

2nd International Congress on **Bacteriology & Infectious Diseases**

November 17-19, 2014 DoubleTree by Hilton Hotel Chicago-North Shore, USA

Phenotypic and genotypic characterization of *Escherichia coli* isolates recovered from treated wastewater effluent and receiving aquatic milieu in Durban, South Africa

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The poor operational status of some wastewater treatment plants (WWTPs) in South Africa results in inadequately treated effluent being discharged into receiving water bodies. These surface waters have therefore been identified as reservoirs for numerous waterborne disease causing organisms. Infections mediated by pathogenic *E. coli* in particular are associated with diseases such as gastroenteritis and haemolytic uremic syndrome. In addition, the use of antimicrobials for the prevention of infection has led to the emergence and spread of multi-drug resistant bacterial pathogens in wastewater. These pathogens harbor resistance determinants on mobile genetic elements such as integrons which can be transferred between bacterial isolates. The current study investigated the treatment efficiency of two independent WWTPs in Durban and determined the impact of treated effluent discharge on the receiving water bodies. Water samples were collected over a six month period and the *E. coli* loads as well as the physico-chemical parameters (BOD, COD, temperature, turbidity, pH etc) were evaluated using standard methods. Presumptive isolates were identified using biochemical tests and detection of the *mdh* gene. Six major virulence genes namely: *eae*, *hly*, *fliC*, *Stx1*, *Stx2* and *rfbE* were defined via PCR while antibiotic resistance profiles of the isolates were determined using the Kirby-Bauer disc diffusion assay. The presence and location of class 1, 2 and 3 integrase genes were determined via PCR while gene cassette arrays were detected via PCR, RFLP and sequence analysis. Conjugation experiments were carried out to determine if plasmid encoded integrons are transferable. The physico-chemical parameters of the water samples varied as follows: between 9-313.33 mg/L, 1.52-76.43 NTUs and 6.30-7.87 for COD, turbidity and pH respectively while the *E. coli* counts ranged between 0 and 31.2 cfu/ml. All values obtained for final effluent at both treatment plants exceeded the acceptable limit of 0 cfu/ml for *E. coli* in water used for domestic and recreational purposes. Of the 200 confirmed isolates the *hly* gene was found in 28%, *fliC* in 20%, *stx2* in 17%, *eae* in 14%, with *stx1* and *rfbE* in only 4% of the isolates. Notable resistance was observed towards Trimethoprim (97%), Tetracycline (56%) and Ampicillin (52.5%). In addition, all isolates tested were identified as multi-drug resistant. All identified integrons (Class 1 in 50.5% of isolates; class 2 in 4% of the isolates) were located on both the plasmid and chromosome. Sequence analysis of gene cassette arrays show an abundance of the *dfrA17* and *dfrA12* gene cassettes encoding resistance to trimethoprim and streptomycin. Conjugation experiments indicate that these resistance determinants can be transferred. These results further highlight the poor operational status of WWTPs in South Africa and outline the need for improved water quality monitoring and stringent guidelines. Further research on the dissemination of antibiotic resistant and virulent *E. coli* in the environment, together with their modes of action and mechanisms of disease will be of extreme importance in water quality studies and provide valuable data to improve the microbiological quality of water in South Africa.

Biography

Leanne Pillay has completed a Bachelor's degree in Medical Science and an honours degree in Microbiology. She is currently completing her Masters degree in Microbiology at the University of KwaZulu Natal.

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