

to obtain the desired 392 base pair fragment (Fig. 1) and of the 57 isolates amplified 46 (80.7%) were positively identified as *E. coli* since they possessed the *mdh* gene fragment (Table 3)

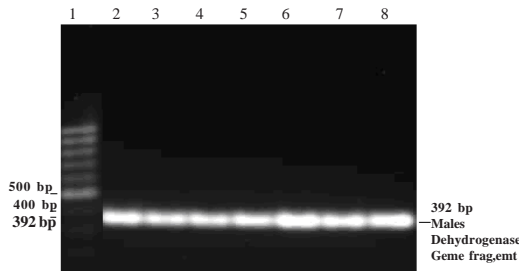


Fig. 1. *mdh* gene amplicons.

Lane 1= DNA marker (100 base pairs); Lanes 2-3= PCR amplification of the *mdh* gene fragments from suspected *E. coli* isolates obtained from water samples in the upstream; Lanes 4-5= PCR amplification of the *mdh* gene fragments from suspected *E. coli* isolates obtained from water samples in the middle-stream; Lanes 6-7= PCR amplification of the *mdh* gene fragments from suspected *E. coli* isolates obtained from water samples in the downstream and Lane 8= PCR amplification of the *mdh* gene fragments from *E. coli* (ATCC 25922) control strain.

Table 3: Proportion of *E. coli* isolates obtained from water samples collected at different points in the dam using PCR.

Upstream	Middle stream	Downstream
24(52.1%)	15(32.6%)	7(15.2%)

DISCUSSION

Water is a vital resource to the society, thus efficient monitoring and management of pathogens from drinking water is very important so as to reduce the number of diarrheal infections that might pose a public health risk to consumers. It is recommended that for water to be considered potable for each 100ml there should not be any bacterial contaminants (WHO 2002). However, the water from the upstream had the highest bacterial load (1624cfu/50ml) compared to samples collected from the mid-stream and the downstream. However, the high fecal contamination observed in the upstream does not necessarily imply that the water treatment procedures are inadequate. The inlet is easily accessible to humans and animal as it is in close proximity to the village and is the main water supply for the villagers, and thus a bee-hive of activities. Animals who drink from the dam, also defecate around it and when rains come their

waste is washed into the dam. The village sanitary system is also very poor. It is therefore suspected that the high level of contamination in the inlet can also be as a result of human and animal activities. The water from this dam therefore, serves as a potential risk for the transmission of pathogens to the villagers.

Several studies have also revealed that the discharge of wastes into dams or rivers is a potential source for transmitting microbial contaminants to crops as well as fish (Tyrrel 1999). Most of the individuals who live in Modimola Village eat fish from the dam and use the same water for watering their gardens. Bacterial concentration in fish has been reported to correlate with level of bacterial contamination in water (Pal and Dasgupta 1992). The consumption of undercooked contaminated fish might therefore, have health implications on the residents of this village

The downstream of the dam is not easily accessible to the villagers and animals. Moreover, the downstream was rich in organic matter and this normally negatively affects the concentration of oxygen. Consequently, mostly facultative anaerobes rather than heterotrophic bacteria will survive in such an environment (Lim and Flint 1989). This may explain why only a small proportion of *E. coli* was isolated from the downstream. However, the water from the downstream is also purified at a nearby Water Purification Plant and distributed to houses in the nearby affluent suburban area of Mmabatho. It is far better to consumers if water that goes to the purification plant has low numbers of bacterial contaminants especially in cases where the treatment protocols are not strictly implemented.

The malate dehydrogenase (*mdh*) is a house keeping gene that is a suitable target gene for identification of *E. coli* species. The variable region of the sequence is flanked by highly conserved regions that are not easily transferred to other species horizontally. In addition, the variable region is relatively short, and contain sufficient allelic polymorphism to differentiate strains (Olive and Bean 1999). The *mdh* gene assay outperforms other phenotypic and genotypic methods for the identification of *E. coli* species (Moore et al. 2005). Amplification of 320 base-pair partial nucleotide sequence of the *mdh* fragment accurately identified *E. coli* isolates from the different sites of the dam and results were highly reproducible. The natural hosts of *E. coli* species are humans and animals, thus their presence in water indicate contamination with faeces from these species. Knowledge of the host specificity for isolates obtained in the present