MA1	−	−	−
MA2	+	+	+
MA3	+	+	+
MA4	+	+	+
MA8	−	−	−
MA12	+	+	−
MA24	+	+	−
MA25	+	+	+
MA28	+	+	+
MK2	−	−	−
MK4	+	+	−
MK5	−	−	−
MK8	−	−	−
MK9	+	+	−
MK12	−	−	−
MK13	+	+	−
MK14	−	−	−
MK15	−	+	−
MK16	−	−	−
MK17	−	−	−
MK18	−	+	−
MK19	+	−	−
MK20	−	−	−
MK22	+	+	−
MK25	+	+	+
MK26	+	+	+
MK29	+	+	+
MK31	−	−	−
MK33	+	+	−
MK34	+	+	+
MK35	−	+	−
MK36	+	+	+

+ positive result, − negative result

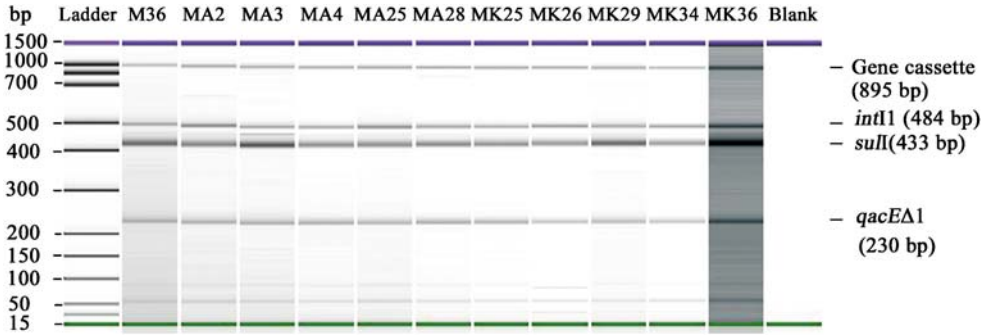

Fig. 2 Gel image generated by Agilent 2100 BioAnalyzer used to analyze PCR products of *int*I1, *sul*I, *qacE*Δ1, and gene cassette carried by 11 LFE isolates from activated sludge

E. coli (EU598449), *Caenorhabditis elegans* (AB291062), *Enterobacter cloacae* (EF571855), *Staphylococcus epidermidis* (AB291061), *Acinetobacter genomo* (EF015498), and *Pseudomonas aeruginosa* (DQ838665). The length of the gene cassette array was beyond the ladder range (0–1,500 bp) of DNA marker of Agilent 2100 BioAnalyzer, thereby a new primer set (GcF and GcR) was designed according to the sequencing result of the common gene cassette array and used for specific amplification of a partial fragment (895 bp) on the gene cassette (Fig. 2).

Antibiotic susceptibility of the isolated bacteria harboring gene cassette array

The 11 isolates carrying the gene cassette array were subject to antimicrobial susceptibility analysis, and all were found to be resistant to at least three antibiotics tested (Table 3). All isolates were resistant to tetracycline and ampicillin, and most of them were resistant to co-trimoxazine, neomycine, and streptomycin. Multiple resistances of the isolates to co-trimoxazine and streptomycin may be associated with the ARGs of *dfr*17 and *aadA*5, which were probably located on the integrons carried by these isolates.

Quantification of class 1 integrons and gene cassettes in activated sludge

Class 1 integronase gene and the gene cassette array detected in activated sludge samples from the five STPs were quantified using qRT-PCR. Figure 4 showed that genes copies of both integrons and gene cassettes varied greatly among the activated sludge samples from different STPs or from the same STP sampled at different time points. The highest gene concentrations of both integrons and gene cassettes occurred in Minhang STP, which were 4.03×10^5 and 1.78×10^2 copy/ng DNA, respectively. The gene copy ratio of gene cassette/integron ranged from 0.04% to 20.83%, indicating that only a small part of integrons carried the gene cassette.

Elimination of class 1 integron and gene cassette in sewage treatment plants

Class 1 integron and the gene cassette array were quantified for the influent, activated sludge, effluent of secondary sedimentation tank, effluent of disinfection tank, and biosolids from Shatin and Stanley STPs. The relative

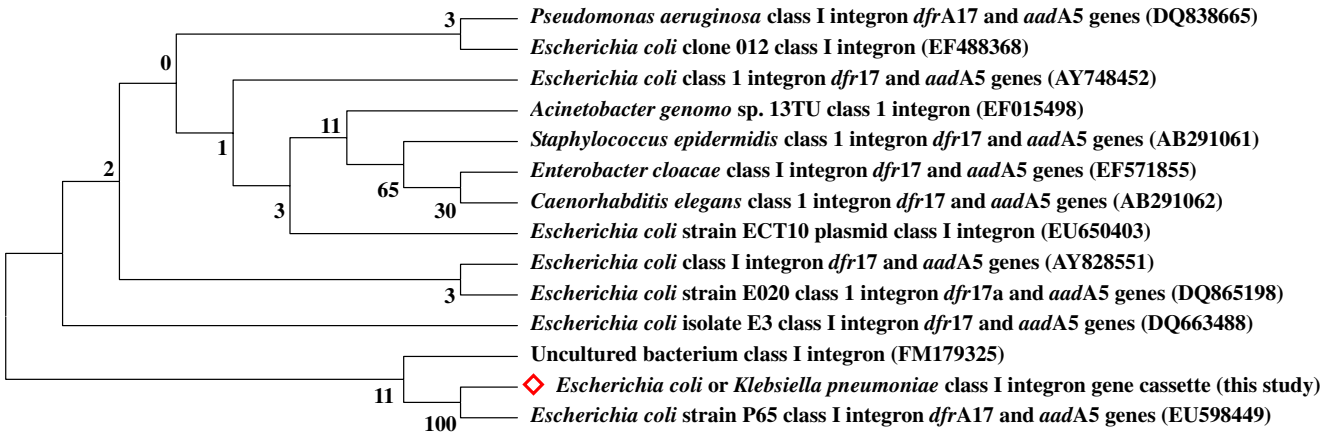

Fig. 3 Phylogenetic tree of the gene cassette identified in LFE isolates from activated sludge constructed with the neighbor-jointing method of MEGA 3.1. GenBank accession numbers of sequences are given in *parenthesis*, and genetic similarity is 87.7% between the gene cassette detected in this study and each corresponding sequence

Table 3 Antibiotic susceptibility of LEF isolates carrying gene cassette array

Antibiotic	M36	MA2	MA3	MA4	MA25	MA28	MK25	MK26	MK29	MK34	MK36
Ampicillin	R0	R0	R0	R0	R0	R0	R0	R0	R0	R0	R0
Cephalothin	R10	M17	M17	R0	S21	R0	M16	M17	S19	R12	R13
Cefuroxime	S24	S21	S21	R0	S25	R0	S24	S24	S22	S21	S23
Gentamicin	S25	S18	S22	S24	S25	R0	R0	R0	S24	R0	R0
Co-trimoxazine	S26	R0	S21	R0	R0	R0	R0	R0	R0	R0	R0
Levofloxazine	S25	S24	S30	R0	S27	R0	R9	R9	S20	R8	R9
Ceftazidime	S29	S28	S28	M16	S30	S29	S30	S31	S26	S31	S28
Augument	R0	S19	S19	M15	S23	S21	S20	S20	S19	S20	S20
Ceftrazine	S29	S30	S29	R10	S33	M18	S31	S30	S30	S31	S31
Tetracyline	R0	R0	R0	R0	R0	R0	R0	R0	R0	R0	R0
Neomycine	S23	S21	S20	R12	S22	S22	R11	R12	R12	R11	R11
Tazocine	S22	S23	S24	S25	S29	S28	S27	S28	S24	S28	S27
Streptomycin	R8	S16	R0	R0	M12	R0	R0	R0	M14	R0	R0

Numbers: diameters (millimeter) of bacteriostatic rings
S sensitive, *R* resistant, *M* moderate

concentrations of class 1 integron and gene cassette were normalized to both DNA mass and original sample volume (Fig. 5). It was found that more than 90% of integron and gene cassette were removed by activated sludge process in the two STPs, in terms of relative concentration based on DNA mass and sample volume. In Stanley STP, the applied disinfection method removed 94% integron and 77% gene cassette. The levels of gene cassettes in Shatin and Stanley biosoilids were below the detection limit (3.12 gene copy/ng DNA).

Discussion

In this study, class 1 integronase gene *int*I1 was found to be present in the sludge samples of all the five STPs, as well as influent and effluent water of the two Hong Kong STPs. Previous reports demonstrated that integrons carrying various ARGs frequently occurred in different water environments, including animal production or aquaculture areas (Schmidt et al. 2001; Moura et al. 2007; Akinbowale et al. 2007; Jacobs and Chenia 2007), sewage treatment plants (Szczepanowski et al. 2004; Tennstedt et al. 2005; Da Silva et al. 2007; Taviani et al. 2008), surface waters (Poppe et al. 2006; Mukherjee and Chakraborty 2006; Lin and Biyela 2005), and sediments (Dalsgaard et al. 2000). These results together with those obtained in this study indicate that class 1 integrons are widespread in environmental samples.

Among the 109 LFE strains isolated from activated sludge, 36 isolates (33%) were found harboring detectable class 1 integrons (11 isolates carrying a common gene cassette array of about 1,600 bp). The proportion of LFE strains carrying integrons is comparable to those of other studies. It was reported that 26% (five of 19) of antibiotic-resistant *Vibrio* spp. isolated from surface urban water (river and sea) contained class 1 integrons (Taviani et al. 2008). da Silva et al. (2007) investigated the resistance characteristics of enterobacteria isolated from raw and treated wastewater of a municipal treatment plant and found that 31 of 343 enterobacteria contained class 1 integrons, with integron-specific variable regions of 0.9–3 kbp. Tennstedt et al. (2003)

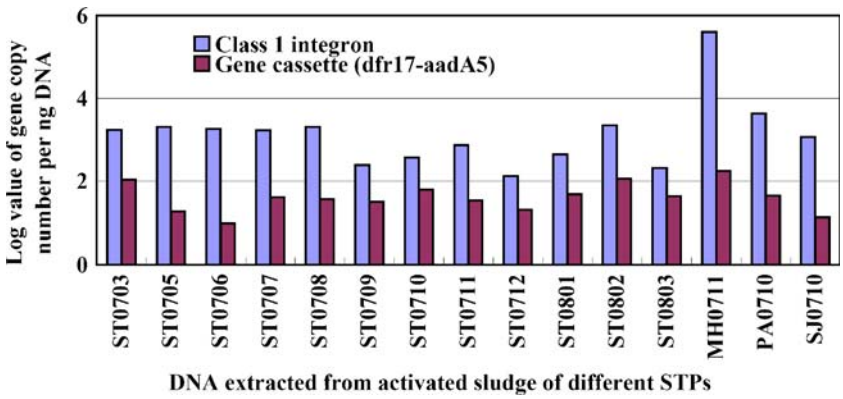

Fig. 4 Abundance of class 1 integron and the gene cassette detected in activated sludge sampled from the STPs of Shatin (*ST*), Minhang (*MH*), San Jose (*SJ*), and Palo Alto (*PA*). *Number* after STP name means sampling time point, 0705 for May of 2007

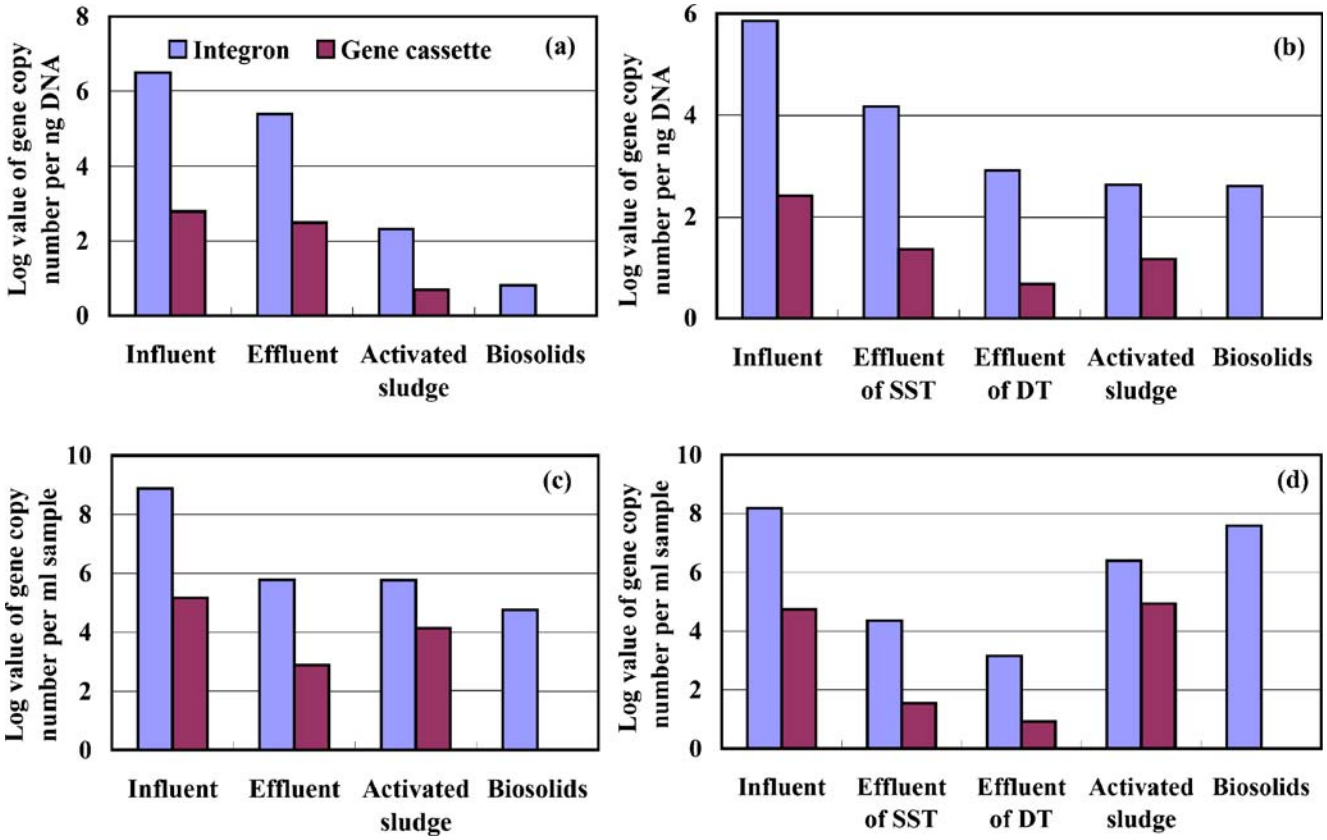

Fig. 5 Abundance of class 1 integron and the gene cassette detected in Shatin (**a** and **c**) and Stanley (**b** and **d**) STPs. **a** and **b** normalized to DNA amount; **c** and **d** normalized to volume of original sample. *SST* secondary sedimentation tank, *DT* disinfection tank

found 12% of 97 multi-resistance plasmids isolated from a wastewater treatment plant carried gene cassettes with sizes of 1.2–3.0 kbp. Different PCR primers were used in these studies of class 1 integron detection, including CS primers and class 1 integrase gene primers. Which primer set is more effective to detect integrons is still unknown. In this study, both CS primers and integrase-directed primers were used to ensure the gene cassettes detected were located on class 1 integrons. Also, the detections were further confirmed by successful PCRs of *sul*I and *qacE*Δ1, the static genes located on 3′ conserved segment of class 1 integron.

In this study, 11 LFE isolates were found to carry class 1 integrons with a common gene cassette array, which was phylogenetically close to ARGs of *dfr*17 and *aad*A5, encoding dihydrofolate reductase mediating resistance to trimethoprim, and adenylyltransferase mediating resistance to spectinomycin/streptomycin. The genotypic results were further confirmed by phenotypic analysis on antimicrobial susceptibility. The 11 LFEs were found to be multi-resistant, and almost all the isolates were resistant to trimethoprim and streptomycin (Table 3). The *dfr* and *aad* genes were frequently found to be acquired on variable regions of class 1 integrons in bacterial isolates from STPs (Tennstedt et al. 2003; Szczepanowski et al. 2004; da Silva et al. 2007). Tennstedt et al. (2003) found eight different gene cassettes (class 1 integron) containing *aad* and seven gene cassettes containing *dfr* located on antibiotic resistance plasmids isolated from one STP, and one gene cassette array was identified to be *dfr*17-*aad*A5, which was just like the one found in this study. Szczepanowski et al. (2004) isolated an integron-containing plasmid named pRSB101 from activated sludge of a wastewater treatment plant and found that the plasmid mediated multiple antibiotic resistances, and the variable region of integrons carried the genes *dfr*1 and *aad*A2. da Silva et al. (2007) found the variable regions of class 1 integrons, detected in *Escherichia* spp. isolates from STPs, contained predominantly the gene cassettes *aad*A1/*dfr*1.

Among the 36 isolates containing *int*I1, only 11 carried gene cassettes, indicating that this type of gene cassette array was only carried by a small part of integrons. The ratio of gene copy number (gene cassette/integron) ranged from 0.04% to 20.83%, demonstrating that most of the integrons are free of gene cassette or carry other types of gene cassettes. Integrons with as many as nine antibiotic resistance genes, typically four or five, are frequently found in clinical environment (Crowley et al. 2008; Labuschagne et al. 2008), agricultural wastewater (Jacobs and Chenia

2007), urban wastewater (Tennstedt et al. 2003; da Silva et al. 2007), and even in the water not exposed to antibiotics (Park et al. 2003; Obst et al. 2006). However, the dispersal of integrons may have begun before the antibiotic resistance since integrons without gene cassettes of antibiotic resistance have been recently recovered from different bacterial species residing in soil and sediments (Stokes et al. 2006).

This is the first study to apply qRT-PCR to quantify class 1 integrons and gene cassettes in STPs. The qRT-PCR is usually used to quantify target DNA on the basis of the principle that initial concentration can be estimated according to the change of PCR product concentration with amplification cycles (Zhang and Fang 2006). Among the several fluorescent reagents developed for qRT-PCR, SYBR Green is the most common method used to quantify ARGs in environmental samples, for example, tetracycline resistance genes (*tet*) in beef cattle farm (Yu et al. 2005), groundwater (Mackie et al. 2006), river sediments (Pei et al. 2006), and STPs (Auerbach et al. 2007), as well as *sul*I genes in river sediments (Pei et al. 2006) and neomycin resistance genes (*npt*) in river water (Zhu 2007). However, qRT-PCR quantification of integrons and gene cassettes in environmental sample was seldom reported. Hardwick et al. (2008) developed a SYBR Green-based qRT-PCR assay and quantified the relative abundance of class 1 integrons in environmental samples normalized to 16S rRNA gene in each sample and found that 2.7% of bacterial cells in creek sediment potentially harbored one class 1 integron.

In this study, most of class 1 integron and the detected gene cassette were found to be removed by activated sludge process in both Shatin and Stanley STPs, in terms of relative concentration based on DNA mass and sample volume (Fig. 5). In addition, the disinfection method used in Stanley STP had a high removal of integron and gene cassette (Fig. 5). So far, no information has been available about the effects of wastewater treatment processes on elimination of gene copy number of integrons and gene cassettes in wastewater. Some researchers investigated the abundance of ARGs in STPs with qRT-PCR. Auerbach et al. (2007) found that *tet*Q concentrations were highest in wastewater influent while *tet*G concentrations were highest in activated sludge, and UV disinfection had no effect on reduction in the amount of detectable *tet* genes in wastewater effluent. Engemann et al. (2006) found the loss rate of *tet*M, O, P, and W in aquatic environment had a significantly positive correlation to simulated sunlight exposure. However, environmental fates of ARGs and integrons have not been reported and should be paid more attention.

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