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REFERENCES

Aggeler, P. M., O'Reilly, R. A., Leong, L., and Kowitz, P. E. (1967). *New England Journal of Medicine*, **276**, 496.
Alvsaker, J. O. (1966). *Scandinavian Journal of Clinical and Laboratory Investigation*, **18**, 227.
Alvsaker, J. O. (1968). *Journal of Clinical Investigation*, **47**, 1254.
Brodie, B. B. (1965). *Proceedings of the Royal Society of Medicine*, **58**, 946.
Elion, G. B., Kovensky, A., Hitchings, G. H., Metz, E., and Rundles, R. W. (1966). *Biochemical Pharmacology*, **15**, 863.
Ertel, N., Omokoku, B., and Wallace, S. L. (1969). *Arthritis and Rheumatism*, **12**, 293.
Gornall, A. G., Bardawill, C. J., and David, M. M. (1949). *Journal of Biological Chemistry*, **177**, 751.
Gutman, A. B., and Yü, T. F. (1958). *Bulletin of the New York Academy of Medicine*, **34**, 287.
Hawkins, D., Pinckard, R. N., Crawford, I. P., and Farr, R. S. (1969). *Journal of Clinical Investigation*, **48**, 536.
Hucker, H. B., Zacchei, A. G., Cox, S. V., Brodie, D. A., and Cantwell, N. H. R. (1966). *Journal of Pharmacology and Experimental Therapeutics*, **153**, 237.
Klinenberg, J. R. (1968). *Arthritis and Rheumatism*, **11**, 828.
Klinenberg, J. R., and Kippen, I. (1969). In preparation.
Liddle, L., Seegmiller, J. E., and Laster, L. (1959). *Journal of Laboratory and Clinical Medicine*, **54**, 903.
Odell, G. B., Cohen, S. N., and Kelly, P. C. (1969). *Journal of Pediatrics*, **74**, 214.
Seegmiller, J. E., and Howell, R. R. (1962). *Arthritis and Rheumatism*, **5**, 616.
Sheikh, M. I., and Møller, J. V. (1968). *Biochimica et Biophysica Acta*, **158**, 456.
Shinaberger, J. H., Pabico, R. C., Shear, L., Knochel, J. P., and Barry, K. G. (1964). *Clinical Research*, **12**, 463.
Yü, T. F., and Gutman, A. B. (1953). *Proceedings of the Society of Experimental Biology and Medicine*, **84**, 21.

Changing Faecal Population of Escherichia coli in Hospital Medical Patients

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Summary: Specimens of faeces were obtained at weekly intervals for one year from patients in a female medical ward and Escherichia coli present were typed. The faecal E. coli population of the patients was constantly changing. No serotypes of E. coli were dominant, but on 31 occasions during the year small clusters of patients carried the same type.

Introduction

Previous workers have found that the serotypes of *Escherichia coli* present in the bowel of an individual may be relatively constant over long periods of time (Sears, Brownlee, and Uchiyama, 1950) and that it may be difficult to change the faecal *E. coli* population (Sears, Janes, Saloum, Brownlee, and Lamoreaux, 1956). Kennedy, Plorde, and Petersdorf (1965) and Winterbauer, Turck, and Petersdorf (1967), however, have shown that certain serotypes of *E. coli* are more commonly found in the faeces of hospital inpatients than in the general population, suggesting that under these conditions the *E. coli* bowel flora may change.

The investigations reported here were carried out to study in some detail the faecal carriage of *E. coli* by patients in one hospital ward.

Material and Methods

The work was done during one year in a female open medical ward which had 24 beds and one further bed in a single side-room. The patients were those seen in a general medical ward, and included particularly diabetic patients and patients with diseases of the bowel.

Specimens of Faeces.—We tried to obtain specimens from each patient as soon as possible after admission and thereafter at weekly intervals. In addition each stool passed by 32 patients during one week was examined.

Specimens of Urine.—These were midstream specimens sent in routinely because urinary tract infection was suspected. The presence of an excess of white cells in the urine and of a bacilluria (of 10^5 or more) were the criteria taken for considering the patient to be infected.

Method of Faeces Examination

The faeces were inoculated on to a MacConkey plate by means of a cotton-wool swab. After overnight incubation the plates were examined and five colonies of each colonial type of coliform present were subcultured on to blood agar. The identity of these coliforms as *Escherichia* was confirmed by determination of the following reactions: fermentation of lactose and glucose, production of indole, and failure to produce urease or to utilize citrate. The *E. coli* were inoculated into 10 ml. of broth which was incubated overnight, steamed for 30 minutes, and then used for serotyping the organism.

Antisera to O groups 1–25, 39, and 75 were prepared by the method of Roschka (1950). The cross-reactions of the antisera with the type strains of 148 *E. coli* O groups were determined, and absorbed sera were prepared as described by Bettelheim and Taylor (1969).

The antisera were grouped into five pools; 0·3 ml. of bacterial suspension was added to 0·3 ml. of antiserum in a Dreyer's tube, incubated at 50° C. for 18 hours, and then read. When a positive result was obtained the organism was tested against the monovalent antisera of the group. The identity of the organisms was confirmed by titration of the antisera, and, when necessary, by use of absorbed antisera.

Results

Examination of 1,136 specimens of faeces from 303 patients was made. From 234 of the patients typable strains of *E. coli*

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were isolated. From 90 of the specimens of faeces *E. coli* was not isolated. Of the 1,046 specimens from which *E. coli* was isolated 592 (56·6%) contained one strain, 397 (37·9%) two strains, 50 (4·8%) three strains, and 7 (0·7%) four strains as judged by the examination of at least five colonies from each stool culture. Non-typable strains in each specimen were counted as one type.

The numbers of serotypes isolated from each patient and the relation of these to the number of examinations made and the length of inpatient stay are shown in Table I.

TABLE I.—*Relationship of Number of E. coli Serotypes Isolated from a Patient to Number of Examinations Made and Length of Inpatient Stay*

No. of *E. coli* Types Isolated from Faeces	No. of Patients	Average Length of Inpatient Stay (in Days)	Average No. of Specimens Examined
1	67	14	2
2	89	23	4
3	43	23	5
4	19	40	9
5	6	35	6
>5	10	74	15

Average length of inpatient stay = 22·4 days (2–180).

Of 100 patients who were inpatients for three weeks or more and from whom three or more specimens of faeces were obtained 10 had a dominant serotype—that is, one present as the only serotype in all specimens examined. Twelve patients had a recurrent serotype. This was defined as one isolated from at least three specimens at weekly intervals or longer, but not necessarily forming the total *E. coli* flora and not necessarily isolated from all specimens examined.

The frequency of isolation of different serotypes of *E. coli* from the faeces of patients on this ward is shown in Table II.

TABLE II.—*Frequency of Isolation of Different Serotypes of E. coli*

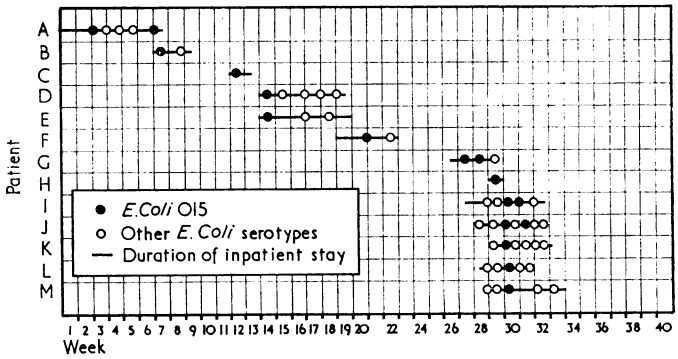
Serotype	No. of Patients from Whom this Serotype was Isolated	% of Patients Studied from Whom this Serotype was Isolated	No. of Urinary Infections Caused by this Serotype
O4	40	13·2	2
O18	32	10·5	3
O3	30	9·9	0
O1	29	9·5	2
O15	28	9·2	0
O2	26	8·5	4
O8	23	7·5	1
O7	21	6·9	1
O21	21	6·9	1
O25	19	6·2	1
O11	17	5·9	2
O6	18	5·9	1
O75	15	5·0	1
O39	14	4·6	0
O12	11	3·9	3
O22	12	3·9	1
O20	10	3·2	2
O5	9	2·9	2
O13	8	2·6	0
O17	6	1·9	0
O10	4	1·3	0
O19	4	1·3	0
O9	4	1·3	1
O14	4	1·3	0
O23	3	0·99	0
O24	1	0·32	0
O16	0	0	0

In 32 patients every specimen of faeces passed during one week was examined, and the results are shown in Table III. The number of serotypes isolated is the total number found in that patient during the week. Not more than two serotypes were found in any one specimen, and all non-typable strains from one patient were counted as one strain.

TABLE III.—*Number of Serotypes of E. coli Isolated from 32 Patients from All Specimens of Faeces Passed During One Week were Examined*

No. of Serotypes Isolated during the Week	No. of Patients	Average No. of Specimens Examined
1	1	7
2	7	6
3	8	6
4	6	6
5	5	8
>5	1	10

The distribution of individual serotypes in the ward throughout the year was studied and clusters of serotypes of *E. coli* were particularly looked for. A cluster was defined as the appearance within five days of each other in three or more patients of a serotype which had never previously been found in the patients. Thirty-one clusters were observed during the year, and a typical cluster is illustrated in the Chart. Other serotypes showed different patterns. Some were constantly present in the ward; others were present in the ward for a number of weeks, were then not isolated for several weeks, and reappeared later.



Distribution of *E. coli* O15, showing a cluster of isolations from seven patients.

Sixty-four urinary tract infections were recorded during the year. Fifteen were not due to *E. coli* and 21 were due to rough strains of *E. coli* or to strains not typable with our sera, leaving 28 due to typable strains. Only 17 of these patients had provided stools for examination before the urinary infection, and in seven the same type as the *E. coli* causing urinary infection had been found.

Discussion

When sampling faecal *E. coli* the number of colonies that can be examined is a very small proportion of the total coliform population. It was, however, shown by Vosti, Monto, and Rantz (1962), who examined up to 25 colonies, that nearly 80% of the total O groups present were discovered by examination of the first five colonies. In the present work also only 27 O groups were looked for, and a greater number of strains could have been identified by the use of more O antisera and H and K antisera.

From our results, however, it is clear that in our patients there is no single dominant type of *E. coli*, and the pattern is one of a constantly changing population with occasional clusters of a single type in a small number of patients. For individual patients the number of serotypes isolated was proportional to the number of specimens examined, whether this was done at weekly or daily intervals. We consider that the pattern of change we have seen is a true one, and that, further, more detailed examination of the faecal flora would only have emphasized the pattern of change and heterogeneity we have found. The patients appear to be steadily acquiring new strains either from other people in the ward or from other sources. Such a view would be compatible with the finding that only 7 of the 17 patients with urinary tract infection whose faeces were examined before the onset of infection carried similar strains in the stool; only very frequent examination of the faeces would ensure that the infecting strain was identified.

We have not compared the serotype present in the first specimen of faeces examined with those subsequently isolated, as we did not consider that, generally, we were obtaining specimens

soon enough after admission of the patients to hospital to be able to compare inpatient and outpatient strains of *E. coli*.

In our work with *Pseudomonas aeruginosa* we have been unable to trace the route by which individual strains spread from one patient to another in the same ward. We have, however, found that some patients acquire intestinal strains from food or medicines (Shooter *et al.*, 1969). Preliminary work suggests that food may also be one way in which our ward patients acquire new types of *E. coli*.

The possible sources of the *E. coli* strains we have found in food are of interest. It has been suggested that "animal" strains of *E. coli* are not so easily established in the bowel as "human" strains (Williams Smith, 1969). In a hospital in which selection of antibiotic resistant strains may operate, however, it is possible that a route is provided by which antibiotic-resistant strains of *E. coli* from animals may reach the human population.

We are particularly indebted to Dr. Joan Taylor, in whose laboratory the antisera were tested and the absorbed antisera prepared. We wish to thank the nursing staff of the wards concerned for their most helpful collaboration, and Dr. K. O. Black and Dr. A. M. Dawson for permission to work on their wards. We should also like to thank Mrs. N. Patel for technical assistance.

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REFERENCES

Bettelheim, K., and Taylor, Joan (1969). *Journal of Medical Microbiology*. In press.
Kennedy, R. P., Plorde, J. J., and Petersdorf, R. G. (1965). *Journal of Clinical Investigation*, **44**, 193.
Roschka, R. (1950). *Klinische Medizin*, **5**, 88.
Sears, H. J., Brownlee, J., and Uchiyama, J. M. (1950). *Journal of Bacteriology*, **59**, 293.
Sears, H. J., Janes, H., Saloum, R., Brownlee, J., and Lamoreaux, L. F. (1956). *Journal of Bacteriology*, **71**, 370.
Shooter, R. A., *et al.* (1969). *Lancet*, **1**, 1227.
Vosti, K. L., Monto, A. S., and Rantz, L. A. (1962). *Proceedings of the Society of Experimental Biology and Medicine*, **111**, 201.
Winterbauer, R. H., Turck, M., and Petersdorf, R. G. (1967). *Journal of Clinical Investigation*, **46**, 21.
Williams Smith, H. (1969). *Lancet*, **1**, 1174.

Abnormal Haemoglobins in Zambia. A New Haemoglobin Zambia α60 (E9) Lysine → Asparagine

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[WITH SPECIAL PLATE]

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Summary: A survey for abnormal haemoglobins in Zambia has demonstrated Haemoglobin S—including 187 cases of sickle-cell anaemia, Haemoglobin J Oxford on one occasion, and a new Haemoglobin Zambia in four unrelated families. No evidence for β-thalassaemia was found, but Haemoglobin H disease and other evidence for α-thalassaemia were found.

Introduction

The results of a survey of some 10,000 blood samples in Zambia differ in several respects from those that have been reported from East and West Africa. Sickle-cell anaemia was diagnosed in 187 cases, most of them in infants, but in contrast to West Africa no Haemoglobin C and no β-thalassaemia were found. On the other hand, again in contrast to both East and West Africa, evidence of Haemoglobin H disease was discovered (Special Plate, Fig. 1), and the presence of the gene for α-thalassaemia was also suggested by the observation of sickle-cell trait carriers with Haemoglobin S proportions of below 20% ; in one case the unusual combination of Haemoglobin H disease with sickling was noted—that is, the red cells both sickled and contained typical Haemoglobin H inclusion bodies and on electrophoresis Haemoglobin H and Haemoglobin S were found. The presence of α-thalassaemia in Zambia was also confirmed by the observation of Haemoglobin Bart's in cord blood. As some cord bloods contain "fast" haemoglobin fractions which are derivatives of Haemoglobin F, we isolated the "Bart's" fraction on two occasions and confirmed by preparing peptide chromatograms that the haemoglobins were indeed consisting of γ-chains only—that is, were Haemoglobin Bart's or γ_4.

A new observation was that in five families an α-chain abnormal haemoglobin was discovered. In one case it was Haemoglobin Jα Oxford (α15 Gly → Asp), previously found occasionally in families of European stock. Our family belonged to the Manyika tribe of Southern Tanzania. A completely new variant was discovered in four other families unrelated to each other (Special Plate, Fig. 2). Three of the families were of the Bemba tribe and the fourth was Tabwa. It was therefore considered appropriate to denote the new haemoglobin as Zambia rather than Bemba.

Identification of Haemoglobin Zambia

The globin from the abnormal haemoglobin was digested with trypsin, and fingerprints were made in the usual way (Sick *et al.*, 1967). The electrophoresis was done at *p*H 6·4. Fig. 3 (Special Plate) is a photograph of the fingerprint. There is a new peptide near the position of α and βTpVII. This peptide stains yellow-brown with ninhydrin, and it stains for histidine. No peptide is seen to be missing.

The abnormal peptide gives the following amino-acid analysis:

Amino-acid	μ Moles	Molar Ratio
Asp	0·106	1·04
Gly	0·205	2·02
His	0·0985	0·97
Lys	0·0985	0·97

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