Escherichia coli Serogroups Isolated from Streams in Pennsylvania, 1965 to 1972

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Of 3,200 cultures of Escherichia coli isolated from streams in Pennsylvania over a 7-year period, 82.46% or 2,639 were O serogrouped. The largest number of cultures (33.4%) belonged to O groups 1 to 26, and the second highest number (16.8%) belonged to O groups 60 to 88. The individual E. coli O groups most frequently isolated were ADO3, 18ac, 2a, 3, 7, 73, 139, and OX13. Practically every known standard E. coli O group was found in the streams. It was not possible to identify the K and H antigen of every E. coli isolate. Serotypes of E. coli O2a:K1:H6, O26:K60:H11, O55:K59:H27, O86:K62:H2, 125ab:K70:H21, 128ab:K67:H2, and O138:K81:H14 known to be pathogenic for humans and animals were identified. Cultures having the same K antigen but a different H antigen for enteropathogenic E. coli O groups 6, 18ab, 18ac, 111ab, 126, 127a, 139, 141, and 147 were also isolated.

Escherichia coli strains are common inhabitants of the intestinal tract of man and warm-blooded animals but also are known to produce disease. The isolation and serological identification of E. coli pathogenic for man and animals have been widely reported (2, 9).

The serological classification of E. coli strains present in the environment, especially in water, and their possible relationship to disease have not been extensively studied. The discharge of potential pathogenic E. coli into streams could be a source of disease for those utilizing such water (6, 8). This report summarizes the frequency and occurrence of E. coli serogroups isolated from water over a 7-year period. Further characterization of the K and H antigens of the E. coli O groups commonly associated with disease was also done.

MATERIALS AND METHODS

Source of samples. The Pennsylvania streams sampled from 1965 to 1972 were located as follows: (i) Centre County—Spring Creek and its tributaries, Slab Cabin Run, Thompson Run, and Cedar Run (5, 6). (ii) Tioga, Lycoming Counties—Pine Creek and two of its tributaries, Marsh Creek and Little Pine Creek (3, 7). (iii) Monroe County—Crawford Lake and two tributaries, Paradise Creek and a fish hatchery, including springs (6).

For a more detailed report on these areas, the reader is referred to the references cited. In all three areas, the streams flowing through unpopulated as well as populated areas were subject to microbial pollution.

Microbiological-serological methods. Water samples were collected in sterile 8-oz. (0.236 liter) plastic containers with caps (Falcon), appropriate volumes were filtered through 45-mm diameter Gelman filters (pore size 0.45 μm), and the filters were incubated on M-coliiform medium (BBL) at 35 C for total coliform, and on M-FC medium (BBL) at 44.5 C for fecal coliform counts (1, 3, 5–7). Typical coliform colonies were then transferred to determine biochemical reactions, and the E. coli isolates were examined serologically (2, 4). E. coli standard O group sera 1 to 149 and unclassified OX sera, OX1 to OX43, were used for slide, tube titer, and cross-absorbed serum tests. The E. coli K sera that were used included K1 to K91 and, for motile forms, H1 to H50 sera. O group sera 150 to 157 were included during 1972.

The E. coli typing sera were prepared in rabbits in this laboratory with standard strains of E. coli. The sera and the cultures were periodically checked for purity, confirmation as to type, and cross-reactions. For preliminary examination of the E. coli cultures, slide agglutination was used, after which tube titer and, when required, reciprocally cross-absorbed sera were also used (4).

Frequency of E. coli serogroups. The frequency of occurrence of an E. coli serogroup was based on the number of isolates obtained from all sampling sites during the period cited. The number of typical colonies examined from each water sample varied from four to eight. The numerical value given each serogroup did not exceed one per sample per site per sampling time. This was necessary, for an individual serogroup sometimes predominated among the E. coli isolated from one sample.

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RESULTS

Of the 3,200 E. coli cultures isolated from water, 2,639 or 82.4% were O serogrouped. The remainder were O negative or rough forms. Table 1 summarizes the frequency of occurrence of the E. coli cultures according to the O antigen groups. The largest number (881 or 33.4%) belonged to O groups 1 to 26, with the second highest (444 or 16.8%) belonging to O groups 60 to 88.

Table 2 lists the individual E. coli O groups identified. Those found most frequently were ADO3 (115 isolates), 18ac (111), 2a (90), 3 (65), 7 and 73 (58), and 139 and OX13 (49). The ADO3 is the O3 group of Alkalescens dispar. Although the latter is an artificial designation and some of the types occur very commonly, it is included in the Escherichia group (2).

The E. coli O groups isolated from water that are associated with disease in humans are listed as follows (the number isolated in parentheses): 2a (90), 6 (47), 18ab (29), 18ac (111), 20 (13), 25 (33), 26 (17), 42 (8), 55 (4), 78 (5), 86a (8), 86ab (3), 111ab (2), 112ab (30), 124 (12), 125 (36), 126 (15), 142 (6), 144 (5), and 146 (17). Those associated with disease in animals are listed as follows (the number isolated in parentheses): 1a (56), 2a (90), 5ab (42), 6 (47), 7 (58), 8 (17), 9 (46), 17 (13), 20 (13), 23 (12), 26 (17), 55 (4), 73 (58), 75 (42), 78 (5), 86 (11), 101 (10), 103 (32), 111 (2), 115 (8), 116 (5), 117 (42), 118 (5), 124 (12), 138 (2), 139 (49), 141 (24), 149 (10), 157 (6), and X28 (40). The E. coli O groups that were not isolated from water in this study include 24, 30, 32, 33, 52, 62, 95, 114, 119, and 131. Because O grouping or OK grouping alone is not sufficient evidence of pathogenicity, the cultures were examined for their K and, if motile, H antigens to confirm their serotype.

The E. coli serotypes identified among the cultures isolated from water are listed in Table 3. Some of these serotypes, such as O2a:K1:H6, O26:K60:H11, O55:K59:H27, O86:K62:H2, O112ab:K68:H2, O125ab:K70:H21, 128ab:K67:H2, and 138:K81:H14, are the same as those reported from disease in humans and animals. In this respect, the serotypes listed for O groups 6, 18ab, 18ac, 111ab, 126, 127a, 139, 141, and 147 have the same K antigen, but not the same H antigen. In three of the O groups, 128ac, 149, and 157, the K antigen could not be identified (K·), and only the H antigen of O128ac matched that of a known enteropathogenic E. coli.

DISCUSSION

It is assumed that the presence of the E. coli O groups in water is an indication of human or animal pollution. If one is to isolate specific enteropathogenic serotypes from a water source, there must be a discharge of that serotype into the water at some point. In addition, the serotype must be able to survive in the water, a habitat that might not be compatible. The prevalence of certain E. coli O groups may be due to the number entering as well as those surviving in the water. Whether there is an adaption to the water environment or a mutation due to the microflora, chemicals, and other pollutants in the stream is not clear. The presence in water of almost every known E. coli O group was confirmed, but it was not possible to complete the serological classification (serotype) of each of the 2,639 O groups identified. This would be expected in polluted water receiving E. coli from a variety of sources.

The presence of E. coli serotypes in water that are known to be associated with pathogenicity for humans and animals does constitute a potential public health hazard (8). With the increased demand for recreational use of
streams, further investigation of the presence of known (and perhaps as yet unknown) pathogenic *E. coli* serotypes is warranted. These *E. coli* isolates should not only be completely serotyped, but should also be examined in laboratory animals for potential pathogenicity or toxicity.

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**Table 3. *E. coli* serotypes isolated from water**

|   | O   | K    | H   |
|---|-----|------|-----|
| 2a| 1   | 6    | 48  |
| 6 | 2ac | 49   | 19,10 |
| 18ab|76 | 8    |  |
| 18ac|77 | 27   | 49  |
| 26 | 60  | 11,8 |  |
| 55 | 59  | 27   |  |
| 86 | 62  | 2,8  |  |
| 111a|58 | 2,18 |  |
| 112ab|68 | 8,21 |  |
| 125ab|70 | .    |  |
| 126 | 71  | .    |  |
| 127a|63  | .    |  |
| 128ab|67 | .    |  |
| 128ac|7  | .    |  |
| 138 | 81  | 14   |  |
| 139 | 82  | 2,19 | 21  |
| 141 | 85  | 2,7  | 10  |
| 147 | 89  | 7    |  |
| 149 | .    | 7,8  | 12  |
| 157 | .    | .    |  |

*a* O., K., or H. means antigen is not identifiable.

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