

## The bacteriological quality of bottled natural mineral waters

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### SUMMARY

Fifty-eight bottles of natural mineral water, taken from the point of sale, were bacteriologically examined. No coliforms or *Aeromonas sp.* were isolated from any sample. High total bacterial counts were found particularly in the still waters. Most of the organisms isolated in the total counts were Gram-negative rods, but Gram-positive organisms were also isolated. Gram-positive cocci were further identified, some of which were known human commensals suggesting contamination of the waters prior to bottling.

### INTRODUCTION

A directive from the Council of the European Communities (European Community, 1980*a*) set maximum limits for coliform counts in potable water and offered guidelines for total viable counts. It applied to all water intended for human consumption, but excluded bottled natural mineral waters which were covered by an additional directive (European Community, 1980*b*). This directive became law in the United Kingdom under the Natural Mineral Waters Regulations, 1985 (Ministry of Agriculture, Fisheries and Food, 1985). Although maximum total viable counts for mineral waters cultured within 12 h after bottling are specified, no guidance is given regarding acceptable microbiological standards for these waters at the point of sale.

This study was undertaken to ascertain the microbiological quality of these waters 'off the shelf' as sold to the consumer.

### MATERIALS AND METHODS

During August and September 1986 Environmental Health Officers were encouraged to submit bottled mineral waters, in their original bottle, to the laboratory for examination. A note was made as to the place of purchase and whether the mineral water was carbonated or still. Both artificially carbonated and naturally sparkling waters were included together as carbonated waters.

The bottles were examined as soon as possible after receipt. Their pH was measured by a pH meter (Data Scientific). Samples of 100 ml were filtered through membranes which were then cultured on Membrane Enriched Teepol Broth, made in the laboratory according to the published recipe (Department of Health and Social Security, 1983). Characteristic yellow coliform colonies would have been

confirmed by culture in lactose peptone water (acid and gas production). Isolation of *Aeromonas* sp. was attempted by culture of a filter from another 100 ml in 25 ml alkaline peptone water (APW) CM9, (Oxoid, Basingstoke, England) at 20 °C. The APW was subcultured at 24 and 48 h onto xylose deoxycholate citrate agar no. 152-1360, (Gibco Europe Ltd, Paisley, Scotland) for incubation overnight at 37 °C.

Total viable counts were estimated by two 1 ml pour plates (DHSS, 1983). This involved the addition of 1 ml volumes of undiluted sample into empty Petri dishes followed by 15 ml of molten Plate Count agar CM325 (Oxoid). Two such plates were made for each sample, these being incubated either at 37 °C for 24 h or 22 °C for 72 h (DHSS, 1983). This enabled counts of up to 1000 organisms per ml. For 52 of the 58 specimens, subcultures of all colonial types from the pour plates were made onto nutrient agar plates. These subcultures were then provisionally classified on the basis of Gram reaction, cytochrome oxidase and the oxidation or fermentation of glucose. Gram-positive cocci were referred to the Division of Hospital Infection for further identification by methods published elsewhere (Kloos & Schleifer, 1975; Marples & Richardson, 1982). Any Gram-negative organisms which were oxidative and oxidase positive were further subcultured on to nutrient agar for examination of colonial morphology as an initial step in the identification of *Pseudomonas aeruginosa*. No further attempts were made with the Gram-negative or Gram-positive rods.

## RESULTS

Fifty-eight bottles of mineral water were examined in the study, 29 were carbonated and 29 still. Of the 29 carbonated waters 14 were naturally carbonated and 15 were artificially carbonated. Thirty-one of the waters were British and 27 imported, all from within the European Community. The pH of the carbonated waters ranged from 4.74 to 7.24 (median 5.48), whilst that of the still waters ranged from 6.38 to 8.82 (median 7.86). Some of the bottles carried labels claiming a specific pH for their contents. The measured pH of these waters did not fall within the claimed range.

No coliforms (yellow colonies on membrane culture) nor *Aeromonas* sp. were isolated from any of the waters examined. The total viable counts per ml after incubation at 22 and 37 °C are shown in Table 1. There was no significant difference between the total viable counts from the domestic and imported waters. Table 2 shows the initial classification of organisms isolated from the pour plates used for total viable counts. There were no differences in the provisional identification of organisms isolated from carbonated and still waters or from those waters yielding high or low counts.

The 17 strains of Gram-positive cocci were isolated from just 11 bottles (6 carbonated and 5 still). Of the 17 strains 2 were identified as *Micrococci* sp., 7 as *Staphylococcus xylosum*, 3 as *S. epidermidis* and 4 as *S. hominis* or *S. warneri*. None of the 4 oxidative positive Gram-negative organisms had colonial morphologies compatible with *Ps. aeruginosa*.

Table 1. Total viable counts at 22 and 37 °C of bottled mineral waters

|            | Organisms per ml |       |         |        |          |       |         |        |
|------------|------------------|-------|---------|--------|----------|-------|---------|--------|
|            | At 22 °C         |       |         |        | At 37 °C |       |         |        |
|            | < 10             | 10-99 | 100-999 | ≥ 1000 | < 10     | 10-99 | 100-999 | ≥ 1000 |
| Carbonated | 25               | 1     | 2       | 1      | 24       | 5     | 0       | 0      |
| Still      | 4                | 5     | 5       | 15     | 14       | 11    | 1       | 3      |
| Total      | 20               | 6     | 7       | 16     | 38       | 16    | 1       | 3      |

Table 2. Preliminary identification of organisms isolated from bottled mineral waters

| Gram reaction         | Oxidase | O/F reaction | Number |
|-----------------------|---------|--------------|--------|
| Gram-negative bacilli | +       | -            | 20     |
|                       | .       | O            | 4      |
|                       | .       | F            | 0      |
|                       | -       | -            | 11     |
|                       | .       | O            | 0      |
|                       | .       | F            | 0      |
| Gram-positive bacilli | .       | .            | 14     |
| Gram-positive cocci   | .       | .            | 17     |
| Total                 | .       | .            | 75     |

## DISCUSSION

It is reassuring that we were unable to isolate coliforms or *Aeromonas* sp. from any of the samples examined. High total viable counts were found particularly after incubation at 22 °C. The E.C. directive on potable water suggests maximum viable counts of 100 colonies per ml after incubation at 22 °C for 72 h and 10 per ml after 24 h at 37 °C (European Community, 1980a). Seventy per cent of the still mineral waters yielded more than 100 organisms per ml after incubation at 22 °C compared to 10% of carbonated waters. After incubation at 37 °C, 52% of still and 17% of carbonated waters yielded more than 10 colonies per ml.

Still waters yielded significantly more high counts than carbonated waters at both 22 °C ( $X^2 = 20.8$ ;  $P < 0.001$ ) and 37 °C ( $X^2 = 7.63$ ;  $P < 0.01$ ). The antibacterial activity of carbon dioxide in water is well known (Koser & Skinner, 1922). This has been shown to be additional to any effect of a fall in pH from associated carbonic acid (King & Nagel, 1967). It has also been shown to increase with partial pressure of CO<sub>2</sub> and decrease with sugar content (Insalata, 1952).

However as previously mentioned the E.C. directive on potable water specifically excludes bottled natural mineral waters. The relevant directive sets a maximum limit for the total counts for the bottled water only within 12 h of bottling. Further, it is stated that the total colony count of a natural mineral water may only be that resulting from the normal increase in the bacteria content which it had at source. An 'acceptable' normal increase in viable count is not defined.

Most of the bacteria isolated were Gram-negative organisms commonly found in water. Gram-positive rods and cocci were also found rather more frequently than in our previous studies (Hunter & Burge, 1986). As it is known that airborne

contamination can be a problem with these waters (Schmidt-Lorenz, 1976), we further identified the Gram-positive cocci to determine whether they were likely to have been airborne contaminants. *S. epidermidis* and *S. hominis* are human commensals, whilst *S. xylosum* appears not to be, at least in UK studies (Noble, 1983). Thus, it is probable that those bottles contaminated by *S. epidermidis* or *S. hominis* were contaminated by human skin scales prior to bottling. These are probably not laboratory contaminants as they were isolated in large numbers from primary culture. It appears that at least 6 of 52 (11.5%) bottles contained organisms which, probably, would not have been present in the source water. These waters may not, therefore, satisfy statutory standards.

The microbiological quality of bottled natural mineral water appears to be variable, although carbonated waters, whether naturally or artificially carbonated, seem generally of good quality. The isolation of human strains of staphylococci suggests that at least in some cases standards of hygiene may not have been as high as one would hope. Bottled natural mineral waters are not as microbiologically 'pure' as some suppliers seem to claim (Anon, 1982). We would certainly agree with Stichel (1982) that these waters should not be used as an alternative drink for infants.

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