Impact Factor: 4.04

JOURNAL OF BASIC AND LIFE SCIENCES
11(1) 2024 JBLS

ISSN:
3067-2651

ASSESSMENT OF ESCHERICHIA COLI O157:H7 IN THE FINAL DISCHARGE OF TWO WASTEWATER TREATMENT PLANTS IN EASTERN CAPE

Thabo Siphesihle Ndlovu
Department of Microbiology, Faculty of Science, University of Fort Hare, Alice, Eastern Cape, South Africa

Abstract: The final effluents of two wastewater plants located in the Eastern Cape of South Africa were tested for the presence of enterohaemorrhagic *Escherichia coli* O157:H7 isolates, and characteristics of the isolates obtained were determined. A total of 23 wastewater samples were collected from the treatment plants at the final effluent point after the disinfectant stages of wastewater processing. Altogether, 540 presumptive *E. coli* isolates were obtained by colony counting on the *E. coli* O157:H7 chromogenic agar base supplemented with cefixime tellurite and were subculture onto sorbitol-MacConkey agar and tested for agglutination using the Prolex *E. coli* O157 latex test reagent kit. The results showed that the 149 suspected colonies from SMAC agar were all negative for the antisera. None of the isolates agglutinated with antisera against *E. coli* O157 and thus no presence of the bacteria can be confirmed from the treated effluents. The likelihood of the receiving water body and the environment being contaminated with *E. coli* O157:H7 is therefore minimal. Future monitoring is however recommended

Keywords: *E. coli* O157:H7, Eastern Cape, Effluent, Wastewater, Latex Agglutination

INTRODUCTION

The most significant pathogenic *E. coli* for the water industry is the enterohaemorrhagic *E. coli* (EHEC). EHEC serotypes, such as *E. coli* O157:H7 and *E. coli* O111, produce large quantities of shiga-like (or vero) toxins that can cause diarrhoea that ranges from mild and non-bloody to highly bloody, which is indistinguishable from haemorrhagic colitis. Between 2% and 7% of cases can develop the potentially fatal haemolytic uraemic syndrome (HUS), which is characterized by acute renal failure and haemolytic anaemia. The infectivity of EHEC strains is substantially higher than that of other strains. As few as 100

EHEC organisms can cause infection (WHO, 2008; NHMRC, 2011). EHEC comprises more than 100 different serotypes, including O157:H7, which has been responsible for a number of waterborne disease outbreaks (NHMRC, 2011). Current bacterial waterborne pathogens have been linked to gastrointestinal illnesses in human populations. Bacteria (e.g. *E. coli* O157:H7, *Salmonella*, *Shigella*, and *Campylobacter jejuni*), enteric viruses (e.g. Hepatitis A), and protozoa (e.g. *Giardia*, *Cryptosporidium*, *Toxoplasmosis gondii*) have all caused waterborne disease outbreaks (The David Suzuki Foundation, 2006).

In the fall of 1991, Animal transmission, particularly from beef had dominated O157:H7 outbreaks infections in South-Eastern Massachusetts provided an opportunity to identify transmission through beef as a seemingly

unlikely vehicle (Cohen, 2008). The *E. coli* O157:H7 was a culprit in a 2002 outbreak in Canada (WHO, 2003). A more recent outbreak was a multi-state outbreak of the organism in November, 2013 in the US but the source remains under investigation (FDA, n.d.). The first case of the *E. coli* O157 in South Africa was reported in the 1990s (Effler *et al*., 2001). Further attempts to isolate the organism in the wastewater treatment plant were performed in the Gauteng area of South Africa and gave low positive confirmation (Muller *et al.,* 2004). Müller *et al* (2001) reported infrequent incidence of *E. coli* O157 in the river water examined for direct and indirect domestic use in the Gauteng region of South Africa. In contrast, in the Eastern Cape of South Africa, Momba *et al.* (2008) reported the isolation of *E. coli* O157:H7 in drinking water from 6 different communities.

The source of this organism has mostly been attributed to animals; cattle and possibly small domesticated ruminants constitute a primary animal reservoir (Ferens and Hovde, 2011). An outbreak in 1999 in Vancouver Washington traced the source of the *E. coli* O157:H7 to duck faeces (US EPA, 2012). Ateba and Bezuidenhout (2008) worked on livestock animals (cattle and swine faeces) and human faeces in South Africa, they reported higher percentage of the organism isolated and confirmed in the livestock faeces as compared to human faeces. The presence of the organism in human faeces was reported by Abong'o *et al.* (2008) who isolated the organism from the stool of HIV/AIDS patients in the eastern cape of South Africa. A 3-year surveillance study revealed that in South Africa, less than 1% of the samples analysed, account for EHEC, concluding that EHEC is rarely isolated from humans in South Africa and is usually a coincidental finding (Smith, Tau, Sooka, & Keddy, 2011). Abong'o, 2008). Abong'o and Momba (2009) reported the isolation of *E. coli* O157:H7 from meat and meat products in the eastern cape of South Africa. A recent work done by Iwu *et al.* (2021) identified Shiga toxigenic *E. coli* O157:H7 strain in irrigation water and agricultural soil samples.

Documentation of the incidence of infection from *E. coli* O157 in South Africa is scarce (Ateba & Bezuidenhout, 2008) and information on environmental detection of the organism is practically non-existent for South Africa in general and specifically for the province of study, the Eastern Cape and this prompted the need to explore the possible presence of the organism in the Eastern Cape.

Therefore, our aim was to evaluate the final effluent from the wastewater treatment plant as a means to identify occurrence of the organism in the environment.

MATERIALS AND METHODS Waste Water Treatment

The monitored wastewater treatment plants are located in the Buffalo City Municipality. Plant B sewage treatment works is situated in the Eastern Cape Province of the Buffalo City Municipality. It receives municipal domestic sewage as well as run-off water. The wastewater treatment plant operates an activated sludge system with design capacity of about 8ML/day which is considered a medium-sized treatment plant (DWAF: Department of Water Affairs, 2009). The plant treats an average dry weather flow of 7,000 m^3/day and an average wet weather flow of 21,000 m^3/day. The plant has two aeration tanks, each equipped with three vertically-mounted mechanical aerators, two anaerobic tanks and two clarifiers. A splitter box controls the flow of the raw sewage and returns activated sludge (RAS) to the aeration tank. Sludge recycling is done through the RAS pump station hauling the sludge from the sedimentation tanks to the aeration tanks. The waste mixed liquid from the aeration tanks is pumped into the sludge lagoons. Chlorination is done by means of a water pressure operated, wall mounted, gas chlorinator in a baffle-resistant concrete contact tank. Thereafter, the final effluent is pumped to a pair of final effluent reservoirs (Osode, 2007; Osuolale & Okoh, 2015) and discharged into a stream that links to the Mdizeni stream, tributary of the Keiskamma River (DWAF: Department of Water Affairs, 2009). Plant A is a medium size plant with treatment design capacity of 5ML/day. The

Bio-filter/Petroleum process treatment system is employed (DWAF: Department of Water Affairs, 2009) and it discharges its final effluent in the Umzonyana stream.

Sample Collection

Samples were collected from the plants (Plant A: 233, Plant B: 307) on a monthly basis for 12 months between September, 2012 – August, 2013 from the final treated effluent (FE) and discharge point (DP) following recommendations of the Department of Water Affairs and Forestry (DWAF, DHE, & WRC, 2000). Samples were collected in one litre Nalgene bottles previously cleaned by washing in non-ionic detergent, rinsed with tap water, finally rinsed with deionised water and autoclaved prior to usage. Sodium thiosulphate was added to sampling bottles. Samples were then transported in cooler boxes containing ice packs to the Applied and Environmental Microbiology Research Group (AEMREG) laboratory at the University of Fort Hare, Alice, South Africa for analyses. Samples were processed within six hours of collection.

Bacteriological Analysis

Test strains were bought from Leibniz-Institut DSMZ (GmBH). All media (Selective media) used for bacterial analyses were performance-checked. Isolation and bacteria count from samples collected were determined by membrane filtration according to SABS (Sans, 2011) and American Public Health Association APHA, AWWA and WEF, (2012). Serial dilutions of the samples were prepared according to methods described by Rogers and Haines (2005). In certain cases where there was excessive chlorine dosage in the effluent, the raw samples were filtered directly. Sample dilutions were homogenate before filtering. The filtered samples were placed on selective agar for the target organism's isolation. Petri dishes were allowed to dry for 15 minutes and inverted, and incubated for 24 h at 37 °C after which counts in the suitable range (0-300 colonies) were done by manual counting and recorded. The sample blanks Trip blanks, Field blanks and Equipment blanks were subjected to same test parameters as samples and served as quality assurance. All bacterial tests were conducted in triplicate with negative and positive controls run simultaneously with each assay.

Isolation and Enumeration of *E. coli* O157:H7 *E. coli* O157:H7 was examined using the membrane filtration method as described. The filters were placed on *E. coli* O157:H7 chromogenic agar base (Conda, Madrid) supplemented with cefixime tellurite and incubated at 37 °C for 24 h. The target colonies appearing pale pink in colour were counted and recorded as CFU/100 ml SABS (Sans, 2011).

Preservation of Isolates

The isolates were taken as presumptive from the selective media on which they were grown based on their phenotypic identification and re-subculture for purification. Presumptive *E. coli* O157:H7 isolates were prepared in Luria broth and stored in 100% glycerol stock at -70°C.

***E. coli* O157 Latex Agglutination Assay**

Each of the presumptive isolate was streaked onto a Sorbitol Mac-Conkey Agar supplemented with cefixime and tellurite plate from a 24 h growth culture and incubated for 18 h at 37°C. Sorbitol-negative or non-sorbitol fermenters (NSF) were tested for agglutination using the Prolex *E. coli* O157 latex test reagent kit (Pro-lab, Canada).

Statistical Analysis

Data from the study was computed as presence or absence of the target organism. The percentage frequency of occurrence of the organism was determined using Microsoft Excel (2013).

RESULTS

Table 1 presents results of the presumptive tests for *E. coli* O157:H7 presence for the treatment plants A and B. Colonies with characteristic pink/pale pink colour on the *E. coli* O157:H7 chromogenic agar were counted and taken as presumptive isolates for immunological testing. Overall, 41.7% of samples were positive for *E. coli* O157:H7 at Plant A treatment works and 45.8% were positive at the Plant B wastewater treatment plant. The bacterial counts varied from 1log10 to 3log10 for Plant B treatment plant and 1log10 to 4log10 for Plant A treatment works. The highest counts were observed in October and December 2012.

Table 1: Presumptive detection of *E. coli* O157:H7 from Eastern Cape wastewater Treatment plant

	2012			2013							
Site	**Sep**	**Oct**	**Nov**	**Dec**	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**
Plant A	ND	+	+	+	-	-	-	-	-	+	+	-
Plant B	-	+	+	-	+	-	-	+	+	-	+	-

ND: not determined; + = positive for *E. coli* O157:H7; **-** = negative for *E. coli* O157:H7

Latex Agglutination Testing

Result of the latex agglutination test is presented in Table 2. Of the 540 isolates, 82 (27%) and 67 (29%) samples from plant B and A respectively yielded the characteristic non-sorbitol fermenting (NSF) colonies which were further tested for latex agglutination. All 149 NSF isolates were negative for the agglutination test. A positive result with the O157 latex reagents is indicated by large clumps of agglutinated latex with partial or complete clearing of the background latex within 1 to 2 min. This however, was not observed for screened waste water isolates but was postive for control test strains.

Table 2: Latex agglutination screening for Eastern Cape Wastewater bacterial isolates

Sampling site	**NSF negative negative**	**Latex agglutination positive**
Plant B	82(27%)	0
Plant A	67(29%)	0

NSF = non-sorbitol fermenting colonies

DISCUSSION

Initial findings in this study observed the waste water isolates were positive for the *E. coli* O157:H7 chromogenic agar. However, further screening on CT-Sorbitol Mac-Conkey agar (CT-SMAC) reduced number of positive cells while NSF negative cells from the CT-SMAC screening were all negative for the latex agglutination. These observations ruled out the possibility of the presumptive isolates being *E. coli* O157:H7. There are other non-O157 EHEC serotypes that are non-sorbitol fermenters (NSF) giving the characteristic growth on SMAC (Rosser, 2010; Johnson *et al*., 2014). While *E. coli* O157 are popularly known to be non-sorbitol fermenting organisms (Rosser, 2010) there are reports of Sorbitol fermenting (SF) *E. coli* O157 with HUS ability (Pollock *et al*., 2010; Marejková, *et al*., 2013). Importantly, studies have shown that *Vibrio, Yersinia, Pseudomonas, Burkholderia* and *Aeromonas* spp. can give the characteristic feature expected on the Sorbitol Mac-Conkey agar (Müller *et al.,* 2000; Müller and Ehlers, 2007). Some other bacteria spp. isolated from food and stool samples have been reported to be non-sorbitol fermenters (Voravuthikunchai *et al*., 2002). Hermos *et al* (2011) and Ngwa *et al.* (2013) suggested in their study that the Sorbitol Mac-Conkey agar may not to be selective enough for screening environmental samples.

Presence of *E. coli* O157:H7 in the Eastern Cape has been previously reported in drinking water (Abong'o, 2008). Drinking water analysed from the Plant B's area location was reported to have prevalence of the organism. Other areas of the Eastern Cape like Kwasaki, Fort Beaufort, Alice and Mdantsane were also reported to have *E. coli* O157:H7 in their drinking water. Likewise, Müller *et al* (2000) previously reported the non-detection of *E. coli* O157:H7 in the sewage plants and environmental samples from the Northern Province of South Africa. Similar findings were reported for the Gauteng Province (Müller and Ehlers, 2007). Much of the reported cases of *E. coli* O157:H7 are attributed to food, dairy and animal products as major sources (Njage, 2012; Ayaz *et al.,* 2014).

CONCLUSION

Occurrence of *E. coli* O157:H7 in the final effluent of the two wastewater plants could not be established which suggests the prevalence of *E. coli* O157:H7 in the sites investigated is low. It is however possible that the variability in the selective media used could not detect other *E. Coli* O157 colonies which may be present in the final effluent samples.

ACKNOWLEDGMENTS

The author would like to thank the Water Research Commission of South Africa (Grant K5/2145), and the South African Medical Research Council for financial support.

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