Bacterial contamination of ground water supplies in Chahaar-Mahaal province (Iran)

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Abstract
Occurrence and distribution of coliform bacteria in drinking water from different sources is investigated. One hundred water samples, collected from wells, rivers and springs in Shahrekord district of Chahar-Mahal province (Iran), were microbiologically examined for total coliforms and recovery of Escherichia coli using a multiple tube test. Coliform spp. formed 58.3, 100 and 22.2%, respectively, for ground water supplies, rivers and springs. Water samples from most of the well sources and all the river sources were regarded as unsuitable for human consumption.

Key Words: water, coliform spp., multiple tube test.

Introduction
The bacteriological examination of water is particularly important as it remains the most sensitive method for detecting faecal and, therefore, potentially dangerous contamination. The key criteria for ideal bacterial indicators of faecal pollution are that they are universally present in large numbers in the faeces of human and other warm-blooded animals. They should also be present in sewage effluent, be readily detectable by simple methods and should not grow in natural waters. Ideally, they should also be of exclusive faecal origin and be present in greater numbers than faecally transmitted pathogens. No single indicator organism fulfils all these criteria, but the member of the coliform group that satisfies most of the criteria for the ideal indicator organism in temperate climates is E. coli. This organism is widely distributed in the intestine of humans and warm-blooded animals and is the predominant facultative anaerobe in the bowel and part of the essential intestinal flora that maintains the physiology of the healthy host (5). The most important of these are the Vero-cytotoxin-producing E. coli (VTEC), in particular VTEC of serogroup O157, but other E. coli serogroups may contain VTEC members. Typical symptoms of people infected with E. coli O157 range from mild diarrhoea, fever and vomiting to severe, bloody diarrhoea and painful abdominal cramps. In 10-15% of cases, a condition known as haemolytic uraemic syndrome which can result in kidney failure. Individuals of all ages can be affected but children up to ten years old and the elderly are most at risk. The infectious dose for E. coli O157 is relatively low compared with other bacterial causes of gastro-enteritis, perhaps as low as 10 organisms. VTEC may not be isolated or may not be recognised by the normal analytical methods for E. coli, and specific isolation methods are required. However, if E. coli is detected in a water supply it should be assumed that VTEC could also be present (2,3,7,11).

The use of indicator organisms, in particular the coliform group, as a means of assessing the potential presence of water-borne pathogens has been paramount to protecting public health.

Materials and Methods
In this investigation 100 samples of water (well, 36; river, 46 and spring, 18) are aseptically collected in sterilized bottles from different areas in Chahaar-Mahaal province. Samples transferred immediately to dark storage conditions and kept at temperatures between 2 - 8 °C for transport to the laboratory and analysed as soon as practicable on the day of collection.
The 5-tube MPN method is used for water examinations (1). Inoculated tubes were incubated at 35 +/- 0.5°C. After 24 +/- 2h for heavy growth, gas, and acidic reaction and, if no gas or acidic growth was formed, reincubated and reexamined at the end of 48 +/- 3h. Production of gas or acidic growth in the tubes within 48 +/- 3h constituted a positive presumptive reaction. Tubes with a positive presumptive reaction submitted to the confirmed phase. The absence of acidic growth or gas formation at the end of 48 +/- 3h of incubation constituted a negative test. For positive tests brilliant green lactose bile broth fermentation tubes used for the confirmed phase.

Results
The details of results have come in Table1. Out of 100 sample of water, *Coliform spp* isolated from 71 samples, which in biochemical tests 56 samples showed the presence of *E.coli* and 15 samples, *Klebsiella*. The number of positive samples for *E.coli* in well water samples was 18, river water samples, 34 and spring water samples was 4. *Klebsiella* isolated from 3 well water and 12 river water samples, but did not isolate from spring water samples. River water was the most contaminated followed by well water and then spring water samples.

Discussion
*E. coli* occurs in the faeces of all mammals, often in high numbers (up to 10⁹ per gram of faeces). This widespread faecal occurrence, coupled with methods that for the recovery and enumeration of *E. coli* are relatively simple to conduct, has contributed to the detection of this bacterium as the cornerstone of microbiological water quality assessment for over 100 years (6,12). The quality of many source waters will depend upon geology, soil type, natural vegetation, climate and run-off characteristics. Disruption of natural geology and heavy rainfall can dramatically affect water quality. Wild animals and birds can also be natural sources of zoonotic pathogens.

All types of water sources may be subjected to contamination by agricultural activity. In an investigation in 1994, Pathak *et al.*, reported that 41-67% of water samples open water sources in India contained coliform and/or faecal coliform bacteria (9). In this investigation 22.2-100% of water samples contained coliform bacteria. Free range animals may excrete faeces into water, and animals like cattle have a habit of wading into water and stirring up sediments. Rainfall can result in the run-off of faecal matter from agricultural and other rural lands into rivers, lakes, reservoirs and springs. Much can be done to reduce the risk of water contamination from slurry spillage, or the use of slurry on land followed by surface run-off, by the adoption of appropriate agricultural practices and aquifer protection policies. Recreational activity may cause pollution through direct contamination of water with faecal material or indirectly by faulty drainage or leakage from sewers and septic tanks provided as part of public access facilities. In a study in Canada, in 1993, Shadix *et al.*, reported out of 119 positive coliform colonies which isolated from 15 water sources (including a lake, 3 rivers, 2 springs, 6 creeks, 2 sewage effluents, and a well) 115 (96.6%) were identified as *E.coli* (10). In my study 86.5% of coliforms were *E.coli*. Proper control of recreational activities or treatment commensurate with the recreational use of water should give adequate protection. Where the public has access to reservoirs, consideration should be given to the provision of toilets and hand-washing facilities. The discharge of effluents from sewage treatment works, septic tanks and cesspools can dramatically increase the microbial content of surface waters. The installation of septic tanks and cesspools should be in accordance with national standards. The discharge of industrial effluents, particularly from abattoirs and cattle markets,
may also contain large numbers of pathogenic micro-organisms which increase the risk of contamination. Slurries and solid waste from sewage treatment and animal waste should be spread on land only with strict control (4).

References

Table 1: distribution of coliform bacteria in drinking water from different sources in Chahar-Mahal province

<table>
<thead>
<tr>
<th>Source</th>
<th>No. of samples</th>
<th>No. of Coliform spp.</th>
<th>Coliform spp. %</th>
<th>E.coli %</th>
<th>Klebsiella %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Well</td>
<td>36</td>
<td>21</td>
<td>58.3</td>
<td>85.7</td>
<td>14.3</td>
</tr>
<tr>
<td>River</td>
<td>46</td>
<td>46</td>
<td>100</td>
<td>73.9</td>
<td>26.1</td>
</tr>
<tr>
<td>Spring</td>
<td>18</td>
<td>4</td>
<td>22.2</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>71</td>
<td>65.7</td>
<td>86.5</td>
<td>13.5</td>
</tr>
</tbody>
</table>