Metagenomic insight into the persistent antimicrobial resistant genes in wastewater treatment plant influent and effluent

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Abstract

Environmental acquisition of antimicrobial resistance is global threat to public health where wastewater treatment plants (WWTP) serve as a hub of carrying diverse range of antimicrobial resistant organism and provide suitable conditions for nonvertical transmission of genetic determinants of antimicrobial resistance. Despite of the advancement in wastewater treatment techniques, certain bacteria and antimicrobial resistant genes (ARGs) persist in the final effluent and are disseminated into the environment. WWTPs are regularly releasing ARGs and pathogenic bacteria into the environment [1]. We aimed at characterizing the core resistome of two WWTP influent and final effluent in a four-year longitudinal metagenomic study. Influent and effluent water samples were collected from JungRang and SeoNam WWTPs for a period of four years. We adopted the methods in for DNA extraction, sequencing and metagenomic data analysis as described in previous studies [2,3]. Briefly, we adapted read based approach to calculate the absolute abundance of ARGs and contig based approach to identify the host of the core ARGs. Moreover, we find the human fecal pollution in both influent and effluent samples and calculated the metagenomic risk score.

We find a diversity of ARGs from 151 to maximum of 654 ARGs per site and the abundance ranges from 1.2 X 10^{-1} to 2.4 copies of ARGs per copy of 16S rRNA gene. The differential abundance analysis revealed that ARGs of class sulfonamide and aminoglycoside were significantly abundant in effluent, while MDR were abundant in influents ($p < 0.05$). Of which,

sul1 and *aadA5* were differentially more abundant in effluent samples. These ARGs have the ability to survive the treatment process due to their chemical structure, stability and their association with mobile elements [2,4]. Of the total 1100 ARGs found in all sites, 73 ARGs in SeoNam WWTP and 75 in JungRang WWTP were found to be persistent in all four years. MDR, aminoglycoside and sulfonamide were the major class of core ARGs. Continuous presence of core ARGs if of particular concern as they can compromise the environment. Abundance of some of the core ARGs got increased in effluent i.e., *sul1* and *aadA5* ($p<0.01$). *CrAssphage* abundance and metagenomic risk score was higher in influent samples. ARGs diversity was negatively correlated to the core resistome while it was positively correlated with the *crAssphage* abundance ($p<0.05$). We observe core resistome was not correlated with either ARG diversity, *crAssphage*, resistome risk and plasmid based ARGs, the factors behind the persistence of these ARGs should be monitored carefully. *Pseudomonas*, *Acinetobacter*, *Aeromonas* and *Mycobacterium* were the major genera carrying core ARGs both in influent and effluent, which are often associated to carry multiple ARGs [5]. The results of our study indicate that core resistome is sharing more than half of the total ARGs abundance and the presence of these ARGs on plasmid make them an environmental hazard. As core ARGs are not correlated to the either plasmids, chromosome or crAssphage, but their constant release in the environment can cause critical public health problems and needs to be addressed carefully.

References

1. Pazda, M.; Kumirska, J.; Stepnowski, P.; Mulkiewicz, E. Antibiotic resistance genes identified in wastewater treatment plant systems – A review. *Science of The Total Environment* **2019**, *697*, 134023, doi:https://doi.org/10.1016/j.scitotenv.2019.134023.
2. Raza, S.; Jo, H.; Kim, J.; Shin, H.; Hur, H.-G.; Unno, T. Metagenomic exploration of antibiotic resistome in treated wastewater effluents and their receiving water. *Science of The Total Environment* **2021**, *765*, 142755.
3. Jo, H.; Raza, S.; Farooq, A.; Kim, J.; Unno, T. Fish farm effluents as a source of antibiotic resistance gene dissemination on Jeju Island, South Korea. *Environmental Pollution* **2021**, *276*, 116764, doi:https://doi.org/10.1016/j.envpol.2021.116764.

4. Thornton, C.N.; Tanner, W.D.; VanDerslice, J.A.; Brazelton, W.J. Localized effect of treated wastewater effluent on the resistome of an urban watershed. *GigaScience* **2020**, *9*, doi:10.1093/gigascience/giaa125.
5. Ju, F.; Li, B.; Ma, L.; Wang, Y.; Huang, D.; Zhang, T. Antibiotic resistance genes and human bacterial pathogens: Co-occurrence, removal, and enrichment in municipal sewage sludge digesters. *Water Res* **2016**, *91*, 1-10, doi:10.1016/j.watres.2015.11.071.