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Use of non-carbonated soft drinks to provide safe drinking water

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SUMMARY Non-carbonated, low-calorie soft drink concentrates (cordials), when diluted according to manufacturers' instructions, had significant antibacterial effects *in vitro*. Bacteria affected include *Vibrio cholerae*, *Aeromonas hydrophila*, *Shigella sonnei*, *Salmonella typhimurium* and *Escherichia coli*. With vibrios, bacterial counts were reduced from 10^6/ml to undetectable numbers in less than 10 min. *Escherichia coli* in an initial concentration of 10^6/ml became undetectable after incubation for 1 h with one brand of cordial. Naturally contaminated water can be rendered potable by incubation with cordials at room temperature for 1 h. This may be a way to reduce the risk of water-borne diarrhoea, particularly where the cleanliness of drinking waters cannot be otherwise assured, for example when making up oral rehydration fluids and for travellers in high-risk areas.

Introduction

In many parts of the world, notably in developing countries in the tropics, provision of clean drinking water is a major problem and polluted water supplies are linked with the high prevalence of infectious diarrhoea, particularly in children. A special problem needing attention is the preparation of clean, clear fluids for children with diarrhoea, with or without signs of dehydration. Oral rehydration therapy is now widely used in developing countries for children with diarrhoea and mild to moderate dehydration (1). If water used to prepare oral rehydration solutions is contaminated with faecal micro-organisms, bacterial multiplication can occur, particularly at tropical temperatures. Some bacteria, such as *Vibrio cholerae* and *Escherichia coli*, multiply more rapidly than others such as *Shigella flexneri*. Studies from Brazil and Central America show that bacteria will also multiply more rapidly in fluids which contain nitrogenous material as well as rehydration salts and water (2, 3).

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It is often very difficult to provide safe drinking water or oral rehydration fluids in places where childhood malnutrition and diarrhoea are prevalent. Metropolitan and town water supplies are often inadequately chlorinated or unchlorinated, often contaminated by sewage and other effluents in pipes or from surface waters (4) and boiling water is often inconvenient, and expensive. Simpler, inexpensive methods are needed to provide drinking water and oral rehydration fluids for children in developing countries.

Early reports about the antibacterial effects of fruit juices and their concentrates (5-7) prompted us to investigate the possible effects of non-carbonated soft drink concentrates on water contaminated with enteric pathogens.

Materials and methods

Concentrated non-carbonated drinks

The concentrates used are known as cordials in Australia. Standard cordials contain at least 25% fruit juice and 25% sugar, usually sucrose, with added flavour, colour, preservatives and suspending agents.

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Low-calorie drinks are similar except that saccharine and/or cyclamate replaces sugar. Preservatives include organic acids (usually citric), sulphur dioxide and benzoic acid. Undiluted, these cordials have pH levels between 2·2 and 2·7. When diluted to the recommended strength of one part of cordial to four parts of water with tap water (pH 6·4), pH ranges from 2·8 to 3·1.

For all experiments, one part of cordial was diluted with four parts of sterile distilled water. Initially, 16 drinks of various flavours from eight manufacturers were tested. As the only difference in bactericidal effects we found were related to whether the cordials were standard or low-caloric, subsequent experiments included only four drinks, two standard cordials and two low-calorie cordials. The low-calorie drinks were found to have a slightly greater anti-bacterial effect and since we wished to avoid the potential risks of hyper-osmolality with sugar-containing drinks, all results given in this paper relate to experiments done with low-caloric drinks. These were produced by two Australian manufacturers and purchased at local supermarkets.

Bacteria

Stock cultures of *V. cholerae*, *Aeromonas hydrophila*, *Pseudomonas aeruginosa*, *Shigella sonnei*, *Salmonella typhimurium* and *E. coli* were used to assess bactericidal capability. All stock cultures were grown on blood agar at 37°C and a series of dilutions were prepared in saline, to contain between 10^3 and 10^7 bacteria/ml.

Method

Samples (4·5 ml) of the diluted drinks were prepared, aseptically, and 0·5 ml of the bacterial dilutions were added to provide concentrations between 10^2 and 10^6 organisms/ml and then incubated at room temperature (about 22°C) or at 4°C. The exact count was obtained at the times indicated by plating 0·001 ml onto blood agar with calibrated loops.

Since the method used would not detect less than 10^2 organisms/ml, enrichment was used for some experiments. Five hundred microlitres of diluted drink, which had been inoculated with bacteria, were added to strontium chloride B broth for *S. typhimurium* (8) or nutrient broth (Oxoid) for *E. coli* and *S. sonnei*.

Field studies

One hundred millilitres of water from a rural, surface-water source was filtered (Gelman), and the filters incubated on McConkey and blood agar. The drinks were diluted with this contaminated water and bactericidal capacity assessed after 20 and 60 min by filtration of 20 ml, direct plating and enrichment in nutrient broth.

Results

At room temperature, the low-calorie cordials diluted one in four reduced bacterial counts from 10^6 organisms/ml to non-detectable levels in times ranging from less than 10 min, for *V. cholerae* and *A. hydrophila*, to more than 1 h with *E. coli* (see Table 1).

At 4°C, killing of bacteria was slower than at room temperature (Table 1) although with low-calorie drinks, *V. cholerae*, *P. aeruginosa* and *A. hydrophila* were all reduced from 10^6 organisms/ml to undetectable levels within 20 min. With *Salmonella* spp., 60 min was required for a comparable bactericidal effect, in contrast with a time of 10 min at room temperature. At 4°C, *S. sonnei* were still detectable in both drinks, but by 2 h *S. sonnei* were not detected in one of the drinks (B), even after enrichment, and in the other (A) the concentration of bacteria had decreased from 10^6/ml to 10^2/ml. *Escherichia coli* were still detected at 4 h in both drinks but counts had fallen from 10^6/ml to 10^2/ml.

One of the diluted cordials studied (Brand B) was very effective *in vitro* against *V. cholerae*, *A. hydrophila* and *P. aeruginosa* and reduced counts of 10^6/ml to undetectable levels in less than 10 min; *Shigella sonnei*

Table 1 *Time in minutes to reduce 10^6 organisms/ml to a non-detectable level*

	At room temp		At 4°C	
	Drink A	Drink B	Drink A	Drink B
V. cholerae	< 10	< 10*	10	10
A. hydrophila	< 10	< 10	10	10
P. aeruginosa	< 10	< 10	20	20
S. sonnei	20	10	> 60	> 60
S. typhimurium	10	10	60	60
E. coli	60	60	> 60	> 60

*Only 10^3 organisms/ml were detected at 15 s.

Table II *Time course of change in bacterial count (LOG_{10}/ml) from an initial value of six to non-detectable levels with brand B low-calorie drink*

Organism	Log$_{10}$ bacterial count				
	15 s	10 min	20 min	60 min	240 min
V. cholerae	3	ND	ND	ND	ND
A. hydrophila	6	ND	ND	ND	ND
P. aeruginosa	5	ND	ND	ND	ND
S. sonnei	6	2	ND	ND	ND
S. typhimurium	6	2	ND	ND	ND
E. coli	6	5	4	ND[a]	ND

ND = not detected with or without enrichment procedures.
[a] = not detected without enrichment but detected after enrichment for 15 h.

and *S. typhimurium* were undetectable within 20 min while *E. coli* could not be detected within 1 h of incubation in this solution (Table II). With an initial concentration of 10^6 organisms/ml, *E. coli* could be recovered after enrichment from samples taken after 1 h, but with starting titres less than 10^6, no *E. coli* were recovered after enrichment from samples which failed to grow on blood agar.

Field studies

The water initially contained 36 coliforms and 17 *E. coli*/100 ml. Coliforms and *E. coli* were undetectable in the first sample after addition of either cordial. No bacteria were recovered after incubation of these samples in nutrient broth for 15 h.

Discussion

This study has shown that commercially available soft drink concentrates, which are known as cordials in Australia, had significant antibacterial activity *in vitro* when diluted in the way suggested for domestic consumption. Bacterial counts were reduced from 10^6/ml to undetectable levels in periods ranging from less than 10 min to more than 1 h.

The organisms tested include *V. cholerae*, *A. hydrophila*, *S. sonnei*, *S. typhimurium* and *E. coli*, all recognised enteric pathogens, and *Pseudomonas aeruginosa*. The vibrios, *V. cholerae* and *A. hydrophila*, were most sensitive to the bactericidal effects of these drinks and became undetectable within 10 min of mixing with the diluted cordials, even at 4°C. *Escherichia coli* was the least sensitive of the bacteria tested. However, with one of these low-calorie preparations, *E. coli* counts of 10^6/ml decreased consistently to undetectable levels within 60 min of addition to diluted cordial, at room temperature; killing was slower at 4°C.

These experiments were carried out with 10^6 organisms/ml, concentrations far higher than those likely to occur in naturally contaminated drinking water. With lower concentrations of bacteria the time required to eliminate micro-organisms was greatly reduced; for example, *E. coli* in a concentration of 10^3 organisms/ml became undetectable in less than 10 min with either of the low-calorie cordials. In a single experiment with naturally contaminated water, no organisms could be recovered after incubation for 60 min with either of the cordials.

The low pH of the drinks appears to be the main factor involved in their bactericidal effects although the type of organic acid used was also significant. The effect of pH has previously been shown for a limited range of micro-organisms, mainly with carbonated beverages (9–11). Differences in bactericidal effects of various organic acids have been reported with several bacteria and yeasts which are potential contaminants of foods (12). Preliminary experiments with the enteric pathogens used in our study have shown that malic acid is a more effective bactericidal agent than citric acid. Malic acid was the only agent we were able to relate to bactericidal activity. Preservatives such as benzoic acid and tartaric acid did not alter bactericidal effects in our system, nor did cyclamate or saccharine in low-calorie preparations account for the difference between these and the slightly lower antibacterial effectiveness of the sugar-containing standard cordials.

The data suggest that water, even when heavily contaminated with bacteria, can be made potable by incubation at an ambient temperature of about 20°C for 60 min with a low-calorie cordial diluted one in five. This dilution does not need to be exact as we have found significant bactericidal activity to be retained in dilutions of one in 10 and even, with vibrios, at dilutions of one in 40. The antibacterial effects are slower at 4°C.

The present findings suggest that non-carbonated concentrates (cordials) when diluted to make soft drinks might have a useful role in making water supplies potable. This could be useful in several situations, for example in making up fluids for oral rehydration therapy and in lowering the risks of water-borne diarrhoeal diseases in travellers (13), hikers and campers where the provision of safe water supplies might otherwise be difficult (14–17). It might also be usueful in protecting healthy children born in a Westernized country when they visit their parental homeland, say, on the Indian subcontinent and are at risk of developing a severe form of traveller's diarrhoea (18). The method is simple, the drinks are palatable and clinical trials seem warranted.

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