Prevalence of antibiotic resistant genes in selected activated sludge processes in Isfahan Province, Iran

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Original Article

Abstract

Wastewater treatment plants are one of the main sources of dissemination of antibiotic resistance genes (ARGs) into the environment. The present study was conducted to determine the prevalence and removal of ARGs in different wastewater treatment processes. A total of 36 samples from raw and final effluent of different activated sludge processes were collected and analyzed. Molecular analysis was conducted on the samples for the detection of encoding genes resistant to three groups of antibiotics (tetracycline, erythromycin, and sulfonamide). The results of this study showed that all ARGs were identified in activated sludge processes (average 70%). Comparison of different activated sludge processed showed that the removal percentage patterns were A-B process, conventional process, and extended aeration process, respectively. The results of this study showed that ARGs were present in relatively high levels in activated sludge process. The results also indicated that the activated sludge process did not contribute to effective reduction of ARGs. However, this revealed the major role of the activated sludge process in the distribution of ARGs in the environment. Thus, it seems that the improvement of the process is necessary for ARGs control in activated sludge process.
KEYWORDS: Activated sludge, Antibiotic resistance genes (ARGs), wastewater, A-B process, Extended aeration

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Introduction

Antibiotics are widely used for human health, and veterinary and agriculture purposes.[1-3] Most antibiotics are released unchanged into the environment.[1,4] Antibiotics can be poorly absorbed and metabolized by humans and animals.[5] The metabolized rate of antibiotics is estimated to be approximately 3%. During recent years, the negative effects of antibiotic residues in the environment have gained more attention.[1,6,7] It is very important to note that the widespread use of antibiotics is the major reason for the emergence, selection, and spreading of antibiotic-resistant bacteria.[5,8] Researches demonstrated the dissemination of resistance genes rapidly transmitted between microorganisms in the environment.[2,9] Resistance to antibiotics can cause many problems such as increased rate of mortality and treatment length.[10] Application of antibiotics in humans, veterinary medicine, and agriculture for nearly 60 years has exerted a major impact on bacterial communities, resulting in various resistances to the antibiotics, which is genetically controlled by antibiotic resistance genes (ARGs).[1] Genetic elements such as integrons, plasmids, and transposons have the main role in the transitions of ARGs between bacteria in the environment.[2,5] Previous studies found resistance genes in different environments (water, wastewater, and soil).[2,5] ARGs and antibiotic resistant bacteria have been detected extensively in wastewater samples.[1,11,12]

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However, infectious diseases by antibiotic resistant bacteria have been a serious concern in hospitals and clinics[13] due to overprescribing of antibiotics to patients.

Wastewater treatment plants (WWTPs) are connected to public and private households, hospitals, and other non-point sources pollution where antibiotics are used.[14] Different investigations have detected resistant bacteria and genes in wastewaters.[11,12,15] However, studies have shown that the elimination of antibiotics and resistant determinants in WWTPs is incomplete and the residues may enter the environment via wastewater discharge and land application of biosolids.[2,12,16,17] Although some researchers indicated an ambiguous role for wastewater treatment in the reduction of ARGs, some reports suggested that certain factors in WWTPs may increase the ARGs.[12,14,18]

The aim of this study was to investigate the prevalence of ARGs, effects of different wastewater treatment processes on the prevalence of ARGs, comparison of ARGs in the influent and effluent of different activated sludge processes, and the possibility of the dispersion of ARGs into the environment. In this study, raw influents and final effluent samples were monitored for the three genes of tetW, sul1, and ermB. These ARGs were chosen because they are commonly used in human and veterinary medicine, and agriculture for different goals.[12]

Materials and Methods

Samples were collected from the inlet and outlet of three different activated sludge processes located in Isfahan Province, Iran, including two-stage activated sludge treatment system (A-B process) (geospatial location: N 32°44' 55.00", E 51°44' 2.12"), conventional activated sludge treatment system (N 32°37' 06.79", E 51°43' 39.32") and extended aeration treatment system (N 31°59' 49.22", E 51°50' 29.68"). A total of 36 samples were collected from raw wastewater and final effluents in 1-liter sterile glasses. Samples were transferred to the laboratory and analyzed immediately after arrival.

DNA was also extracted from the original wastewater samples to determine the presence/absence of the selected genes in raw wastewater and effluent samples. For this purpose, 50 ml of each sample was centrifuged at 6000 rpm for 15 minutes. The supernatant was discarded, and the pellet was resuspended in 300 μl of distilled water. The resuspended pellets were frozen in liquid nitrogen and heated in boiling water three times.[19] DNA was extracted and purified using Promega DNA Extraction kit (Promega Wizard Genomic DNA Purification Kit, Madison, WI, USA) according to the manufacturer's manual. The 3 resistance encoding genes of tetW, sul1, and ermB were selected. Three primers pairs were used for the amplification of these genes. The characteristics of the primers are given in table 1.

Polymerase chain reaction (PCR) amplification was conducted in a total volume of 25 μl containing 2.5 μl of 10 X PCR buffer, 0.2 μM of each primer, 0.2 mM of each dNTP, 2 units of Taq DNA polymerase, and 1 μl of DNA. All assays contained a positive and a negative control. The PCR process is performed using an initial denaturation at

Table 1. Characteristics of primers used in the study

Adsorbate	Target gene	Sequence (5'- 3')	Amplified size (bp)	Annealing temperature (°C)	Reference
TetW-FV	TetW	GAGAGCCTGCTATATGCCAGC	168	64	12, 27
TetW-RV		GGCGTATCCACAATGTTAAC			
Sul(I)-FW	Sul1	CGCACCGGAAACATCGCTGCAC	163	55.9	12, 28
Sul(I)-RW		TGAAGTTCCGCCGCAAGGCTCG			
ErmB-F	ermB	AAAACTTACCCGCCATACCA	193	60	14, 29, 30
ErmB-R		TTTGGCGTGTTTCATTGCTT			

94°C for 10 minutes, denaturation at 94°C for 45 seconds, annealing at varied temperatures for 30 seconds, and extension at 72°C for 45 seconds for 30 cycles, followed by a final extension at 72°C for 10 minutes. PCR products were analyzed through 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).[19]

To compare the presence and absence of genes in different sites and before and after treatment, Excel and SPSS software (version 20, SPSS Inc., Chicago, IL, USA) were used.

Results and Discussion

ARGs are a global concern.[8] Environmental compartment is one of the main route for prevalence and dissemination of these pollutants. WWTPs can enhance or reduce ARGs in the environment.[3,20,21] This study documents the prevalence of ARGs at different WWTPs in Isfahan. All genes were detected in WWTPs (Figure 1).

The overall prevalence of ARGs in different WWTPs are presented in figure 2. This shows that the abundance of ARGs in A-B process was higher than other WWTPs and the abundance of ARGs was lowest in extended aeration process. However, the pattern of ARGs in different WWTPs is A-B process > conventional process > extended aeration process. Although, A-B process has a double process for the treatment of wastewaters, the results show that the efficiency of this process is very low. Previous studies have reported that the low performance of the A-B process in WWTPs is related to types of operation and raw wastewater inlet. Munir et al. showed that these genes are found in abundance in WWTPs (activated sludge, membrane bio reactor (MBR), rotating biological contactors (RBCs), and oxidation ditch). Their findings confirm the results of the present study.[12] However, other researchers showed that these genes can be transferred by different bacteria (Aeromonas, escherichia, listeria, and etc., microbial community, bacillus, and enterococcus).[1,22]
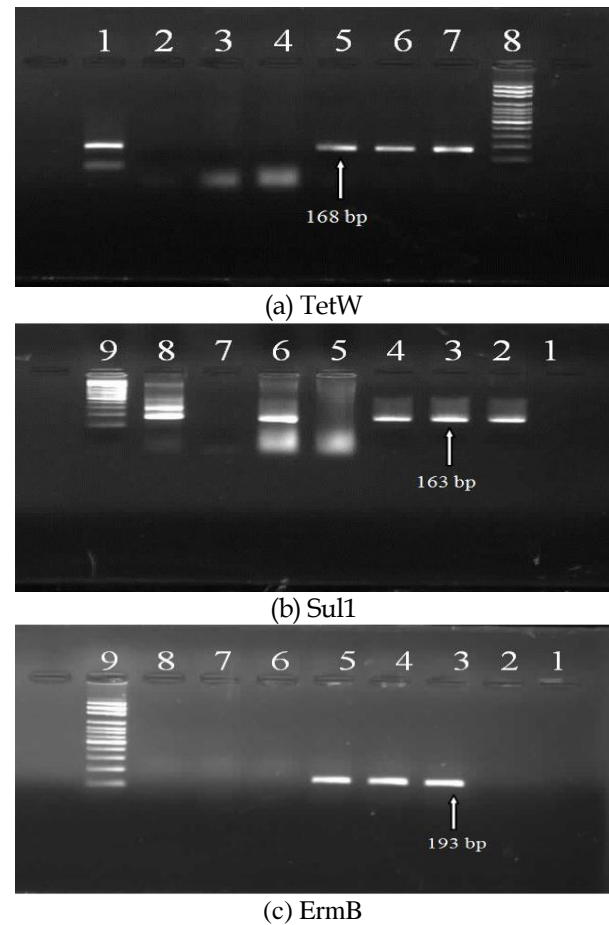

(a) TetW

(b) Sul1

(c) ErmB

Figure 1. Polymerase chain reaction (PCR) product profiles of TetW (a), Sul1 (b), and ErmB (c)

As shown in figure 3, the frequencies of ARGs in influent and effluent are sul1 > ermB > tetW and sul1 > tetW > ermB, respectively. Munir et al. found that the sul1 gene had the highest prevalence (100%) in the WWTPs.[12]
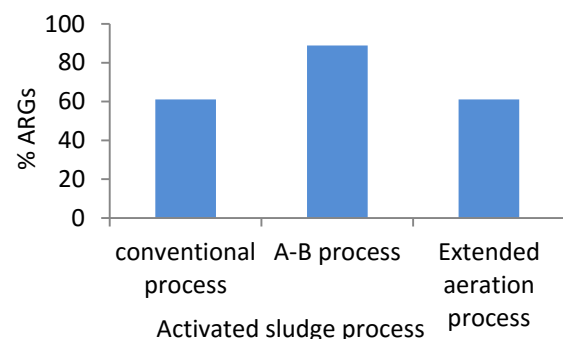

Figure 2. Overall antibiotic resistance genes (ARGs) detected in different wastewater treatment plants (WWTPs)

The high prevalence of this gene can be due to a high prevalence of the genetic elements (integron) in the activated sludge process.[19]
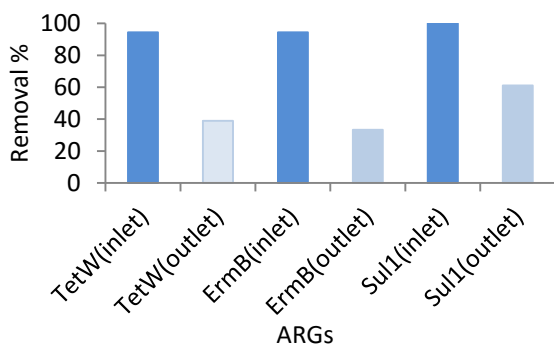

Figure 3. Antibiotic resistance genes (ARGs) detected in influent and effluent of wastewater treatment plants (WWTPs)

However, there was a variation in the prevalence of different ARGs. This variation was also observed in other studies.[8,12] The variation in the prevalence of ARGs in wastewater treatment may be due to the nature of wastewater, type of bacteria that carry ARGs, and environmental factors (such as temperature, pH, oxygen, and etc.).[23] As seen in figure 4, ARG reduction for Sul1, tetW, and ermB was 29, 49, and 54 percent, respectively.
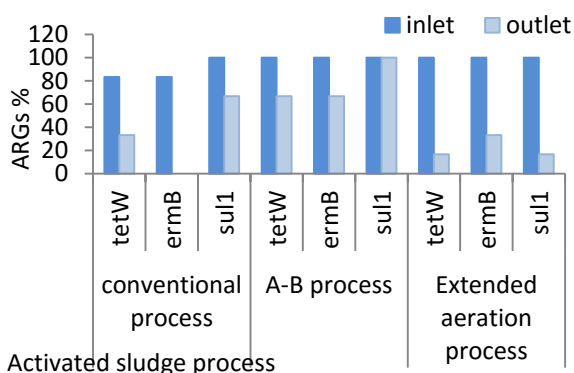

Figure 3. Percentage of antibiotic resistance genes (ARGs) in inlet and outlet of different activated sludge processes

The percentage of reduction shows that the impacts of the process on ermB is the highest and on Sul1 is the lowest. Rodriguez-Mozaz et al. and Samadi et al. reported that the effects of WWT on the reduction of ARGs were not uniform.[24,25] These findings were in agreement with those of the present study. For example, ermB and tetW decreased during wastewater treatment, but increased in the case of bla_{TEM}, sul1, and qnrS.[25] Furthermore, Szczepanowski et al. detected 64% resistance genes in the final effluent of WWTPs.[14] Aydin et al. showed that these genes are found in abundance in anaerobic treatment facilities.[26]

Conclusion

The present research findings indicate that activated sludge process is a hotspot for the release of ARGs into the environment. Dissemination of ARGs into the environment is a growing public health concern. ARGs in the environment can be transferred from pathogenic bacteria to non-pathogenic bacteria. However, this impaires water ecology through the change in population dynamics and physiology and also poses a hazard to public health.

Conflict of Interests

Authors have no conflict of interests.

Acknowledgements

References

1. Zhang XX, Zhang T, Fang HH. Antibiotic resistance genes in water environment. Appl Microbiol Biotechnol 2009; 82(3): 397-414.
2. Gao P, Munir M, Xagoraraki I. Correlation of tetracycline and sulfonamide antibiotics with corresponding resistance genes and resistant bacteria in a conventional municipal wastewater treatment plant. Sci Total Environ 2012; 421-422: 173-83.
3. Bouki C, Venieri D, Diamadopoulos E. Detection and fate of antibiotic resistant bacteria in wastewater treatment plants: a review. Ecotoxicol Environ Saf 2013; 91: 1-9.
4. Kummerer K. Significance of antibiotics in the environment. J Antimicrob Chemother 2003; 52(1): 5-7.
5. Tao R, Ying GG, Su HC, Zhou HW, Sidhu JP. Detection of antibiotic resistance and tetracycline

resistance genes in Enterobacteriaceae isolated from the Pearl rivers in South China. Environ Pollut 2010; 158(6): 2101-9.

6. Sarmah AK, Meyer MT, Boxall AB. A global perspective on the use, sales, exposure pathways, occurrence, fate and effects of veterinary antibiotics (VAs) in the environment. Chemosphere 2006; 65(5): 725-59.

7. Wright GD. The antibiotic resistome: the nexus of chemical and genetic diversity. Nat Rev Microbiol 2007; 5(3): 175-86.

8. Aali R, Nikaeen M, Khanahmad H, Hassanzadeh A. Monitoring and comparison of antibiotic resistant bacteria and their resistance genes in municipal and hospital wastewaters. Int J Prev Med 2014; 5(7): 887-94.

9. Wang HH, Schaffner DW. Antibiotic resistance: how much do we know and where do we go from here? Appl Environ Microbiol 2011; 77(20): 7093-5.

10. Huang JJ, Hu HY, Lu SQ, Li Y, Tang F, Lu Y, et al. Monitoring and evaluation of antibiotic-resistant bacteria at a municipal wastewater treatment plant in China. Environ Int 2012; 42: 31-6.

11. Auerbach EA, Seyfried EE, McMahon KD. Tetracycline resistance genes in activated sludge wastewater treatment plants. Water Res 2007; 41(5): 1143-51.

12. Munir M, Wong K, Xagoraraki I. Release of antibiotic resistant bacteria and genes in the effluent and biosolids of five wastewater utilities in Michigan. Water Res 2011; 45(2): 681-93.

13. Rosenblatt-Farrell N. The landscape of antibiotic resistance. Environ Health Perspect 2009; 117(6): A244-A250.

14. Szczepanowski R, Linke B, Krahn I, Gartemann KH, Gutzkow T, Eichler W, et al. Detection of 140 clinically relevant antibiotic-resistance genes in the plasmid metagenome of wastewater treatment plant bacteria showing reduced susceptibility to selected antibiotics. Microbiology 2009; 155(Pt 7): 2306-19.

15. Pruden A, Pei R, Storteboom H, Carlson KH. Antibiotic resistance genes as emerging contaminants: studies in northern Colorado. Environ Sci Technol 2006; 40(23): 7445-50.

16. Munir M, Xagoraraki I. Levels of antibiotic resistance genes in manure, biosolids, and fertilized soil. J Environ Qual 2011; 40(1): 248-55.

17. Iwane T, Urase T, Yamamoto K. Possible impact of treated wastewater discharge on incidence of antibiotic resistant bacteria in river water. Water Sci Technol 2001; 43(2): 91-9.

18. Reinthaler FF, Posch J, Feierl G, Wust G, Haas D,

Ruckenbauer G, et al. Antibiotic resistance of E. coli in sewage and sludge. Water Res 2003; 37(8): 1685-90.

19. Czekalski N, Berthold T, Caucci S, Egli A, Burgmann H. Increased levels of multiresistant bacteria and resistance genes after wastewater treatment and their dissemination into lake geneva, Switzerland. Front Microbiol 2012; 3: 106.

20. Wright GD. Antibiotic resistance in the environment: a link to the clinic? Curr Opin Microbiol 2010; 13(5): 589-94.

21. Chee-Sanford JC, Aminov RI, Krapac IJ, Garrigues-Jeanjean N, Mackie RI. Occurrence and diversity of tetracycline resistance genes in lagoons and groundwater underlying two swine production facilities. Appl Environ Microbiol 2001; 67(4): 1494-502.

22. Kummerer K. Resistance in the environment. J Antimicrob Chemother 2004; 54(2): 311-20.

23. Samadi N, Aali R, Asgari E, Mirhosaeini H, Shahriari A, Mahmoodi F, et al. Identification of clinically antibiotic resistant genes Aac(3)-IIa and Aac(6')-Ib in wastewater samples by multiplex PCR. Environmental Health Engineering and Management Journal 2015; 2(2): 47-52.

24. Rodriguez-Mozaz S, Chamorro S, Marti E, Huerta B, Gros M, Sanchez-Melsio A, et al. Occurrence of antibiotics and antibiotic resistance genes in hospital and urban wastewaters and their impact on the receiving river. Water Res 2015; 69: 234-42.

25. Aydin S, Ince B, Ince O. Development of antibiotic resistance genes in microbial communities during long-term operation of anaerobic reactors in the treatment of pharmaceutical wastewater. Water Res 2015; 83: 337-44.

26. Lee DY, Seo YS, Rayamajhi N, Kang ML, Lee SI, Yoo HS. Isolation, characterization, and evaluation of wild isolates of Lactobacillus reuteri from pig feces. J Microbiol 2009; 47(6): 663-72.

27. Negreanu Y, Pasternak Z, Jurkevitch E, Cytryn E. Impact of treated wastewater irrigation on antibiotic resistance in agricultural soils. Environ Sci Technol 2012; 46(9): 4800-8.

28. Knapp CW, Zhang W, Sturm BS, Graham DW. Differential fate of erythromycin and beta-lactam resistance genes from swine lagoon waste under different aquatic conditions. Environ Pollut 2010; 158(5): 1506-12.

29. Knapp CW, Dolfing J, Ehlert PA, Graham DW. Evidence of increasing antibiotic resistance gene abundances in archived soils since 1940. Environ Sci Technol 2010; 44(2): 580-7.

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