FAECAL CARRIAGE OF AEROBIC GRAM-NEGATIVE BACILLI AND DRUG RESISTANCE OF *ESCHERICHIA COLI* IN DIFFERENT AGE-GROUPS IN DUTCH URBAN COMMUNITIES

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SUMMARY. Faecal carriage rates for aerobic gram-negative bacilli and for antibiotic-resistant *Escherichia coli* were determined in samples of the Dutch urban population. Of the 741 people studied, 64 were under 1 year old (infants), 53 were 1–5 years old, and there were approximately 200 in each of the age-ranges 6–17 years, 18–49 years and 50–80 years. Carriage rates of *E. coli* were similar (87–93%) in all age groups, but *Klebsiella* and *Enterobacter* species were found more often in specimens from infants and young children than in those from older people. *E. coli* strains resistant to tetracycline, ampicillin, or sulphamethoxazole or to any one or more of them were detected in 42%, 26%, 46% and 66% respectively of the specimens found to contain *E. coli*. The corresponding figures for the finding of *E. coli* populations that were predominantly resistant to tetracycline, ampicillin or sulphamethoxazole were 12%, 6% and 20%. The frequency of resistance to any of these drugs was not related to age or sex of the subjects. All *E. coli* isolates were sensitive to gentamicin. Among the 577 subjects aged 6–80 years from whose samples *E. coli* was isolated were 19 who had taken antibacterial drugs in the previous 30 days and 11 who were involved in cattle farming; carriage rates for tetracycline-resistant and sulphonamide-resistant *E. coli* were significantly higher among these 30 than in the other 547.

INTRODUCTION

The alimentary tract is the normal habitat of enterobacteria and *Pseudomonas* species. It is the main source from which infections with these organisms originate. In general practice these are usually urinary tract infections. Intra-abdominal infections, wound sepsis and pneumonia occur mainly in hospital. If antibiotic-resistant gram-negative bacilli colonise the alimentary tract, they may be responsible for such infections (Brumfitt *et al.*, 1971; Grüneberg and Shaw, 1976; Cooke *et al.*, 1979, 1980). There is much concern about the emergence of resistant enterobacterial strains

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in the hospital environment, but little is known of their prevalence in the community. We have tried to define the present level of resistant faecal flora by an epidemiological survey in two urban communities in The Netherlands. The results are discussed in the light of comparable surveys in Great Britain and The Netherlands.

MATERIALS AND METHODS

Population samples. This study was part of a large epidemiological survey in a Dutch town of c. 60 000 inhabitants (Hofman and Valkenburg, 1980). Between 1975 and 1978 invitations to participate in that survey were issued to 13 500 people > 6 years old in two districts of the town, and 10 500 (78%), belonging to 4600 households, responded. By random selection 917 of these, all from different households, were chosen for the present survey in such a way as to give roughly equal numbers of children (6–17 years), young adults (18–49 years) and older people (≥ 50 years). For each of these we had information about (1) the use of antibacterial drugs within the 30 days before the faecal sampling, (2) hospital discharge within the 3 months before the survey, and (3) connections with cattle breeding. The investigations described here were performed in a 26-month period between September 1978 and November 1980, by which time some of the 917 people were no longer available (see later). An additional study population of children < 6 years old was obtained from an infant and child welfare clinic in another town, comparable to the first town in size and setting but c. 10 km from it and nearer to the laboratory. Consecutive mothers bringing their children to this clinic between April and November 1980 were asked to allow us to examine faecal samples from their children; all cooperated. Mothers and children visiting a child welfare clinic are to some extent self-selected, but in The Netherlands the attendance rate is > 90% for children under one year of age and approx. 75% for children above that age.

Faecal samples. One faecal sample was collected from each subject by swabbing a freshly passed stool. Swabs were stored in Amies's transport medium for no more than 24 h. Each swab was transferred to a tube containing 10 ml of 0.9% NaCl, and dilutions of 10^{-3} – 10^{-5} were made. Standard drops (0.05 ml) of these dilutions were placed on MacConkey agar without antibiotic, MacConkey agar with tetracycline 30 μ g/ml or ampicillin 40 μ g/ml, DST Agar (Oxoid) with 0.5% lysed horse red cells and sulphamethoxazole 100 μ g/ml, and Iso-Sensitest Agar (Oxoid) with gentamicin 10 μ g/ml. Colony counts were made after incubation overnight at 37°C. From the drug-free medium as well as from the drug-containing agar one colony of each morphologically distinct type was selected and identified by the methods of Cowan (1974). If colonies looked similar, they were assumed to be of the same strain. Tetracycline- and ampicillin-containing plates were used for all samples, but for technical reasons sulphamethoxazole- and gentamicin-containing plates were not introduced until the third and tenth month of the survey respectively. Therefore, only 83% of the samples from people aged 6–80 years were examined for the presence of sulphamethoxazole-resistant organisms and only 26% for gentamicin-resistant organisms. All specimens obtained from children < 6 years old were tested with the four drugs.

RESULTS

Participants. Samples were obtained from 64 welfare clinic children < 1 year old (infants) and 53 children aged 1–5 years. Of the infants, 41 (64%) had been born in hospital, 15 (23%) in an out-patient clinic and eight (13%) at home. Four were breast-fed. Of the 917 people > 6 years old invited to participate, 114 had moved out of town or had died before the start of this study, and 179 (22%) of the remaining 803 failed to participate (19%, 26%, 21% of those in the three age groups 6–17, 18–49 and 50–80 years). This left 624 participants > 6 years old, fairly evenly divided between the three age groups (see table I). Of these, 23 (4%) had used antibacterial drugs in the

TABLE I

Numbers of faecal samples from people in different age groups that yielded aerobic gram-negative bacilli

Species	Number (percentage) of subjects from whom the given species was isolated in age groups					
	< 1 (n = 64)	1-5 (n = 53)	6-17 (n = 193)	18-49 (n = 221)	50-80 (n = 210)	total (n = 741)
All aerobic gram-negative bacilli	61 (95)	50 (94)	181 (94)	207 (94)	196 (93)	695 (94)
<i>Escherichia coli</i>	57 (89)	46 (87)	178 (92)	205 (93)	194 (92)	680 (92)
<i>Klebsiella</i> spp.	46 (72)	16 (30)	23 (12)	18 (8)	26 (12)	129 (17)
<i>Enterobacter</i> spp.	15 (23)	9 (17)	17 (9)	12 (5)	13 (6)	66 (9)
<i>Proteus mirabilis</i>	1 (2)	0	0	2 (1)	3 (1)	6 (1)
<i>Proteus</i> , indole-positive	3 (5)	1 (2)	0	0	0	4 (1)
<i>Pseudomonas</i> spp.	0	0	3 (2)	4 (2)	6 (3)	13 (2)

previous 30 days and 11 (2%) were involved in cattle farming. Nine had been discharged from hospital recently.

Distribution of bacterial species in faeces. Table I shows the frequency of isolation of gram-negative aerobic bacilli from the faeces of people in different age groups. In all groups, *E. coli* was found in 87-92% of the faecal samples. *Klebsiella* and *Enterobacter* species were found more often in samples from those < 6 years old than in the rest ($p < 0.001$). *Klebsiella* species were isolated from eight out of 12 samples from breast-fed infants—the four mentioned above and eight others not included in the rest of the study. This isolation rate of 67% is similar to that for all of the infants (72%).

Prevalence of drug-resistant E. coli. Table II shows the prevalence of resistant *E. coli* in the five age groups. Calculations were confined to faecal samples that contained *E. coli*, and a sample was classified as containing *E. coli* resistant to a drug if there was any growth of that species on the plate containing that drug. No relationship was found between age or sex and carriage of drug-resistant *E. coli*. The overall prevalence of resistant *E. coli* strains was 42% for tetracycline, 26% for ampicillin, 46% for sulphamethoxazole, and 66% for any one or more of these. Gentamicin resistance was not encountered in this study.

Predominance of drug-resistant E. coli. Table III shows the numbers of samples

TABLE II

Antibiotic-resistant faecal *E. coli* in samples from different age groups

Antibacterial drug	Number (percentage) of subjects from whom resistant <i>E. coli</i> strains were isolated in age groups					
	< 1 (n = 57)	1-5 (n = 46)	6-17 (n = 178)	18-49 (n = 205)	50-80 (n = 194)	total (n = 680)
tetracycline	19 (33)	26 (57)	76 (43)	90 (44)	74 (38)	285 (42)
ampicillin	18 (32)	17 (37)	42 (24)	49 (24)	51 (26)	177 (26)
sulphamethoxazole*	29 (51)	26 (57)	79 (51)	86 (48)	56 (36)	276 (46)
one or more of the three drugs*	40 (70)	34 (74)	106 (68)	116 (64)	96 (62)	392 (66)

* The percentages shown in these lines are of the numbers tested for sulphamethoxazole resistance in each age group—< 1 year 57, 1-5 years 46, 6-17 years 156, 18-49 years 181, 50-80 years 154, total 594.

TABLE III
Predominance of antibiotic-resistant E. coli in samples from different age groups

Antibacterial drug	Number (percentage) of subjects in whom <i>E. coli</i> strains resistant to the given drug were predominant in age groups					
	< 1 (n = 57)	1-5 (n = 46)	6-17 (n = 178)	18-49 (n = 205)	50-80 (n = 194)	total (n = 680)
tetracycline	6 (11)	10 (22)	22 (12)	24 (12)	21 (11)	83 (12)
ampicillin	10 (18)	4 (7)	8 (4)	9 (4)	11 (6)	42 (6)
sulphamethoxazole*	9 (16)	14 (30)	30 (19)	36 (20)	27 (18)	116 (20)

* see footnote to table II.

from which colony counts of *E. coli* on the drug-containing agar were at least 50% of those on the control drug-free medium. No differences in frequency of such predominance between the five age-groups were found for tetracycline or sulphamethoxazole, but predominance of ampicillin-resistant *E. coli* was commoner in samples from infants (18%) than in those from the other age groups (4-7%; $p < 0.01$).

Prevalence of drug-resistant E. coli in high-risk groups. As shown in table IV, both tetracycline resistance and sulphamethoxazole resistance were more common among *E. coli* strains from those who had recently taken antibacterial drugs or were involved in cattle farming than among those from the rest of people > 6 years old. *E. coli* was isolated from only four of the nine samples from people who had recently been in hospital.

TABLE IV
Antibiotic-resistant faecal E. coli from samples from high-risk groups and from the rest of those aged 6-80 years

Antibacterial agent	Number (percentage) of subjects from whom <i>E. coli</i> strains resistant to the given drug were isolated in		
	High-risk groups		
	Antibacterial treatment (n = 19)	Cattle farming (n = 11)	Others aged 6-80 years (n = 547)
tetracycline	12 (63)	9 (82)	219 (40)
ampicillin	3 (16)	5 (45)	134 (24)
sulphamethoxazole*	11 (73)	6 (55)	204 (44)

* The percentages shown are of the numbers tested for sulphamethoxazole resistance in each group—"antibacterial" group 15, "cattle farming" group 11, "others" 465.

DISCUSSION

A number of earlier studies of the incidence of resistant intestinal flora used methods comparable with our own. Davis and Anandan (1970) showed that antibiotic-resistant coliforms were significantly less common in an isolated ethnic

TABLE V
Prevalence of faecal aerobic gram-negative bacilli resistant to tetracycline, ampicillin and sulphonamides in various population studies using comparable methods

Author (year of study)	Place	Population studied	Number of samples examined	Species studied	Percentage of samples containing strains resistant to			Drugs tested
					T	A	S	
Datta <i>et al.</i> , (1971)	London (UK)	Patients in general practice without antibiotic treatment	53	<i>E. coli</i>	17	2	23	T (10), A (25), S (100)
Linton <i>et al.</i> , (1972)	Bristol (UK)	1. Healthy adults; families of children visiting a welfare centre 2. Healthy children visiting a welfare centre 2. Healthy adults in a rural community 4. Healthy children in a rural community	97	coliforms	19	13	...	
		2. Healthy children visiting a welfare centre	96	"	24	36	...	T (25), A (25)
		2. Healthy adults in a rural community	43	"	47	42	...	
		4. Healthy children in a rural community	28	"	68	57	...	
Guinée <i>et al.</i> , (1970)	Utrecht (Netherlands)	Office employees;	86	<i>E. coli</i>	38	...	...	T (30)
Degener <i>et al.</i> , (1978-1980)	Zoetermeer (Netherlands)	General population, 6-80 years	624	<i>E. coli</i>	35-41	22-24	27-41	T (30), A (40), S (100)
(this paper)*	Overschic (Netherlands)	Healthy children visiting a welfare centre, 0-5 years	117	<i>E. coli</i>	30-49	28-32	45-49	

T=tetracycline, A=ampicillin, S=sulphonamide. ()=drug concentration in plate in $\mu\text{g/ml}$.

* For comparability, the percentage figures are based upon the total numbers of samples from each group.

group in Borneo than in Western populations. Unfortunately no figures of antibiotic resistance are available from the pre-antibiotic era in Western Europe. Since the introduction of antibiotics, resistance of intestinal flora has become a common finding both in normal people and in patients not treated with antibiotics. Datta *et al.* (1971) studied patients who consulted a general practitioner in London and who were not using antibiotics at the time. Her results are listed in table V. Guinée, Ugueto and van Leeuwen (1970), using an agar medium containing tetracycline, observed resistant *E. coli* in samples from 38% of a group of office employees in Utrecht, a percentage similar to our findings. Linton *et al.* (1972), in Bristol, isolated more resistant strains from children than from adults, and more from the rural than from the urban population; but they did not differentiate between the various genera of enterobacteria. Our study revealed a significantly higher prevalence of *Klebsiella* and *Enterobacter* species in infants. Moreover, we found a higher frequency of ampicillin-resistant *E. coli* strains in the stools of infants than in older children and adults. In general our results, although obtained in urban populations, most closely resemble Linton's findings in the rural population group.

Our most remarkable finding was the lack of correlation between age and the presence of drug-resistant faecal flora, although the age groups must have differed in their exposure to therapeutic antibiotics. The origins of drug-resistant human faecal flora are still obscure. Large scale use of antibiotics in livestock farming has led to multiresistant flora in cattle (Anderson, 1965; Smith, 1968). These resistant strains are readily transmitted to human subjects (Levy, FitzGerald and Macone, 1976). Person-to-person spread can also occur (Petrocheilou, Richmond and Bennet, 1977). Other means of transmission are by faecal contamination of surface waters (Guinée, Sekhuis and Ugueto, 1974) and food products (Shooter *et al.*, 1971; Casewell and Phillips, 1978; Cooke *et al.*, 1979). Finally the use of antibiotics in medical practice can also contribute to carriage of resistant strains (Richmond and Linton, 1980). During our study period general practitioners in the town from which we drew our older children and adults provided, in one year, 33 460 sick-fund patients with a total of 18 000 prescriptions for antibiotics. These 33 000 patients represented approximately 11 000 families. Therefore, on average in one year in each family, one or two family members received a full course of antibiotics that might have induced the circulation of resistant faecal strains. Tetracyclines were included in 20% of these prescriptions. If we assume that excretion of tetracycline-resistant strains lasted for 2 weeks after each prescription, this represents an annual total of 50 400 patient-days or an average of 4.5 days of excretion per household. This seems insufficient alone to account for our finding that 42% of *E. coli* strains were tetracycline-resistant.

Another interesting finding was that the prevalence of *Klebsiella* and *Enterobacter* species, which are commonly drug-resistant, was highest among infants and decreased with age. Feeney, Cooke and Shinebaum (1980) reported a higher incidence of resistant flora, including *Klebsiella* species, in bottle-fed than in breast-fed children. We could not confirm this, but our number of breast-fed infants was small.

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