J. Hyg., Camb. (1972), **70**, 99
Printed in Great Britain

Antibiotic resistance and transmissible R-factors in the intestinal coliform flora of healthy adults and children in an urban and a rural community

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(*Received* 29 *July* 1971)

SUMMARY

Faeces of healthy adults and of children under the age of 5, none of whom were attending hospital or receiving antibiotics, were examined for the presence of antibiotic resistant coliform bacilli.

A higher proportion of children (67 %) than of adults (46 %) carried resistant strains and this difference was observed in both the rural and urban groups.

Rural members of both age groups more often carried resistant organisms than urban members. Among rural adults, the incidence of drug-resistant strains was 63 % in those whose occupation involved close contact with farm animals, compared with 29 % in those with other occupations. The survey took place before the implementation of the Swann Report could have influenced the use of antibiotics in animal foodstuffs.

Transmissible R-factors were demonstrated in 61 % of the resistant strains. The incidence of transmissible resistance was similar among adults and children in town and country.

INTRODUCTION

Most studies of transmissible antibiotic resistance have been made with pathogenic Enterobacteriaceae or commensal coliform bacilli isolated from patients attending hospital. There have been fewer studies in normal populations. Smith & Halls (1966) found 20 drug-resistant *Escherichia coli* strains in the faeces of 15 of 24 healthy persons; 19 of these strains possessed R-factors. Datta (1969) found drug-resistant *E. coli* in the faeces of 52 of 100 adults admitted to hospital for non-urgent surgery; 60 % of the strains carried R factors. Moorhouse (1969) found drug-resistant faecal enterobacteria in 81 of 100 healthy Dublin infants below the age of 2; 71 of these were *E. coli* and 84 % of all the resistant strains possessed R-factors.

We report here a study of antibiotic-resistant coliform bacilli in the faeces of healthy adults and of young children in urban and rural populations. Our principal aim was to observe the effects of living in the country and of contact with farm animals on the prevalence of drug resistance, transmissible and non-transmissible, in normal human intestinal bacteria. The study took place before the results could have been much influenced by the recommendations of the Swann Report (1969) to restrict the use of antibiotics in animal foodstuffs.

MATERIALS AND METHODS

From June 1968 until July 1969, and again from May to July 1970, specimens of faeces were obtained from healthy children under the age of 5 years who lived in Bristol and attended infant welfare centres or were visited in their homes. Specimens were also obtained from adults some of whom were relatives of the children; but usually only one sample was obtained from one household. No one was receiving antibiotics or sulphonamides. Specimens from adults and children under 5 who lived in the country were obtained by visiting households in North Somerset, between July 1969 and April 1970. No one in the rural group had received antibiotics or sulphonamides for at least 6 months.

Faeces was plated on MacConkey agar. The investigation was confined to lactose-fermenting Gram-negative bacilli, hereafter referred to as coliform bacilli. Most strains were investigated by further tests (Cowan & Steel, 1965) which showed that 91 % of them were *E. coli* and the remainder *Klebsiella* spp. and *Citrobacter*.

The proportion of the faecal samples that contained large numbers of drug-resistant coliform bacilli was estimated during the first 12 months of the investigation by subculturing one lactose-fermenting colony chosen at random from MacConkey plates inoculated with faeces. (If colonial appearances suggested that there was more than one type present, one of each type was taken.) Sensitivity tests were made on nutrient agar containing 5 % haemolysed horse red cells, using Oxoid 'Multodisks' with ampicillin (25 μg.), streptomycin (25 μg.), tetracycline (50 μg.), kanamycin (30 μg.), nalidixic acid (30 μg.), chloramphenicol (50 μg.), nitrofurantoin (200 μg.) and sulphafurazole (500 μg.). A standard sensitive strain of *E. coli* was included with each batch of tests. All resistant strains were then tested for the presence of transmissible R-factors as described below.

Two methods were used to detect drug-resistant strains even when present in small numbers.

(i) Weighed samples of faeces were diluted 1/10 with sterile physiological saline and shaken for 1 min. in an M.S.E. homogenizer: 0·08 ml. of this suspension was spread evenly on a MacConkey plate and a 'Multodisk' placed on it. After incubation overnight, drug-resistant coliform colonies were selected, retested on lysed horse blood agar plates and examined for transmissible resistance.

(ii) Drops of 0·02 ml. of ten-fold dilutions of each faecal suspension were placed on 4 MacConkey agar plates; one plate was plain and the others contained 25 μg. per ml. of ampicillin, tetracycline or streptomycin respectively. Resistant coliform colonies were re-tested and examined for transmissibility.

Detection of transmissible drug resistance

Each resistant strain was tested by two methods:

(i) A mixture was prepared of 0·5 ml. of overnight broth culture of the resistant (potential donor) strain with 4·5 ml. of overnight broth culture of a non-lactose-fermenting nalidixic acid-resistant strain of *E. coli K. 12* and 5 ml. of sterile broth. After incubating this 'mating broth' at 37° C. for 20 hr., 0·02 ml. was spread on MacConkey agar containing nalidixic acid (50 μg./ml.) and the drug to which the donor was resistant (25 μg./ml.). A similar subculture was made after incubating the mating broth for a further 24 hr. Finally, the deposit obtained by centrifuging the mating broth was spread on a third MacConkey plate containing the drugs. Any non-lactose fermenting colonies growing on the plates were re-tested for resistance on haemolysed horse red cell agar.

(ii) R-factor transfer was studied quantitatively using a phosphatase-derepressed strain of *E. coli W. 3110* (Lee & Richmond, 1969).

RESULTS

In most specimens the predominant coliforms were sensitive to all the antibiotics (Table 1) but small numbers of resistant bacilli were present in more than half of the faecal samples. The more laborious method of plating several dilutions of faecal suspensions gave results only slightly higher than the simple procedure of placing a 'Multodisk' on a single plate inoculated with faeces. In determining the incidence of resistant strains in faeces, the results of both methods were combined together.

The incidence of antibiotic resistant strains was significantly higher in the faeces of children than of adults, in both rural and urban groups (Table 2). The effect of family occupation on the results can also be seen in Table 2. In the rural group 63 % of faeces from adult members of farming families contained resistant coliform bacilli compared with 29 % of adults in non-farming rural families, a significant difference. The difference between children of the farming and non-farming groups was slight, and not statistically significant. Similar differences were found between the proportions of individuals carrying strains resistant to each of the antibiotics separately (Table 3). Nearly all the resistant organisms were strains of *E. coli*.

Table 1. *Incidence of drug-resistant coliform bacilli in the faeces of healthy people (adults and children), tested by three methods (see text)*

Method	Total samples	Number with drug-resistant organisms
(i) Resistance of predominant organism	121	20 (17 %)
Detection of small numbers of resistant organisms		
(ii) Direct plating, with disk	264	142 (54 %)
(iii) Viable counts on antibiotic agar	264	154 (58 %)
(ii) and (iii) combined	264	156 (59 %)

https://doi.org/10.1017/S0022172400022130 Published online by Cambridge University Press

R-factors. One hundred and ninety-eight drug-resistant strains were tested for transmissibility by both methods, with identical results. There was no statistically significant difference between any of the population groups; the proportion of resistance that was transmissible lay betweeen 53% and 72% (average 61%). The R-factors that were transferred are shown in Table 4.

DISCUSSION

Many different methods have been employed in surveys of antibiotic resistant bacteria and few workers have used identical techniques. The comparison of methods reported here indicates that simply placing a 'Multodisk' on a MacConkey plate inoculated heavily with faeces is remarkably efficient as a screening method. In experienced hands it can even provide reliable semi-quantitative data. The much more elaborate method of inoculating serial dilutions of homogenized faeces on a range of media incorporating different antibiotics does not appear worth while

Table 2. *Incidence of drug-resistant coliform bacilli in faeces of adults and children from an urban and a rural area. Influence of family occupation*

	Adults		Children		Total	
Source	Total samples	Samples with resistant organisms	Total samples	Samples with resistant organisms	Total samples	Samples with resistant organisms
Urban	97	41 (42%)	96	61 (64%)	193	102 (53%)
Rural						
Employed with livestock	43	27 (63%)	28	22 (79%)	71	49 (69%)
Other occupations	28	8 (29%)	17	11 (65%)	45	19 (42%)
Total rural	71	35 (49%)	45	33 (73%)	116	68 (59%)

Tests of significance for differences between groups:

Urban: Adults *vs.* children — $\chi^2 = 8{\cdot}75$, $0{\cdot}005 > P > 0{\cdot}001$

Rural: Adults *vs.* children — $\chi^2 = 6{\cdot}56$, $0{\cdot}025 > P > 0{\cdot}01$

Adults employed with livestock *vs.* other adults — $\chi^2 = 7{\cdot}94$, $0{\cdot}005 > P > 0{\cdot}001$

Table 3. *Resistance to individual antibiotics in coliform bacilli in faeces of healthy people*

	Adults with resistant organisms			Children with resistant organisms		
Antibiotic	Urban	Rural (farming*)	Rural (non-farming)	Urban	Rural (farming*)	Rural (non-farming)
Ampicillin	13 (13%)	18 (42%)	4 (14%)	35 (36%)	16 (57%)	8 (47%)
Streptomycin	17 (17%)	17 (39%)	6 (21%)	24 (25%)	13 (46%)	6 (35%)
Tetracycline	18 (19%)	20 (47%)	8 (29%)	23 (24%)	19 (68%)	10 (59%)
Sulphonamide	17 (17%)	14 (33%)	4 (14%)	16 (17%)	11 (39%)	3 (18%)
Chloramphenicol	4 (4%)	4 (9%)	0	5 (5%)	6 (21%)	1 (6%)
Total samples	97	43	28	96	28	17

* Employed with livestock.

https://doi.org/10.1017/S0022172400022130 Published online by Cambridge University Press

for surveys of the kind described here, although it allows the determination of viable counts (and can provide suitable culture plates for replica plating techniques).

Considering this survey as a whole, resistant coliform bacilli were found in 49% of faecal samples from normal adults, an almost identical figure to that reported by Datta (1969) in pre-admission specimens from patients at Hammersmith Hospital and similar also to the figure obtained by Datta *et al.* (1971) in pre-treatment faecal samples from adults with urinary infections. In faecal samples from children under 5, we found resistant coliforms in 67%, similar to the results of Moorhouse (1969) from infants below the age of 2 years. The marked difference which we found between people of different ages within the same community and often within the same family is difficult to explain. One possible explanation might be a greater use of antibiotics among children than adults and so a short preliminary survey was made of the antibiotics prescribed during one week in September 1970 by 15 general practitioners from widely different parts of Bristol. The greatest use of antibiotics was among children of school age (antibiotics prescribed at a rate of 7·31 per 1000 patients on register), the least was in children of 0–4 years (4·71 per 1000) and adults received antibiotics at an intermediate rate (5·30 per 1000). This limited comparison suggests that the high incidence of resistant organisms in the bowel flora of young children may not be due to frequent antibiotic therapy.

The much higher incidence of resistant flora in the faeces of healthy rural families concerned with livestock, than in other rural inhabitants, was of particular interest,

Table 4. *Drug-resistance patterns in faecal coliform bacilli of healthy people, and the R-factors demonstrated in the resistant strain*

Antibiotic resistance patterns	Number of strains	R-factors (numbers transferred shown in parentheses)
A	43	A (8)
T	56	T (38)
S	4	0
A.T	24	A.T (21); T (1); A (1)
T.S	5	T.S (2); T (2)
T.Su	1	T (1)
S.Su	16	S.Su (11)
A.Su	1	0
A.Ne	1	A.Ne (1)
A.S.Su	12	A.S.Su (7)
A.C.T	1	A.C.T (1)
T.S.C	1	T.S.C (1)
T.S.Su	18	T.S.Su (13); T (1)
A.T.S	1	A.T.S (1)
A.T.S.Su	8	A.T.S.Su (4); A.T (1)
A.C.S.Su	1	A.C (1)
T.C.S.Su	3	T.C.S.Su (1); T.C (1)
A.T.S.Su.Ne	1	A.T.S.Su.Ne (1)
A.T.C.S.Su	1	A.T.C.S.Su (1)

Key

A	Ampicillin	Su	Sulphonamide
T	Tetracycline	Ne	Neomycin
S	Streptomycin	C	Chloramphenicol

https://doi.org/10.1017/S0022172400022130 Published online by Cambridge University Press

although perhaps not surprising. The rural study was undertaken just after the publication of the Swann Report (1969) but it is almost certain that the restrictions in antibiotic use recommended by that report would have had little if any effect within the period of the survey. The occurrence of resistance to antibiotics, e.g. chloramphenicol, not used as foodstuff additives might be attributed to their use in treating sick animals. Whether the substitution of alternative growth promoters, as recommended in the report, will have the desired effect of reducing the antibiotic-resistant gut flora of farm animals or whether the substitutes will still be able to select bacteria carrying R-factors remains to be seen. We intend to make a further study of the intestinal flora of normal people living in the same regions, when the provisions of the Swann Report have been implemented.

We wish to acknowledge the co-operation of the health visitors and inspectors of the Public Health Departments in Bristol and Long Ashton who collected the samples for us, and in particular, Mr A. P. D. Nichols whose enthusiastic help was of great value.

We wish to thank Professor E. C. Moorhouse for providing the culture of *E. coli E 711*.

The work was supported by grants from the Research Committee of the United Bristol Hospitals and from the Medical Research Council.

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