Prevalence of Antibiotic Resistant Enterococci in Treated Wastewater Effluent in Durban, South Africa and Characterization of Vancomycin and High-Level Gentamicin-Resistant Strains

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Abstract : Wastewater treatment plants (WWTPs) have been implicated as the leading reservoir for antibiotic resistant bacteria (ARB), including Enterococci spp. and antibiotic resistance genes (ARGs), worldwide. Enterococci are a group of clinically significant bacteria that have gained much attention as a result of their antibiotic resistance. They play a significant role as the principal cause of nosocomial infections and dissemination of antimicrobial resistance genes in the environment. The main objective of this study was to ascertain the role of WWTPs in Durban, South Africa as potential reservoirs for antibiotic resistant Enterococci (ARE) and their related ARGs. Furthermore, the antibiogram and resistance gene profile of Enterococci species recovered from treated wastewater effluent and receiving surface water in Durban were also investigated. Using membrane filtration technique, Enterococcus selective agar and selected antibiotics, ARE were enumerated in samples (influent, activated sludge, before chlorination and final effluent) collected from two WWTPs, as well as from upstream and downstream of the receiving surface water. Two hundred Enterococcus isolates recovered from the treated effluent and receiving surface water were identified by biochemical and PCR-based methods, and their antibiotic resistance profiles determined by the Kirby-Bauer disc diffusion assay, while PCR-based assays were used to detect the presence of resistance and virulence genes. High prevalence of ARE was obtained at both WWTPs, with values reaching a maximum of 40%. The influent and activated sludge samples contained the greatest prevalence of ARE with lower values observed in the before and after chlorination samples. Of the 44 vancomycin and high-level gentamicin-resistant isolates, 11 were identified as E. faecium, 18 as E. faecalis, 4 as E. hirae while 11 are classified as "other" Enterococci species. High-level aminoglycoside resistance for gentamicin (39%) and vancomycin (61%) was recorded in species tested. The most commonly detected virulence gene was the gelE (44%), followed by asa1 (40%), while cylA and esp were detected in only 2% of the isolates. The most prevalent aminoglycoside resistance genes were aac(6')-Ie-aph(2''), aph(3')-IIIa, and ant(6')-Ia detected in 43%, 45% and 41% of the isolates, respectively. Positive correlation was observed between resistant phenotypes to high levels of aminoglycosides and presence of all aminoglycoside resistance genes. Resistance genes for glycopeptide: vanB (37%) and vanC-1 (25%), and macrolide: ermB (11%) and ermC (54%) were detected in the isolates. These results show the need for more efficient wastewater treatment and disposal in order to prevent the release of virulent and antibiotic resistant Enterococci species and safeguard public health.

Keywords : antibiogram, enterococci, gentamicin, vancomycin, virulence signatures

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