Occurrence of tetracycline resistant bacteria and resistance gene (fetW) in hospital and municipal wastewaters

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OCCURRENCE OF TETRACYCLINE RESISTANT BACTERIA AND RESISTANCE GENE (*tet*W) IN HOSPITAL AND MUNICIPAL WASTEWATERS

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ABSTRACT

The occurrence and spread of antibiotic resistant bacteria (ARB) and genes in the environment is a public health concern. Wastewater is a potential source for dissemination of ARB and antibiotic resistance genes (ARGs) in the environment. The present study was conducted to determine the abundance of tetracycline resistant bacteria (TC^r) and gene (*tet*W) in hospital and municipal wastewaters. The influence of wastewater treatment plants (WWTPs) on the fate of TC^r bacteria and *tet*(W) gene was also investigated. A total of 66 samples from raw and final effluent of hospital and domestic WWTPs were analyzed. TC^r bacteria as a major part of the heterotrophic plate count (HPC) bacteria were found in the raw wastewater and final effluent samples in a concentration ranged from 1.28×10^8- 5.19×10^6 to 4.60×10^6 - ND (not detected) CFU/ 100ml, respectively. *Tet*(W) gene concentration in the raw wastewaters were found to be in the range of 3.87×10^7-6.23×10^{13} copies/100ml and WWTPs did not contribute in effective reduction of *tet*(w) gene. No significant correlation was found between the levels of *tet*(W) gene and TC^r bacteria in either raw wastewater or final effluent. The results of this study showed that TC^r bacteria and *tet*(W) gene were present in relatively high levels in both municipal and hospital wastewaters. The results also indicated that conventional wastewater treatment plants didn't contribute in effective reduction of *tet*(w) gene and wastewater effluents are a potential source for dissemination of *tet*(w) gene into the natural environment.

KEYWORDS: TC^r bacteria, *tet*(W), Municipal wastewater, Hospital wastewater, wastewater treatment plant

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1. INTRODUCTION

Today, antibiotics play an important role in human and livestock health, but raising the resistance of microorganisms to antibiotics represents a serious cosmopolitan concern [1-5]. Increase of ARB in the environment may pose a threat to public health. Wastewater is a major source of ARB and ARGs in the environment which acts as a pathway for introduction of ARB and ARGs from anthropogenic sources into natural system [6-9].

Tetracyclines were the first major class of antibiotics defined as "broad spectrum" [10]. These antibiotics have been widely used for more than four decades as antimicrobial agents and as growth promoters in agriculture [11, 12]. Before the 1950s, there was no reported pathogen resistance to tetracycline [10], but recently, the emergence of tetracycline-resistant microorganisms has led to limited usage of these antibiotics.

The emergence and distribution of tetracycline resistant pathogenic bacteria have caused serious concerns. Tetracycline resistance results from different mechanisms such as tetracycline efflux pump proteins, ribosome protection proteins (RPPs), and tetracycline modification [10, 13]. At least 38 different TC^r genes have been identified and characterized [10, 14-16]. TC^r genes including *tet*M, *tet*O,*tet*S, *tet*Q, and *tet*W have been detected in microbial communities of sewage and wastewater treatment systems[1, 7, 17], hospital or animal produced wastewaters, even in natural surface and ground water environments [13, 18], sediments [19-21], and soil [22]. Their ability to spread among the microbial population could complicate the situation [19, 23]. These genes disseminate through a variety of agents, including plasmids, transposons, and bacteriophages [13, 24, 25].

There is controversy about the fate of ARB and ARGs in wastewater treatment plants. A few studies have shown that wastewater treatment plants (WWTPs) are efficient in the removal of ARB and ARGs [2, 26, 27].

whereas, some studies have reported very low or no efficiency of WWTPs in removing of ARB and ARGs [7, 17, 27-29],However, the fate of ARB and ARGs in the WWTPs could be influenced by many factors, such as the type of wastewater, microorganisms, organic loading, sunlight and design and operation of wastewater treatment systems [26]. Furthermore, determination of the occurrence and fate of antibiotic resistant bacteria and genes in municipal and hospital wastewaters is necessary as a basic step toward preventing the dissemination of ARB and ARGs into the environment. Therefore, this study was carried out to investigate the abundance of TC^r bacteria and *tet*(W) gene in hospital and municipal wastewaters. Furthermore, the influence of WWTPs on the fate of TC^r bacteria and *tet*(W) gene was investigated.

2 MATERIALS AND METHODS

2.1. Sample collection

Wastewater samples were taken from six different sites between September 2012 and April 2013. A total of 66 samples from raw and final effluent of hospital and domestic wastewaters were analyzed. Characteristics of the different sites are given in Table 1. Samples were collected in 1 liter sterile glasses, transferred to the laboratory in an insulated box with cooling packs and were analyzed immediately after arrival at the laboratory.

2.2. Enumeration of heterotrophic plate count bacteria and TC^r bacteria

To determine the number of heterotrophic plate count (HPC and TC^r bacteria, wastewater samples were serially diluted, and 0.1 ml of each serial dilution was plated on R2A (Merck) medium without and with tetracycline (16 $\mu g.ml^{-1}$), respectively. Nystatin was also added to all plates as antifungal [1, 17, 30]. Plates were incubated for 48h at 37˚C. Following incubation, the results were calculated and expressed as colony-forming units per milliliter (CFU/ml) and TC^r percentages were derived by comparing heterotrophic and TC^r bacteria numbers. All the experiments were carried out in duplicates and the mean values were considered.

2.3. DNA extraction

For detection of *tet*(W) gene in TC^r bacteria, predominant TC^r bacteria with regard to the apparent characteristics were isolated and subcultured onto R2A agar plates with tetracycline. For detection of *tet*(W) gene, the isolated colonies were suspended in 100 µl of deionized water, and genomic DNA was extracted by boiling for 15 min and centrifugation at 13,000 rpm for 5 min. 0.1 (v/v) of 3M sodium acetate and 2.5 (v/v) of 95% ethanol were added to the supernatant, and then the suspensions were mixed and centrifuged at 13000 rpm for 10 min. The supernatant was discarded, and 500 µl of 80% ethanol was added to the pellets. Suspensions were centrifuged at 13000 rpm for 5 min. DNA was suspended in 100 µl of distilled water and extracted DNA was stored in a freezer at -20˚C until use.

Tet(w) gene was quantified in wastewater samples by real-time PCR. In order to determine the quantity of *tet*(W) gene in wastewater samples, DNA was extracted from wastewater samples. 50 ml of the all samples were centrifuged at 6000 rpm for 15 min. The supernatant was discarded, and the pellet was resuspended in 300µl of distilled water. The resuspended pellets were frozen in liquid nitrogen and heated in boiling water three times. The DNA was extracted and purified using Promega DNA Extraction kit (Promega Wizard Genomic DNA purification kit, Madison, WI) according to the manufacturer's manual.

2.4. Conventional PCR detection of tet(W) gene in TC^r bacteria

Two sets of primers 5'-gagagcctgctatatgccagc-3' and 5'-gggcgtatccacaatgttaac-3' were used to amplify a fragment of 168 base pairs regarding the *tet*(W) gene [1, 13, 31-36]. The total volume of the reaction mixture (25 µl) contained 2.5 µl of 10x PCR buffer, 0.2 µM of each primer, 0.2 mM of each dNTPs, 2 units of Taq DNA polymerase, and 1 µl of DNA. All PCR assays contained a positive and a negative control. PCR amplification was performed using a thermal cycler (Corbett, Australia). The PCR profile included initial denaturation at 94˚C for 10 min, denaturation at 94˚C for 45s, annealing at 64˚C for 30 s, and extension at 72˚C for 45 s for 30 cycles,

TABLE 1 – Characteristics of hospital and municipal wastewaters.

	Municipal wastewater			Hospital wastewater		
	MW1	MW2	MW3	HW1	HW2	HW3
Types of WWTP	Conventional activated sludge(two step)	Conventional activated sludge	Stabilization pound	*	*	Extended aeration+ high speed sand filtration
Capacity(m^3/d)	250000	130000	90000	807	84	90
Disinfection process	Chlorine	Chlorine	Chlorine	Chlorine	Chlorine	Chlorine+ UV
Final effluent receiving field	Land application	River	Agricultural application	Municipal wastewater collection system	Absorption well	Absorption well

*Influent sample only

followed by a final extension at 72˚C for 10 min. PCR products were analyzed by agarose gel electrophoresis using 1.5% gels containing ethidium bromide together with a DNA molecular weight marker. Gels were viewed on a UV transilluminator (UV Tech, France).

2.5. Real- time PCR

In order to quantify the concentrations of *tet*(W) gene in wastewater samples, a real-time PCR was performed to specifically amplified the *tet*(W) gene by primers described in conventional PCR. Real-time PCR assay using SYBR Green was carried out on ABI prism equipment (Applied Biosystems, USA) as describe previously [1, 17, 19]. Plasmid DNA containing *tet*(W) gene sequence insert was used as a standard control in real-time PCR. The amplified target DNA product from a conventional PCR was separated on an agarose gel, and the products were excised from the gel. The gel fragments were purified with a gel extraction kit (Gel purification kit, Cat NO:K-3035-1, Bioneer, USA) and cloned into a PTZ 57R plasmid vector, transformed into *Escherichia coli* Top 10 using $CaCl_2$ and heat-shock and recombinant bacteria were selected on LB agar containing ampicillin. Plasmids were screened for the presence of target gene by colony PCR and sequencing of the amplified gene. Plasmids were purified and then plasmid concentration was determined by spectroscopy (Nanodrop® ND1000). To generate the standard curve, plasmids were prepared in a series of 10-fold dilutions and CT value for each respective concentration was used.

The presence/ absence of inhibitors in all conventional and real-time PCR samples was verified using amplification of 16S rRNA gene region of bacteria with primer set Eubac27F and 1492R.

2.6. Statistical analysis

Results were statistically analyzed using the T-test to compare quantity averages in raw wastewaters and final effluents. ANOVA analysis was used to compare the variables of different sites. The correlations between HPC, TC^r bacteria, and *tet*(W) gene were calculated by Spearman correlation coefficients (SPSS 20 for Windows, SPSS Inc., Chicago, IL). A p-value of <0.05 was considered statistically significant.

3. RESULTS

3.1. HPC and TC^r bacteria in raw wastewater and final effluent

Abundance of HPC and TC^r bacteria in raw wastewater and final effluent samples is shown in Figure 1. Concentration of TC^r bacteria in raw wastewater and final effluent samples were found to range from 5.19×10^6-1.28×10^8 to4.60×10^6-ND (not detected) CFU/100ml. In general, no significant difference was observed in HPC of raw wastewater from different sources, but there was a significant difference in the final effluents. In this study, no significant difference was observed between HPC levels in inlet and outlet wastewaters from the MW1 and MW3 treatment plants, but a significant difference was seen in those of the MW2 and HW3treatment plants. The comparison of TC^r bacteria in inlet wastewaters showed no significant differences among six various wastewater sources. Among municipal and hospital wastewater plants, HW3 was the most efficient in removing of HPC (96.3%) and TC^r bacteria (100%). The removal percent of HPC and TC^r bacteria in various wastewater treatment plants was HW3>MW2> MW3>MW1 and HW3>MW2> MW1>MW3, respectively.

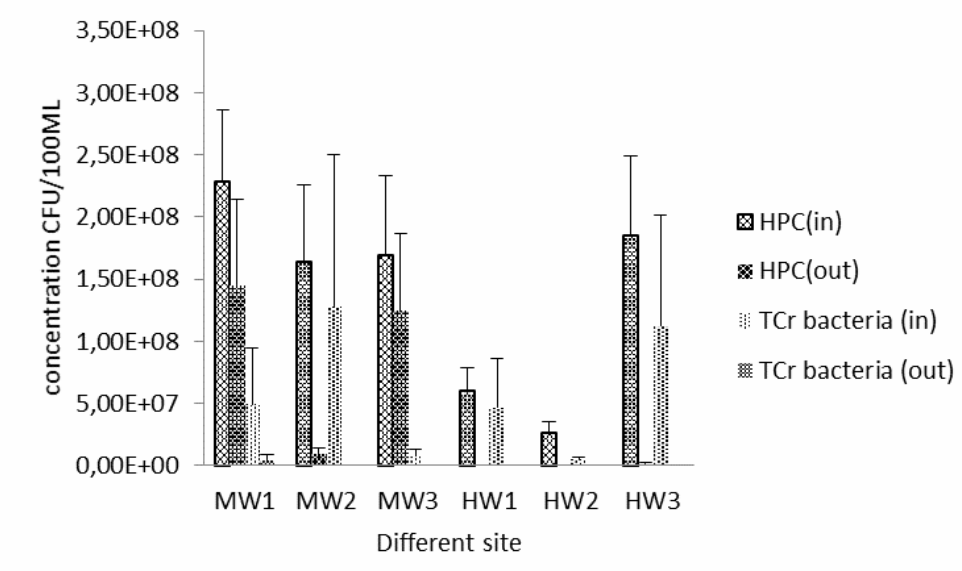

FIGURE 1 - Average concentration of HPC and TC^r bacteria in raw wastewater and final effluent of municipal and hospital wastewaters. Error bars are indicating standard errors.

TABLE 2 – Presence (+) or absence (-) of *tet*(W) gene in isolated TC^r bacteria from raw wastewater and final effluent samples.

Site	Municipal wastewaters			Hospital wastewaters		
	MW1	MW2	MW3	HW1	HW2	HW3
Raw wastewater	+	+	+	-	-	-
Final effluent	-	-	-			-

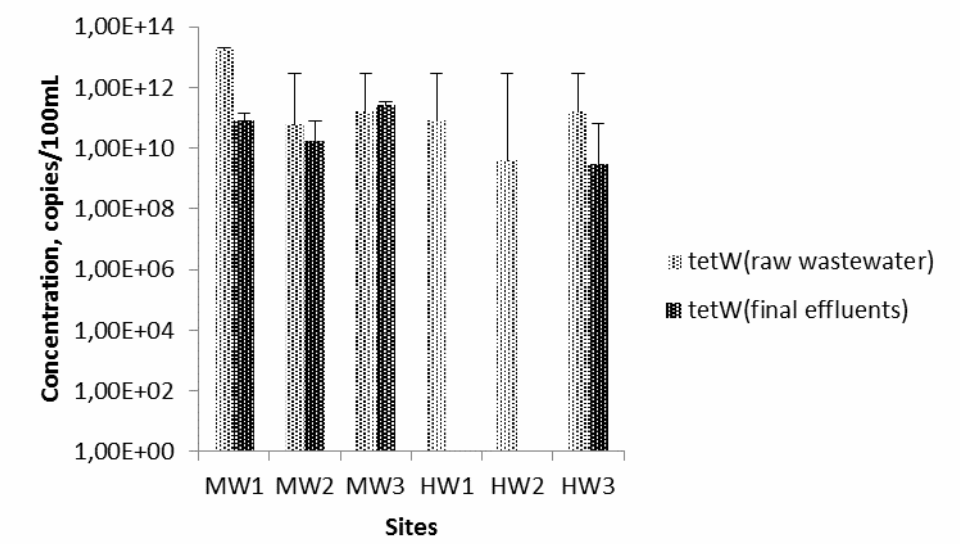

FIGURE 2 - Log concentration (copies/100mL) of *tet*(W) gene in municipal and hospital wastewaters. Error bars are indicating standard errors.

3.2. PCR detection of *tet*(w) gene in TC^r bacteria

The presence/absence of *tet*(w)gene in isolated colonies from positive samples of raw wastewater and final effluents of municipal and hospital wastewaters is given in Table 2. The results showed that *tet*(W) gene were only identified in TC^r bacteria of municipal raw wastewaters. Likewise, this gene was not found in any of TC^r bacteria of hospital wastewaters.

3.3. Quantification of *tet*(W) gene

The logarithmic concentration values of *tet*(W) in raw wastewater and final effluent of municipal and hospital wastewaters are given in Figure 2. Concentration of *tet*(W) gene in raw wastewater and final effluent were found to range from 3.87×10^{7}-6.23×10^{13} to1.13×10^{7}-7.6×10^{10} copies/100ml, respectively. The results indicated that *tet*(W) gene are more frequently found in influent of municipal wastewaters than in hospital wastewaters. The values of *tet*(W) gene in raw wastewaters follow up the front pattern: MW1>MW3>HW3>HW1>MW2>HW2. The maximum *tet*(W) gene value was identified in inlet and outlet wastewater at the MW1 site. The Pearson correlation test indicated that *tet*(W) gene levels in raw wastewater were significantly correlated with the levels in final effluent (r=0.38). Similarly, the Pearson correlation test characterized that *tet*(W) gene levels and HPC concentration were correlated in the final outlet of wastewater plants (r= 0.416).

Overall, no significant correlation was seen between TC^r bacteria and *tet*(W) gene in raw wastewater and final effluent samples.

4. DISCUSSION

Tetracycline is one of the most commonly used antibiotics in human and veterinary medicine. It is also used to improve agricultural growth [37, 38]. Previous studies have mainly focused on identifying the genes resistance to tetracycline in municipal wastewater treatment plants [1, 7]. In this study, the concentration of TC^r bacteria and *tet*(W) gene in raw wastewater and final effluent of municipal and hospital wastewater treatment plants were investigated. The results showed that TC^r bacteria exist in both municipal and hospital wastewaters. These findings correspond with the results of studies done by other researchers, including Kümmerer [3] and Schulter et al. [39]. However, the mean concentration of TC^r bacteria in the study was higher than in other similar studies. Reinthaler et al. [40] determined the concentration of resistant bacteria as $10^{3.9}$ – $10^{5.45}$ and $10^{0.78}$ – $10^{3.15}$CFU/100 ml in inlet and outlet samples of wastewater treatment plants. Similarly, in another study carried out by Munir et al. [1], the mean concentrations of TC^r bacteria in raw wastewater and effluent parts of wastewater treatment plants were $10^{0.7}$ – $10^{2.48}$ and $10^{4.18}$ – $10^{5.36}$ CFU/100ml, respectively.

High concentration of TC^r bacteria in the study could be related to the heavy use of this antibiotic in the country. Based on the findings of this study, there is a relationship between TC^r and HPC bacteria in raw wastewaters which indicates that TC^r bacteria make up a major part of HPC. The results showed that the number of HPC and TC^r bacteria decrease from the inlet to the outlet in all 3 municipal and 1 hospital wastewater treatment plants. However, the decrease in bacteria was only significant for MW2 and HW3 wastewater treatment plants with the maximum removal rate for HW3.The reason for this impact could be related to the efficiency of various wastewater treatment processes on removing of bacteria. In HW3 wastewater treatment plant, the disinfection system includes a chlorination process supported by a UV system. Likewise, this treatment plant also benefits from high speed sand filtration after disinfecting. It seems that high removal efficiency of HPC and TC^r bacteria in the wastewater plant is related to disinfection system as well as high speed sand filtration. After HW3, the MW2 plant has the highest efficiency in removing HPC and TC^r bacteria. This treatment plant become equipped with an integral operation system and, in comparison with other municipal wastewater plants in the study, is in a better condition from BOD and suspended solids removal point of view.

The results of this study showed that the concentration of HPC and TC^r bacteria in raw wastewater and effluents are greater in municipal treatment plants than in hospital sites. This finding does not correspond with the dominant idea that the levels of ARB might be higher in hospital wastewaters [3]. Wiethan et al. [41] found no difference between outlet resistance parameters in municipal treatment plants and those of hospital treatment plants. Some researchers indicated that although, hospital wastewaters constitute only about 1% of the municipal wastewaters, but they maybe the main sources of the inlet of resistant bacteria into the environment [3]. However, due to the various sources of municipal wastewaters, these may play a more crucial role in the dissemination of resistant bacteria throughout the environment [3, 42, 43].

According to the results derived from isolated colonies, *tet*(W) gene were found only in TC^r bacteria isolated from raw wastewater of municipal treatment plants. This gene was not found in either the final effluent isolated colonies at municipal wastewater treatment plants or in hospital wastewaters; possibly, because it is induced by other genes resistant to tetracycline.

Research findings showed that *tet*(W) gene Similar to TC^r bacteria was found in higher concentration in raw wastewaters of municipal treatment plants than in hospital wastewaters. This is due to the fact that tetracycline is found more often in non-medical environments than in medical areas. The mean concentration of *tet*(w) gene in inlet and outlet wastewater samples was1.83×10^{12} and 9.85×10^{10} copies/100ml, respectively. In comparison to the other studies, as TC^r bacteria the levels of *tet*(w) gene in the study were higher than reported in others. For example, in a study conducted by Munir et al. [1], the given results are $10^{7.4} - 10^{5.37}$ and ND -$10^{3.63}$copies/100ml of *tet*(w) gene. Other researchers, including Auerbach et al. [7] and Zhang et al. [44], reported lower values for *tet*(W). The highest concentration of *tet*(W) gene in raw wastewater belonged to wastewater from the MW1 treatment plant. This treatment plant is situated in an area adjacent to agriculture and veterinary medical regions. For this reason, *tet*(W) gene has been found abundantly in this area. According to the profile of *tet*(w) gene (Fig 2), the highest gene level in influent belonged to the MW3 site after MW1. The location of this treatment plant is almost the same as the MW1 site. As seen in Figure 2, the MW3 treatment plant with stabilization pond system had no effect on the removal of *tet*(W) gene. This result contradicts the findings of Engemann et al. (2006) which based on *tet*(W) gene might be intensified after being exposed to sunlight [45]. In this study, the lowest level of genes in municipal wastewater influents was seen in the MW2 treatment plant; because this treatment plant receive wastewater from limited sources than two other municipal wastewater plants. A high level of *tet*(W) gene in all effluent samples confirm that conventional wastewater treatment processes couldn't efficiently reduce the *tet*(W) gene. In the study of Munir et al. [1], among different types of WWTPs, the highest removal of *tet*(W) gene was observed in a membrane biological reactor (MBR). They also observed a significant difference in the log removal of *tet*(W) gene between conventional wastewater utilities such as activated sludge and MBR facility. The result of our study also showed that UV disinfection couldn't significantly reduce the values of *tet*(W) gene. This result is consistent with the results of Munir et al. [1] and Auerbach et al. [7] which demonstrated disinfection process (chlorination and UV process) don't contribute in significant reduction of ARGs.

However, conventional treatment processes could significantly reduce ARB [1].

The results of our study showed no or small numbers of TC^r bacteria in effluent of WWTPs, whereas the high values of *tet*(W) gene were detected in the effluents. As a result of disinfection process some bacteria may be in a viable but non-culturable (VBNC) state and therefore, it seems possible that detected *tet*(W) gene was part of VBNC bacteria.

4. CONCLUSION

In conclusion this study demonstrated that TC^r bacteria and *tet*(W) gene were present in relatively high levels in both municipal and hospital wastewaters. It was also found that, despite primary presuppositions, municipal wastewaters play a more important role in the dissemination of resistant bacteria into the environment. The results also indicated that conventional wastewater treatments did not contribute in significant reduction of *tet*(W) gene and wastewater effluents are a potential source for dissemination of *tet*(W) gene into the natural environment.

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REFERENCES

[1] Munir M., Wong K., Xagoraraki I. (2011) Release of antibiotic resistant bacteria and genes in the effluent and biosolids of five wastewater utilities in Michigan. Water Research, 45, 681-693.

[2] Huang J.J., Hu H.Y., Tang F., Li Y., Lu S.Q., Lu Y. (2012) Monitoring and evaluation of antibiotic-resistant bacteria at a municipal wastewater treatment plant in China. Environment International, 42, 31-36.

[3] Kümmerer K. (2004) Resistance in the environment. Journal of Antimicrobial Chemotherapy, 54, 311-320.

[4] Pruden A., Amézquita A., Collignon P., Kristian K., Graham D.W., Lazorchak J.M., Suzuki S., Silley P. (2013) Management Options for Reducing the Release of Antibiotics and Antibiotic Resistance Genes to the Environment. Environmental Health Perspectives, 121, 878-885..

[5] Jia R., Jing D., Li H.,Xie K., Hunag D., Zhang Z., Xu X. (2011) Membrane bioreactor process for removing selected antibiotics from wastewater at different solids retention times. Fresenius Environmental Bulletin, 20, 754-763

[6.] Kim S., Aga D.S. (2007) Potential Ecological and Human Health Impacts of Antibiotics and Antibiotic-Resistant Bacteria from Wastewater Treatment Plants. Journal of Toxicology and Environmental Health, Part B, 10, 559-573.

[7.] Auerbach E.A., Seyfried E.E., McMahon K.D. (2007) Tetracycline resistance genes in activated sludge wastewater treatment plants. Water Research, 41, 1143-1151.

[8] Pruden A., Storteboom H., Carlson K.H. (2006) Antibiotic resistance genes as emerging contaminants: studies in northern Colorado. Environmental Science Technology, 407, 445-450.

[9] Rizzo L., Manaia C., Merlin C., Schwartz T., Dagot C., Ploy M.C. (2013) Urban wastewater treatment plants as hotspots for antibiotic resistant bacteria and genes spread into the environment: A review. Science of The Total Environment, 447, 345-360.

[10] Van hoek A.H.A.M., Mevius D., Guerra B., Mullany P., Roberts A.P., Aarts H.J.M. (2011) acquired antibiotic resistance gene: an overview. Frontiers in Microbiology, 2, 1-27.

[11] Dang H., Ren J., Song L., Sun S., An L. (2008) Diverse Tetracycline Resistant Bacteria and Resistance Genes from Coastal Waters of Jiaozhou Bay. Microbial Ecology, 55, 237-246.

[12] Kümmerer K., (2009) The presence of pharmaceuticals in the environment due to human use - present knowledge and future challenges. Journal of Environmental Management, 90, 2354-2366.

[13] Koike S., Oliver H.D., Yannarell A.C., Chee-Sanford J.C., Aminov R.I., Mackie R.I. (2007) Monitoring and source tracking of tetracycline resistance genes in lagoons and groundwater adjacent to swine production facilities over a 3-year period. Applied Environmental Microbiology, 73, 4813-23.

[14] Zhang X.X., Zhang T., Fang H. (2009) Antibiotic resistance genes in water environment. Applied Microbiology and Biotechnology, 82, 397-414.

[15] Tao R., Ying G.G, Su H.C., Zhou H.W., Sidhu J.P.S. (2010) Detection of antibiotic resistance and tetracycline resistance genes in Enterobacteriaceae isolated from the Pearl rivers in South China. Environmental Pollution, 158, 2101-2109.

[16] Srinivasan V., Nam H.M., Sawant A.A., Headrick S.I., Nguyen L., Oliver S. (2008) Distribution of Tetracycline and Streptomycin Resistance Genes and Class 1 Integrons in <i>Enterobacteriaceae</i> Isolated from Dairy and Nondairy Farm Soils. Microbial Ecology, 55, 184-193.

[17] Gao P., Munir M., Xagoraraki I. (2012) Correlation of tetracycline and sulfonamide antibiotics with corresponding resistance genes and resistant bacteria in a conventional municipal wastewater treatment plant. Science of The Total Environment, 421–422, 173-183.

[18] Xu, H., Chen W.,Whang C., Yang J. (2012) Distribution of antibiotics and antibiotic resistant fecal coliform in influent and effluents in a wastewater treatment plants and its receiving water. Fresenius Environmental Bulletin, 21, 1619-1625.

[19] Pei R., Kim S.C., Carlson K.H., Pruden A. (2006) Effect of River Landscape on the sediment concentrations of antibiotics and corresponding antibiotic resistance genes (ARG) . Water Research, 40, 2427-2435.

[20] Suzuki S., Kobayashi T., Suehiro F., Tuyen B.C., Tana T.S. (2008) High Occurrence Rate of Tetracycline (TC) -Resistant Bacteria and TC Resistance Genes Relates to Microbial Diversity in Sediment of Mekong River Main Waterway. Microbes and Environments, 23, 149-152.

[21] Kümmerer K., (2009) Antibiotics in the aquatic environment - A review - Part II. Chemosphere, 75, 435-441.

[22] Walsh F., Ingenfeld A., Zampicolli M., Hilber-Bodmer M., Frey J.E., Duffy B. (2011) Real-time PCR methods for quantitative monitoring of streptomycin and tetracycline resistance genes in agricultural ecosystems. Journal of Microbiological Methods, 86, 150-155.

[23] Munir M., Xagoraraki I. (2011) Levels of antibiotic resistance genes in manure, biosolids, and fertilized soil. Journal of Environmental Quality, 40, 248-255.

[24] Chopra I., Roberts M. (2001) Tetracycline antibiotics: mode of action, applications, molecular biology, and epidemiology of bacterial resistance. Microbiology and Molecular Biology reviews, 65, 232-260.

[25] Salyers A.A., Shoemaker N.B., Li L.Y. (1995) In the driver's seat: the Bacteroides conjugative transposons and the elements they mobilize. Journal of Bacteriology, 177, 5727-5731.

[26] Bouki C., Venieri D., Diamadopoulos E. (2013) Detection and fate of antibiotic resistant bacteria in wastewater treatment plants: A review. Ecotoxicology and Environmental Safety, 91, 1-9.

[27] Guardabassi L., Lo Fo Wong D.M.A., Dalsgaard A. (2002) The effects of tertiary wastewater treatment on the prevalence of antimicrobial resistant bacteria. Water Research, 36, 1955-1964.

[28] Huang J.J., Hu H.Y., Tang F., Li Y., Lu S.Q., Lu Y. (2011) Inactivation and reactivation of antibiotic-resistant bacteria by chlorination in secondary effluents of a municipal wastewater treatment plant. Water Research, 45, 2775-2781.

[29] Kümmerer K. (2003) Significance of antibiotics in the environment. Journal of Antimicrobial Chemotherapy, 52, 5-7.

[30] Brooks J.P., Rensing C., Gerba C.P., Pepper I.L. (2007) Occurrence of antibiotic-resistant bacteria and endotoxin associated with the land application of biosolids. Canadian Journal of Microbial, 53, 616-622.

[31] Aminov R. I., Garrigues N., Teferedegne B., Krapac I. J., White B. A., Mackie R. I. (2002) Development, Validation, and Application of PCR Primers for Detection of Tetracycline Efflux Genes of Gram-Negative Bacteria. Applied and Environmental Microbiology, 68, 1786-1793.

[32] Aminov R.I., Chee-Sanford J.C., Garrigues N., Mehboob A., Mackie R.I. (2004) Detection of tetracycline resistance genes by PCR methods. Methods in Molecular Biology-Clifton Then Totowa, 268, 3-14.

[33] Srinivasan V., Nam H.M., Sawant A.A., Headrick S.I., Nguyen L., Oliver S. (2008) Distribution of tetracycline and streptomycin resistance genes and class 1 integrons in Enterobacteriaceae isolated from dairy and nondairy farm soils. Microbial Ecology, 55, 184-193.

[34] Devirgiliis C., Caravelli A., Coppola D., Barile S., Perozzi G. (2008) Antibiotic resistance and microbial composition along the manufacturing process of Mozzarella di Bufala Campana. International Journal of Food Microbiology, 128, 378-384.

[35] Rysz M., Alvarez P.J.J. (2004) Amplification and attenuation of tetracycline resistance in soil bacteria: aquifer column experiments. Water Research, 38, 3705-3712.

[36] Lee D.Y., Seo Y.S., Rayamajhi N., Kang M.L., Lee S.I., Yoo H.S. (2009) Isolation, characterization, and evaluation of wild isolates of Lactobacillus reuteri from pig feces. The Journal of Microbiology, 47, 663-672.

[37] Castanon J.I.R. (2007) History of the Use of Antibiotic as Growth Promoters in European Poultry Feeds. Poultry Science, 86 2466-2471.

[38] Sarmah A.K., Meyer M.T., Boxall A.B.A. (2006) A global perspective on the use, sales, exposure pathways, occurrence, fate and effects of veterinary antibiotics (VAs) in the environment. Chemosphere, 65, 725-759.

[39] Schlüter A., Szczepanowski R., Pühler A., Top E.M. (2007) Genomics of IncP‐1 antibiotic resistance plasmids isolated from wastewater treatment plants provides evidence for a widely accessible drug resistance gene pool. FEMS Microbiology Reviews, 31, 449-477.

[40] Reinthaler F.F., Posch J., Feierl G., Wüst G., Haas D., Ruckenbauer G. (2003) Antibiotic resistance of E. coli in sewage and sludge. Water Research, 37, 1685-1690.

[41] Wiethan J., Unger J., Brunswik Titize A., Kummerer K. (2001) Occurrence and reduction of antibiotic resistant (pathogenic) bacteria in municipal sewage treatment plants. in Proc. International Water Association 2nd World Water Congress, Berlin.

[42] Kümmerer K., (2009) Antibiotics in the aquatic environment - A review - Part I. Chemosphere, 75, 417-434.

[43] Martinez J.L., (2009) Environmental pollution by antibiotics and by antibiotic resistance determinants. Environmental Pollution, 157, 2893-2902.

[44] Zhang T., Zhang M., Zhang X., Fangh H. (2009) Tetracycline resistance genes and tetracycline resistant lactose-fermenting Enterobacteriaceae in activated sludge of sewage treatment plants. Environmental Science & Technology, 43, 3455-3460.

[45] Enegemann C.A., Adams L., Knapp C.W., Graham D.W., (2006) Disappearance of oxytetracycline resistance genes in aquatic systems. FEMS Microbiology Letters, 263, 176-182.

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