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Removal of bacterial contaminants and antibiotic resistance genes by conventional wastewater treatment processes in Saudi Arabia: Is the treated wastewater safe to reuse for agricultural irrigation?

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ARTICLE INFO

Article history:
Received 30 October 2014
Received in revised form
22 January 2015
Accepted 23 January 2015
Available online 3 February 2015

Keywords:
Water reuse
Conventional activated sludge processes
Microbial contaminants
Antibiotic resistance
Microbial risk

ABSTRACT

This study aims to assess the removal efficiency of microbial contaminants in a local wastewater treatment plant over the duration of one year, and to assess the microbial risk associated with reusing treated wastewater in agricultural irrigation. The treatment process achieved 3.5 logs removal of heterotrophic bacteria and up to 3.5 logs removal of fecal coliforms. The final chlorinated effluent had 1.8×10^2 MPN/100 mL of fecal coliforms and fulfils the required quality for restricted irrigation. 16S rRNA gene-based high-throughput sequencing showed that several genera associated with opportunistic pathogens (e.g. *Acinetobacter*, *Aeromonas*, *Arcobacter*, *Legionella*, *Mycobacterium*, *Neisseria*, *Pseudomonas* and *Streptococcus*) were detected at relative abundance ranging from 0.014 to 21 % of the total microbial community in the influent. Among them, *Pseudomonas* spp. had the highest approximated cell number in the influent but decreased to less than 30 cells/100 mL in both types of effluent. A culture-based approach further revealed that *Pseudomonas aeruginosa* was mainly found in the influent and non-chlorinated effluent but was replaced by other *Pseudomonas* spp. in the chlorinated effluent. *Aeromonas hydrophila* could still be recovered in the chlorinated effluent. Quantitative microbial risk assessment (QMRA) determined that only chlorinated effluent should be permitted for use in agricultural irrigation as it achieved an acceptable annual microbial risk lower than 10^{-4} arising from both *P. aeruginosa* and *A. hydrophila*. However, the proportion of bacterial isolates resistant to 6 types of antibiotics increased from 3.8% in the influent to 6.9% in the chlorinated effluent. Examples of these antibiotic-resistant isolates in the chlorinated effluent include *Enterococcus* and *Enterobacter* spp. Besides the presence of antibiotic-resistant bacterial isolates, tetracycline resistance genes tetO, tetQ, tetW, tetH, tetZ were also present at an average 2.5×10^2, 1.6×10^2, 4.4×10^2, 1.6×10^1 and 5.5×10^3 copies per mL of chlorinated effluent. Our study highlighted that potential risks associated with the reuse of treated wastewater arise not only from conventional fecal indicators or known pathogens, but also from antibiotic-resistant bacteria and genes.

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http://dx.doi.org/10.1016/j.watres.2015.01.036

1. Introduction

Agricultural irrigation accounts for ~70% of the freshwater use worldwide (FAO, 2005), and imposes a serious burden on water supplies in water-stressed countries like Saudi Arabia. To mitigate the stress on water resources, Saudi authorities have commenced a countrywide initiative to develop the local market for treated wastewater to meet the needs of agricultural irrigation (Al-Jassem, 2012). Despite intense efforts to develop treated wastewater as an alternative water resource for agricultural irrigation, reuse of wastewater is still not widely acceptable in Saudi Arabia as exemplified from the low reuse rates (Abu-Rizaiza, 1999; KICP, 2010).

The reluctance to reuse treated wastewater is, in part, due to a lack of understanding on the efficacy of wastewater treatment plants (WWTPs) in removing contaminants. The general perception among the local public is that the WWTPs in a developing country like Saudi Arabia may have inefficient treatment processes that would lead to incomplete removal of microbial contaminants (e.g. pathogens and fecal indicators). There are at least 30 major sewage treatment facilities in Saudi Arabia (UNEP, 1999). The majority of these facilities utilize secondary treatment that involves biological processes like activated sludge trickling filters, aerated lagoons and rotating biological contactors. Agricultural workers can be directly exposed to these microbial contaminants through occupational contact if wastewater were not well-treated and reused for irrigation. Public consumers can also be indirectly exposed through consumption of food produce that is contaminated by insufficiently treated wastewater. In order to promote acceptance of the use of these waters, it would be essential to first implement systematic monitoring efforts on the treatment efficiency of local WWTP, and assess any potential microbial risks associated with reusing the treated water.

Evaluation of treatment efficiency is generally based on monitoring conventional water quality parameters that include heterotrophic bacterial counts and the abundance of coliforms. However, emerging contaminants like antibiotic-resistant bacteria (ARB) and antibiotic resistance genes (ARGs) are also of increasing concern. Surveillance efforts to document the incidence of ARB revealed that *Pseudomonas aeruginosa*, *Acinetobacter* spp., *Staphyloccoccus aureus* and *Klebsiella pneumoniae* were the most prevalent resistant pathogens reported among nosocomial infections in Saudi Arabia (Aly and Balkhy, 2012; Balkhy et al., 2006; Memish et al., 2012). Although there are limited epidemiological studies that detail the death burden arising from antibiotic-resistant pathogens in Saudi Arabia, local practitioners have reported that pediatric patients are most susceptible to infections caused by antibiotic-resistant pathogens (Bukhari and Al-Otaibi, 2009; Shibl et al., 2009). To illustrate, 5 out of 80 children with community-onset methicillin-resistant *S. aureus* (MRSA) succumbed to the infection, while fatalities arising from invasive pneumococcal meningitis ranged from 0 to 22% among children in the Arabian Peninsula and Egypt (Shibl et al., 2009).

Infections by antibiotic-resistant pathogens are not limited to the hospital settings. Since 1990s, there is a statistically significant increase in community-acquired infections caused by antibiotic-resistant *Escherichia coli* (Al-Tawfiq, 2006b) and *Staphylococcus aureus* (Al-Tawfiq, 2006a; Yezli et al., 2012). The increase in ARB in community settings has been attributed in part to the ready availability of over-the-counter antibiotics, a lack of public knowledge towards responsible use of antibiotics and antibiotic misuse in animal husbandry (Al-Tawfiq et al., 2010). In recent years, there is increasing global recognition that wastewater treatment processes can become hotspots for antimicrobial resistance and the subsequent dissemination of the resistance genes into environment (Michael et al., 2013; Pruden, 2014; Rizzo et al., 2013). A WWTP that does not effectively remove ARB is likely to further compound antimicrobial resistance threats if such partially treated wastewater were to be reused. As such, microbial contaminants related to ARB and ARGs should also be monitored alongside with the conventional indicators.

The overall aims of this study are **(i)** to assess the removal efficiency of microbial contaminants in wastewater by a local WWTP, and **(ii)** to evaluate the microbial risk associated with reusing the wastewater. To achieve these aims, wastewater samples were collected at different stages of the treatment process over the duration of one year. The approaches undertaken to evaluate the efficacy of this WWTP were to; firstly, determine the removal efficiency of heterotrophic bacterial counts, total and fecal coliforms. Secondly, high-throughput sequencing was performed to characterize the microbial community in wastewater samples, with emphasis given to determine the occurrence of bacterial genera associated with opportunistic pathogens and those that persist throughout the wastewater treatment process. Thirdly, ARB and tetracycline resistance genes were also evaluated using culture-based approaches and quantitative PCR (qPCR) to provide insight into the occurrence of these emerging contaminants. These approaches provide datasets related to the abundance of opportunistic pathogens determined in the wastewater samples, which can be used in quantitative microbial risk assessment (QMRA). The QMRA approach, in turn, provides estimates on the microbial risk faced by the agricultural workers if the wastewater were to be reused for irrigation.

2. Materials and methods

2.1. Sampling site and samples collection

Wastewater was obtained from a wastewater treatment plant (WWTP) in Jeddah, Saudi Arabia. The exact location of the WWTP cannot be disclosed due to a confidentiality agreement with the plant operators. The treatment process in the WWTP consisted of initial settling of solids in a primary clarifier, followed by conventional aerobic activated sludge treatment. The hydraulic retention time (HRT) and sludge retention time (SRT) in the activated sludge tank was 6 h and 15 d, respectively. Biomass from the activated sludge tank was then channeled for further settling of solids in a secondary clarifier prior to disinfection. The amount of treated wastewater that flowed through this WWTP was