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# Further studies of *Escherichia coli* in babies after normal delivery

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## SUMMARY

Previous work showed that on the basis of O serotyping alone of *Escherichia coli*, the majority of babies acquired the same O serotype as was found in the stools of their respective mothers. Further characterization of the *E. coli* by H serotyping, determination of their antibiotic resistance and ability to ferment six carbohydrates showed that in the majority of cases the previous results were confirmed. In a minority of cases this further testing showed that the strains were not identical. In some instances a number of strains isolated from the same pair showed different combinations of the markers used.

## INTRODUCTION

Previous work (Bettelheim *et al.* 1974) showed that from the faecal flora from 22 out of 28 babies *Escherichia coli* carrying the same O antigen as that found amongst the *E. coli* of the maternal faeces were isolated. This work describes the further characterization of these 2525 strains. Strains were typed according to their H antigen, their ability to ferment six carbohydrate substrates and their antibiotic resistance. The maternal and baby strains were then again compared.

## MATERIALS AND METHODS

The collection of strains and O serotyping has been described previously (Bettelheim *et al.* 1974). The numbers used to identify the mothers and their babies are the same as those used previously.

### *H antigen determination*

The H antigens of all strains were determined after achieving full motility (Chandler & Bettelheim, 1974). Strains consistently unable to develop motility as judged by microscopical observation were considered non-motile.

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Table 1. *Isolation of identical types of* Escherichia coli *from mothers and their babies*

| Patient no. | Common strain in maternal and neonatal stool | | | |
|---|---|---|---|---|
| | O antigen | H antigen | Antibiotic resistance | Carbohydrates fermented |
| 2 | 3 | 2 | Su | Du, Ma |
| | 81 | 27 | F/S | Ma, Rh |
| 21 | 18 | 14 | F/S | Ma, Rf, Rh, Sc |
| | 58 | 40 | F/S | Ma, Rf, Rh |
| | R | 14 | F/S | Ma, Rf, Rh, Sc |
| 35 | 11 | 4 | S, Su | Du, Ma, Rh |
| | R | 4 | S, Su | Du, Ma, Rh |
| 37 | 25 | 1 | F/S | Ma, Rh |
| | NT | 8 | F/S | Du, Ma, Rf, Rh, Sc |
| 1 | 1 | NM | F/S | Du, Ma, Rh, Ss |
| 26 | 1 | NM | F/S | Ma, Ss |

*Carbohydrate fermentation*

The ability of the strains to ferment dulcitol (Du), maltose (Ma), raffinose (Rf), rhamnose (Rh), sorbose (Ss) and sucrose (Sc) were examined (hereafter the abbreviations in brackets are used to label these markers). Bettelheim & Taylor (1969) found that of 16 carbohydrates used, the above six gave most differentiation between strains. Strains were inoculated by a multiple point inoculator onto agar plates of Oxoid Blood Agar Base (CM 55) containing 1 % of the substrate (w/v) and neutral red ($6 \times 10^{-5}$ %, w/v, final concentration). Plates were incubated at 37° C. and examined after 24, 48 and 72 hr.

*Antibiotic resistance*

Approximately ten organisms of each strain were inoculated by a multiple point inoculator on agar plates (Oxoid DST agar, CM 261) containing 4 % lysed blood and one of the following drugs: ampicillin (A), 25 μg./ml., streptomycin (S) 50 μg./ml., tetracycline (T) 10 μg./ml., chloramphenicol (C) 25 μg./ml., kanamycin (K) 10 μg./ml., sulphadimidine (Su) 100 μg./ml., nalidixic acid (Nal) 25 μg./ml., trimethoprim (Tr) 2 μg./ml. and gentamicin (G) 8 μg./ml. The characters shown in brackets are used as abbreviations throughout. Strains which are sensitive to all drugs tested are designated fully sensitive (F/S).

## RESULTS

The further testing of the strains from six mothers and their babies showed no differences with respect to any of the characters tested (Table 1).

Strains from six further mothers and babies still showed that the majority of babies' stool strains were identical with the maternal ones. However, there were a minority of strains from the babies which differed from the maternal ones by one or more markers (Table 2).

Table 2. *Patients in whom the majority of strains of* Escherichia coli *isolated from mothers and their babies were identical but related strains were also isolated in small numbers*

| Patient no. | Common O antigen | Strains isolated | Strains isolated from: Mother stool | Baby mucus | Baby stool |
|---|---|---|---|---|---|
| 5 | 42 | O42:H–:F/S:Du, Ma, Rf, Rh, Ss, Sc | 1 | 0 | 1 |
| | | O42:H8:F/S:Du, Ma, Rf, Rh, Ss | 0 | 2 | 0 |
| | 78 | O78:H–:F/S:Du, Ma, Rf, Rh, Ss, Sc | 30 | 11 | 48 |
| | | O78:H8:F/S:Du, Ma, Rf, Rh, Ss | 0 | 1 | 0 |
| | | O78:H18:S, Su:Du, Ma, Rf, Rh, Ss, Sc | 0 | 2 | 0 |
| | | O78:H18:F/S:Du, Ma, Rf, Rh, Ss, Sc | 1 | 0 | 0 |
| 17 | 71 | O71:H11:F/S:Ma, Rh | 7 | 0 | 42 |
| | 81 | O81:H–:F/S:Ma, Sc | 3 | 0 | 1 |
| | | O81:H–:F/S:Ma, Rh, Ss | 2 | 5 | 0 |
| | | O81:H11:F/S:Ma, Rh | 0 | 0 | 1 |
| 18 | 48 | O48:H32:F/S:Du, Ma, Rf, Rh, Ss | 2 | 8 | 24 |
| | | O48:H32:F/S:Ma | 0 | 0 | 1 |
| 33 | 84 | O84:H2:Su:Du, Ma, Rf, Rh | 5 | 0 | 5 |
| | | O84:H2:Su:Du, Ma, Rf, Sc | 0 | 0 | 2 |
| 13 | 7 | O7:H30:F/S:Ma | 1 | NS* | 0 |
| | | O7:H–:F/S:Ma, Rh | 1 | — | 0 |
| | | O7:H–:F/S:Ma, Rf, Rh, Ss, Sc | 6 | — | 44 |
| | 82 | O82:H31:A:Du, Ma, Rf, Rh | 1 | — | 0 |
| | | O82:H31:F/S:Du, Ma, Rf, Rh | 0 | — | 6 |
| 22 | 6 | O6:H1:F/S:Du, Ma, Rh, Ss | 14 | 0 | 30 |
| | | O6:H–:A:Du, Ma, Rf, Rh | 1 | 0 | 0 |

* NS = no specimen obtained.

Table 3. *Mothers and their babies colonized with identical O serotypes of* Escherichia coli. *Further typing showed the majority of strains to be related but not identical*

| Patient no. | Common O antigen | Strains isolated | No. of strains isolated from: Mother stool | Baby stool |
|---|---|---|---|---|
| 6 | 38 | O38:H25:F/S:Ma, Rf, Rh, Ss, Sc | 1 | 0 |
| | | O38:H37:F/S:Du, Ma, Rh, Ss | 9 | 0 |
| | | O38:H–:F/S:Du, Ma, Rh, Ss | 1 | 4 |
| | | O38:H4:F/S:Du, Ma, Rh, Ss | 0 | 56 |
| 7 | 42 | O42:H7:F/S:Du, Ma, Rf, Rh, Ss | 1 | 0 |
| | | O42:H19:F/S:Du, Ma, Rf, Rh, Sc | 1 | 68 |
| | | O42:H19:A:Du, Ma, Rf, Rh, Sc | 7 | 0 |
| | | O42:H19:F/S:Du, Ma, Rh, Sc | 12 | 0 |
| | | O42:H19:A:Du, Ma, Rh, Sc | 1 | 0 |
| | R | R:H7:F/S:Du, Ma, Rf, Rh, Ss | 6 | 0 |
| | | R:H19:F/S:Du, Ma, Rf, Rh, Sc | 0 | 1 |

No strains of *E. coli* were isolated from the mucus of these babies.



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Table 4. *Identification of rough and non-typable* Escherichia coli *from mothers and their babies*

| Patient no. | Common O antigen | Strain isolated | No. of strains isolated from: Mother stool | Baby mucus | Baby stool |
|---|---|---|---|---|---|
| 15 | R | R:H−:F/S:Ma, Rh, Ss | 5 | 0 | 0 |
| | | R:H−:S:Ma, Rh, Ss | 2 | 0 | 0 |
| | | R:H−:Su:Ma, Rh, Ss | 7 | 0 | 0 |
| | | R:H7:F/S:Du, Ma, Rh, Ss | 0 | 0 | 50 |
| 19 | R | R:H7:F/S:Du, Ma, Rf, Rh, Ss | 8 | 0 | 50 |
| | | R:H7:T:Du, Ma, Rf, Rh, Ss | 1 | 0 | 0 |
| 10 | NT | OX*:H10:F/S:Ma | 1 | NS† | 3 |
| | | OX:H10:T:Ma | 37 | — | 37 |
| 29 | 42 | O42:H2:F/S:(strain died) | 1 | 0 | 0 |
| | | O42:H37:F/S:Ma, Rh, Sc | 0 | 0 | 1 |
| | 79 | O79:H7:F/S:Ma, Rf, Rh, Sc | 1 | 0 | 0 |
| | | O79:H7:F/S:Du, Ma, Rh, Sc | 0 | 0 | 1 |
| | R | R:H37:T:Ma | 1 | 0 | 0 |
| | | R:H37:T:Ma, Rh, Sc | 1 | 0 | 0 |
| | | R:H37:F/S:Ma, Rh, Sc | 1 | 0 | 0 |
| | | R:H7:F/S:Ma, Rh, Sc | 1 | 0 | 0 |
| | | R:H52:F/S:Du, Ma, Rf, Rh | 2 | 0 | 0 |
| | | R:H7:F/S:Du, Ma, Rf, Rh | 0 | 1 | 0 |
| | | R:H37:F/S:Du, Ma, Rf, Rh | 0 | 1 | 0 |
| | | R:H7:F/S:Du, Ma, Rh, Sc | 0 | 1 | 1 |
| | | R:H−:F/S:Ma, Rh, Ss | 0 | 12 | 21 |
| | NT | OY:H2:F/S:Ma, Rh, Sc | 5 | 0 | 0 |
| | | OY:H2:F/S:Du, Ma, Rh, Sc | 9 | 0 | 32 |
| | | OY:H2:F/S:Du, Ma, Rf, Rh | 2 | 1 | 0 |
| | | OY:H−:F/S:Du, Ma, Rf, Rh | 3 | 0 | 0 |
| | | OY:H−:F/S:Du, Ma, Rh, Sc | 0 | 0 | 3 |
| | | NT:H2:F/S:Du, Ma, Rh, Sc | 0 | 0 | 1 |
| 23 | R | R:H7:F/S:Du, Ma, Rh | 7 | 4 | 0 |
| | | R:H7:F/S:Du, Ma, Rf, Rh, Sc | 0 | 1 | 0 |
| | | R:H52:F/S:Ma | 1 | 0 | 0 |
| | | R:H5:F/S:Du, Ma | 1 | 0 | 0 |
| | | R:H−:F/S:Du, Ma, Rf, Rh, Sc | 4 | 0 | 1 |
| | | R:H−:F/S:Ma, Sc | 1 | 2 | 0 |
| | NT | NT:H4:F/S:Ma, Rh, Ss, Sc | 2 | 0 | 0 |
| | | NT:H−:F/S:Du, Ma, Rf, Rh, Sc | 1 | 0 | 0 |
| | | NT:H−:F/S:Du, Ma, Rf, Rh, Ss, Sc | 1 | 0 | 0 |
| | | NT:H−:F/S:Du, Ma, Rf, Sc | 1 | 0 | 0 |
| | | NT:H−:F/S:Du, Ma, Rf, Rh | 1 | 0 | 0 |
| | | NT:H−:F/S:Ma | 1 | 0 | 0 |
| | | OX:H10:F/S:Ma | 0 | 0 | 1 |
| | | OX:H10:T:Ma | 0 | 0 | 18 |
| 27 | 27 | O27:H−:F/S:Ma, Rh | 1 | 2 | 35 |
| | 108 | O108:H19:F/S:Du, Ma, Sc | 2 | 0 | 0 |
| | | O108:H−:F/S:Du, Ma, Sc | 0 | 1 | 1 |
| | | O108:H−:F/S:Du, Ma, Rf, Sc | 0 | 0 | 1 |
| | R | R:H4:F/S:Du, Ma, Sc | 1 | 0 | 0 |
| | | R:H−:F/S:Ma, Rf, Rh, Sc | 1 | 0 | 0 |
| | | R:H−:F/S:Ma | 0 | 1 | 6 |

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Table 4 (*cont.*)

| Patient no. | Common O. antigen | Strain isolated | No. of strains isolated from: Mother stool | Baby mucus | Baby stool |
|---|---|---|---|---|---|
| 27 | NT | NT:H34:F/S:Ma, Sc | 3 | 1 | 1 |
| | | NT:H4:F/S:Du, Ma, Sc | 2 | 3 | 0 |
| | | NT:H−:F/S:Du, Ma, Sc | 1 | 0 | 0 |
| | | NT:H4:F/S:Ma, Ss, Sc | 1 | 1 | 6 |
| | | NT:H11:F/S:Ma, Sc | 0 | 1 | 1 |
| | | NT:H8:F/S:Ma, Rh | 0 | 1 | 0 |
| | | NT:H9:F/S:Ma, Ss, Sc | 0 | 0 | 1 |
| | | NT:H−:F/S:Ma | 0 | 0 | 2 |
| 30 | 141 | O141:H2:F/S:Du, Ma, Rh, Sc | 8 | 0 | 27 |
| | NT | NT:H30:F/S:Du, Ma, Rf, Rh, Sc | 1 | 0 | 0 |
| | | NT:H12:F/S:Ma, Rf, Rh, Sc | 1 | 0 | 0 |
| | | NT:H2:F/S:Du, Ma, Rh, Sc | 0 | 0 | 3 |

* OX = O162 – see Discussion.
† NS = no specimen obtained.

From another two mothers and babies identical strains were found but only in minority numbers. The majority of strains isolated varied from one another by one or more markers, although the O type was the same (Table 3).

In one case (patient 31) there were three strains all with the O9 antigen isolated from the mother: (i) O9:H14:F/S:Du, Ma, Rf, Rh; (ii) O9:H−:F/S:Du, Ma, Rf, Rh; and (iii) O9:H−:F/S:Du, Ma, Rh – no strains of *E. coli* were isolated from the baby's mucus and the only strain isolated from the baby's faeces, although serotype O9 was O9:H10:F/S:Du, Ma, Rf, Rh. Many strains from both mother and baby were tested against both H antisera, which are not related (Edwards & Ewing, 1972) and no cross-reaction was found.

In the previous study (Bettelheim *et al.* 1974) rough or non-typable strains were considered similar if they were found in specimens from one mother and her baby. There were two patients (15 and 19) where rough strains were the only strains common to mother and baby. Further studies showed that in patient 19 the strains were in fact similar but in patient 15 the strains were quite different (Table 4). In two patients (10 and 29), whose predominant strains were non-typable by O serotyping, it was noted that the strains carried the same H antigen. O antiserum was, therefore, raised to an isolate from each of these patients and all other non-typable strains tested against them. This led to the identification of two more O serotypes called X and Y for the purpose of this paper. In the other patients (23, 27, 30) with non-typable strains, the other markers were too dissimilar (Table 4) to warrant preparing further sera.

## DISCUSSION

O serotyping has been extensively used in the study of the spread of *Escherichia coli* in man (Linzenmeier, Freislederer, Apak & Metz, 1961; Linzenmeier, 1962; Ewing, 1962; Turck, Petersdorf & Fournier, 1962; Nejedla, Srajbr & Lanc, 1967;

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Table 5. *Examples in which further typing indicated that strains thought to be different merely showed O–R variation*

| Patient no. | Strains isolated | No. of strains isolated from: Mother stool | No. of strains isolated from: Baby stool |
|---|---|---|---|
| 18 | O48:H32:F/S:Du, Ma, Rf, Rh, Ss | 2 | 24 |
| | R:H32:F/S:Du, Ma, Rf, Rh, Ss | 0 | 32 |
| | O48:H32:F/S:Ma | 0 | 1 |
| | R:H32:F/S:Ma | 1 | 1 |
| 35 | O11:H4:S, Su:Du, Ma, Rh | 16 | 54 |
| | R:H4:S, Su:Du, Ma, Rh | 13 | 6 |

Gruneberg, Leigh & Brumfitt, 1968; Cooke, Ewins & Shooter, 1969), animals (Sojka & Carnagham, 1961; Glantz, Narotsky & Bubash, 1962; Soderlind, 1965; Gossling & Rhoades, 1966; Hemsley, Barnum & Ingram, 1967; Shooter, Cooke, Rousseau & Breaden, 1970) and the environment (Muller, 1967; Cooke *et al.* 1970). At first only a limited number of O antisera were available but nevertheless this initiated these studies. As more types were identified, the studies could be extended. Recently, with 152 O antisera, it seemed that extensive epidemiological investigation might be more successful. This study shows that although O serotyping alone gave meaningful answers in a majority of cases and no further information was obtained from examining strains in more detail, there was a significant minority of cases in which a common O serotype was found but this did not indicate spread of the same strain.

The use of further O antisera prepared against untypable wild strains led to further differentiation of strains. Both X and Y antisera were tested against all known O antigens and no significant cross-reactions were detected. Strain X, representatives of which were found in a number of patients, has been forwarded to Dr F. Ørskov (International Escherichia Centre, Copenhagen) who has confirmed that it is a new serological type and will be designated O162. Strain Y, which was only found in one patient, has not been sent to Dr F. Ørskov, as current policy of the International Centre is not to put up new antigen numbers unless these are of special importance in clinical medicine or science (Ørskov, Ørskov & Rowe, 1973).

Furthermore, the use of both H and biotyping of some rough strains showed them to have the same markers as O types isolated from the same mother or baby. This O–R variation is well established and therefore the further typing enabled the detection of further common strains. Examples of this (patients 35 and 18) are shown in Table 5.

In other cases a number of strains isolated from the same pair showed different combinations of markers (Table 6). Furthermore, the same phenomenon was seen in strains isolated in a 10-day period from several patients (Table 7). In the latter case the markers H38 and H52 in our experience are most unusual (Bettelheim, 1969, Ph.D. thesis; Bettelheim, in preparation). On the other hand, there have been a number of instances of colonization of different mothers and babies with

Table 6. *Examples of strains showing some markers in common from mother and baby pairs*

| Patient no. | Strains isolated | No. of strains isolated from: Mother stool | Baby mucus | Baby stool |
|---|---|---|---|---|
| 5 | O78:H–:F/S:Du, Ma, Rf, Rh, Ss, Sc | 30 | 11 | 48 |
| | O78:H18:F/S:Du, Ma, Rf, Rh, Ss, Sc | 1 | 0 | 0 |
| | O78:H18:S, Su:Du, Ma, Rf, Rh, Ss, Sc | 0 | 2 | 0 |
| | O78:H8:F/S:Du, Ma, Rf, Rh, Ss | 0 | 1 | 0 |
| | O42:H–:F/S:Du, Ma, Rf, Rh, Ss, Sc | 1 | 0 | 1 |
| | O42:H8:F/S:Du, Ma, Rf, Rh, Ss | 0 | 2 | 1 |
| | R:H–:F/S:Du, Ma, Rf, Rh, Ss, Sc | 3 | 0 | 0 |
| | R:H18:A:Du, Ma, Rf, Rh, Ss, Sc | 1 | 0 | 0 |
| 7 | O42:H19:F/S:Du, Ma, Rf, Rh, Sc | 1 | 0 | 68 |
| | O42:H19:A:Du, Ma, Rf, Rh, Sc | 7 | 0 | 0 |
| | O42:H19:F/S:Du, Ma, Rh, Sc | 12 | 0 | 0 |
| | O42:H19:A:Du, Ma, Rh, Sc | 1 | 0 | 0 |
| | O42:H7:F/S:Du, Ma, Rf, Rh, Ss | 1 | 0 | 0 |
| | O20:H19:F/S:Du, Ma, Rf, Rh, Sc | 0 | 0 | 1 |
| | O96:H19:A:Du, Ma, Rf, Rh, Sc | 1 | 0 | 0 |
| | R:H19:F/S:Du, Ma, Rf, Rh, Sc | 0 | 0 | 1 |
| | R:H7:F/S:Du, Ma, Rf, Rh, Ss | 6 | 0 | 0 |
| 17 | O71:H11:F/S:Ma, Rh | 7 | 0 | 42 |
| | O81:H–:F/S:Ma, Rh, Ss | 2 | 5 | 0 |
| | O81:H–:F/S:Ma, Sc | 3 | 0 | 1 |
| | O81:H11:F/S:Ma, Rh | 0 | 0 | 1 |
| | O147:H–:F/S:Ma, Rh, Ss | 0 | 1 | 0 |
| | R:H–:F/S:Ma, Sc | 0 | 0 | 2 |

Table 7. *Complex variation of selected strains isolated during a ten-day period*

| Date | Patient | Strain |
|---|---|---|
| 8 May 1972 | 24 mother | O9:H38:F/S:Du, Ma, Rf, Rh |
| 13 May 1972 | 24 mother | R:H52:F/S:Du, Ma, Rf, Rh, Sc |
| 13/15 May 1972 | 24 mother | O46:H52:F/S:Du, Ma, Rf, Rh, Sc |
| 14 May 1972 | 23 mother | O46:H52:F/S:Du, Ma, Rf, Sc |
| 14 May 1972 | 23 mother | R:H52:F/S:Ma |
| 14/16 May 1972 | 26 mother | O46:H38:F/S:Du, Ma, Rf, Sc |
| 15 May 1972 | 30 mother | R:H52:F/S:Du, Ma, Rh, Sc |
| 15 May 1972 | 24 mother | R:H52:F/S:Du, Ma, Rf, Rh, Ss, Sc |
| 15 May 1972 | 29 mother | R:H52:F/S:Du, Ma, Rf, Rh |
| 16 May 1972 | 27 mother | O46:H52:F/S:Du, Ma, Rf, Sc |
| 18 May 1972 | 30 mother | O46:H52:F/S:Du, Ma, Sc |
| 18 May 1972 | 30 mother | R:H52:F/S:Du, Ma, Sc |

strains possessing identical markers indicating classical cross-infection (Bettelheim *et al.* 1974).

To date, it has been assumed from epidemiological investigation of *E. coli* spread that the antigenic markers exhibit only limited variations, e.g. O–R and H+/H–. There has been one report of serial study of serological typing of organisms causing chronic urinary infection which showed complex variations of sero- and biotypes

(Bettelheim & Taylor, 1969). The present study suggests that this phenomenon may be more widespread than previously assumed. The genetic basis for this is under investigation.

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