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REMOVAL OF *SALMONELLA TYPHI, SHIGELLA DYSENTERIAE, VIBRIO CHOLERAE* AND ROTAVIRUS FROM WATER USING A WATER TREATMENT TABLET

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ABSTRACT

Chlor-floc tablets are intended for purification of small quantities of water for individual use by combined flocculation and disinfection. Removal of *Escherichia coli* E25, various viruses and *Giardia* cysts from polluted water by Chlor-floc has been previously demonstrated. This study evaluated the efficiency of removal of *Salmonella typhi, Shigella dysenteriae, Vibrio cholerae* and rotavirus from simulated hard water of high organic content and colour. All four pathogenic microorganisms were successfully removed within the recommended treatment time. Log removals of 7-8 log were achieved for bacterial pathogens, and a removal of 6.5 log was obtained for rotavirus.

KEYWORDS

Disinfection, flocculation, water treatment tablet, *Salmonella typhi, Shigella dysenteriae, Vibrio cholerae*, rotavirus

INTRODUCTION

Despite technological advances in water treatment and supply, waterborne disease remains a persistent problem. It is estimated that 50 000 people die daily worldwide as a result of waterborne illness (Schalekamp, 1990). Diseases such as typhoid fever, bacillary dysentery, and cholera are associated with polluted water (Rakotvahiny and Arrestat, 1982; Craun, 1986) and contribute significantly to these statistics. Rotavirus is foremost amongst causative agents of viral gastroenteritis, particularly among infants. Gastroenteritis is a major contributor to infant morbidity and mortality in underdeveloped and developing regions (Craun, 1986; Genthe *et al.*, 1991).

In developed areas, towns and cities safe drinking water is provided by treatment plants. However, in developing regions and in rural areas, where facilities for water treatment are limited or absent, water treatment for individual use is of great importance. Even in developed countries, natural disasters such as floods may disrupt water treatment. Backpackers, campers or military units operating in areas where treated water is not available may also require individual water treatment. Point-of-use water treatment tablets provide a rapid and simple method for purifying small quantities of raw water to potable quality (Kfir *et al.*, 1989).

Chlor-floc water treatment tablets are formulated to purify water by combined flocculation and disinfection, and have been found to remove *Escherichia coli* E25, various viruses and *Giardia* cysts effectively from polluted water (Kfir *et al.*, 1989; Hamilton and Jackson, 1990). Increasing urbanisation and development of peri-urban communities, both in southern Africa and worldwide, carries the risk of water pollution as a result of inadequate sanitation and runoff. This creates potential for widespread waterborne illness. Diseases which may occur as epidemic outbreaks include typhoid, dysentery, cholera and viral gastroenteritis. Adequate protection or disinfection of water supplies has been identified as a major factor in preventing such outbreaks (Rakotvahiny and

Arrestat, 1982; Glass *et al.*, 1991). A water treatment tablet capable of removing the corresponding infectious agents would be of great value in limiting waterborne disease outbreaks. This study evaluated the capability of Chlor-floc tablets to remove *Salmonella typhi, Shigella dysenteriae, Vibrio cholerae* and rotavirus from water.

MATERIALS AND METHODS

Preparation of Test Water

Simulated hard water with organic colouring was prepared by the addition of 10 mℓ of bicarbonate salt solutions (0.4 M $NaHCO_3$; 0.24 M $KHCO_3$) and 10 mℓ of chloride salt solutions (0.1 M $CaCl_2$; 0.6 M $MgCl_2.6H_2O$) to 980 mℓ of 0.15% tea infusion water. Tea infusion water was prepared by the addition of 1.5 mℓ highly concentrated tea to sterile distilled water, to yield a final volume of 1 ℓ. This provided a water with high chlorine demand. The simulated hard coloured water was used for seeding experiments at room temperature.

Bacterial and viral cultures

Pure bacterial cultures were obtained from the South African Institute for Medical Research (Johannesburg, South Africa). Aliquots of 1 mℓ (*S. typhi, V. Cholerae*) or 10 mℓ (*S. dysenteriae*) of overnight cultures were used to inoculate 1ℓ final volumes of simulated hard, coloured water. Bacteria were assayed by membrane filtration (APHA, AWWA, WPCF, 1989) and cultivation on selective media (ISO/DIS 6340, 1986; APHA, AWWA, WPCF, 1989). Average inocula and recovery conditions are shown in Table 1.

A laboratory strain of rotavirus SA 11, obtained from the National Institute of Virology (Johannesburg, South Africa), was utilised in all studies. Virus stocks were kept at -70° C and aliquots of 1 mℓ were introduced into 1ℓ final volumes of simulated hard coloured water. Virus was assayed by cell culture on MA 104 cells (Genthe *et al.*, 1991). The average inoculum and the recovery method is shown in Table 1.

TABLE 1 Mean Inocula Concentrations and Summarised Recovery Methods

Microorganisms	Mean stock culture concentration/mℓ	Recovery method
S. typhi	1.9×10^8 [a]	Membrane filtration Bismuth sulphite agar (37° C, 24 hrs)
S. dysenteriae	3.3×10^7 [a]	Membrane filtration XLD agar (37° C, 24 hrs)
V. cholerae	4.6×10^8 [a]	Membrane filtration TCBS agar (37° C, 24 hrs)
Rotavirus	1.7×10^7 [b]	Cell culture (MA 104 cells)

a CFU/mℓ
b MPN $TCID_{50}$/mℓ

Tablets

Chlor-floc treatment tablets were supplied by Control Chemicals Ltd (South Africa). Each tablet contained approved potable water grade flocculant aids, with sodium dichloro-s-triazine-trione (2.5%; 1.4% available chlorine) as the chlorine source.

Test procedure

One litre volumes of simulated hard coloured water were seeded with the test microorganisms. Tests were conducted separately for each organism. One Chlor-floc tablet was added to each flask, which was shaken for one minute, swirled for 10 seconds and allowed to stand for 4 minutes (as recommended by manufacturer), 10 minutes or 20 minutes. Each flask was then swirled for 10 seconds, whereafter the contents were filtered through a cotton bag into a sterilised beaker. All samples were neutralised with sodium thiosulphate and samples analyzed for rotavirus were chloroformed.

RESULTS AND DISCUSSION

Water treated with Chlor-floc tablets was clear and without objectionable odour. All seeded organisms were completely inactivated after a standing time of 4 minutes. No microorganisms were recovered after standing times of 4, 10 or 20 minutes. Figures 1, 2, 3 and 4 show mean results for *S. typhi, S. dysenteriae, V. cholerae* and rotavirus, respectively. Treatment with Chlor-floc effected an 8 log removal of *S. typhi* and *S. dysenteriae*, a 7.5 log removal of *V. cholerae* and a 6.5 log removal of rotavirus. Observed removals were slightly lower than, but similar to, removals reported for *E. coli* E25, coliphage V_1 and poliovirus 1 by Kfir *et al.* (1989). The latter investigation evaluated lower dilutions than were used in this study, hence it is possible that log removal of the same order occurred.

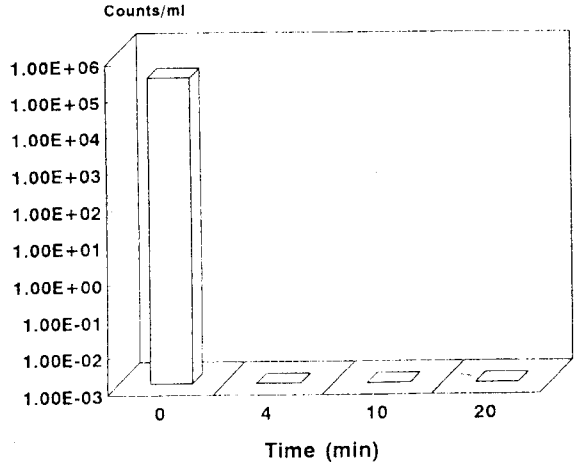

Fig. 1. Removal of *S. typhi* from water by Chlor-floc tablet. Simulated hard coloured water seeded with 1.9×10^8 organisms. Results are mean of three experiments.

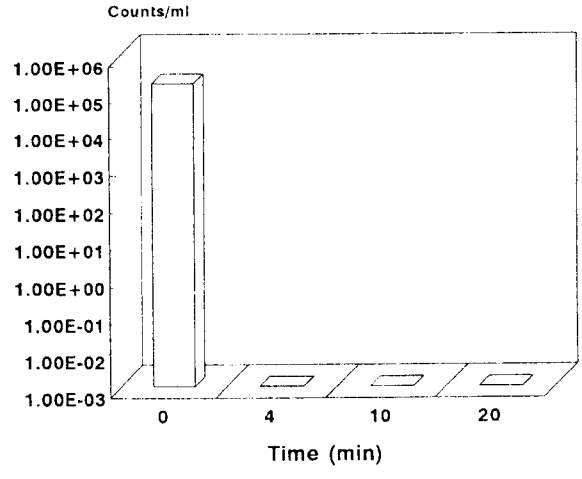

Fig. 2. Removal of *S. dysenteriae* from water by Chlor-floc tablet. Simulated hard coloured water seeded with 3.3×10^8 organisms. Results are mean of three experiments.

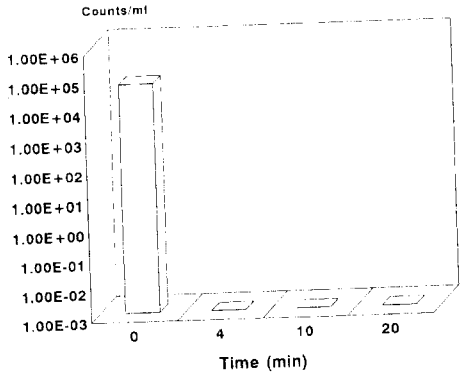

Fig. 3. Removal of *V. cholerae* from water by Chlor-floc tablet Simulated hard coloured water seeded with 4.6×10^8 organisms. Results are mean of three experiments.

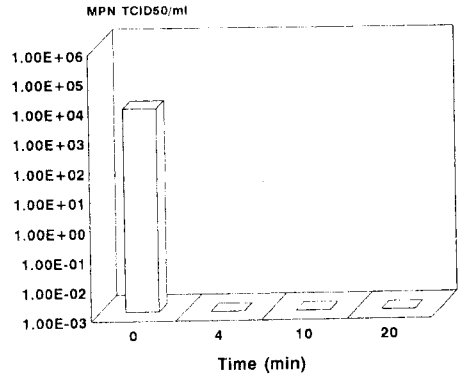

Fig. 4. Removal of rotavirus from water by Chlor-floc tablet Simulated hard coloured water seeded with 1.7×10^7 organisms. Results are mean of three experiments.

From the results it may be concluded that Chlor-floc treatment tablets efficiently remove the microbial pathogens *S. typhi, S. dysenteriae, V. cholerae* and rotavirus from water with high disinfectant demand and high microbial concentration within the time recommended by the manufacturer. This corroborates and extends the findings of Kfir *et al.* (1989) and Hamilton and Jackson (1990). The tablets fulfil the requirements of clarification and disinfection outlined for water treatment in rural areas (Rakotvahiny and Arrestat, 1982), and yields water which is highly likely to be free of microbial agents of gastrointestinal disease.

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