# The occurrence of pathogenic *Escherichia coli* in South African wastewater treatment plants as detected by multiplex PCR

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## Abstract

The aim of this study was to investigate the use of PCR to detect commensal and diarrhoeagenic *Escherichia coli* concentrated from water samples using membrane filtration. To achieve this, culture-based and PCR-based methods were compared for the detection of *E. coli* in raw sewage and primary, secondary and tertiary effluents from 6 wastewater treatment plants around Johannesburg, Gauteng. *E. coli* was concentrated from the samples using standard filtration techniques with subsequent incubation on *E.* coli/coliform chromogenic media to determine the *E. coli* levels. Bacterial DNA was isolated from bacterial colonies trapped on polyethersulphone membranes after filtration using a celite/guanidium thiocyanate method. A single multiplex PCR (m-PCR) assay was used that targeted the *mdh*, *eaeA, stx1, stx2, st, lt, ial* and *eagg* genes associated with diarrhoeagenic *E. coli*. The *mdh* gene was detected in all of the samples even if no culturable *E. coli* was detected. All the diarrhoeagenic *E. coli* types were detected in one or more of the raw sewage samples from the various plants. EPEC was present in 20% (2/10) of the samples, EHEC in 50% (5/10), ETEC in 80% (8/10), EIEC in 10% (1/10) and EAEC in 90% (9/10) of the samples. In the case of the primary and secondary treatment only ETEC (5/5; 100%) and EAEC (5/5; 100%) were detected in all of the samples. The results demonstrate that molecular techniques such as PCR have the potential to be used for the monitoring of water samples for the presence of pathogenic *E. coli,* without the need to culture the organisms.

**Keywords:** *E. coli*, multiplex PCR, wastewater treatment plant effluent