

# *Escherichia coli* with Resistance Factors in Vegetarians, Babies, and Nonvegetarians

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The prevalence of *Escherichia coli* carrying resistance factors (R factors) was examined in meat-consuming individuals and in those not consuming meat (vegetarians and babies below the age of 6 months). Assuming that the transport of resistant *E. coli* from animals through meat and meat products to the human consumer is most important, with regard to the incidence of resistant *E. coli* in man, we expected a significant difference in the proportions of people with resistant *E. coli* between the two groups. However, the percentage with resistant *E. coli* was larger in the group of vegetarians and babies than in the group of meat-eating individuals.

In many countries, small amounts of antibiotics (penicillin, streptomycin, tetracycline) are added to feeds to promote the growth of the animals. These amounts usually do not exceed 20 µg/g. Larger amounts (up to 100 µg/g) are used to prevent certain diseases in particular groups of animals, for instance, fattening calves. The nonmedical use of antibiotics has created a reservoir of *Enterobacteriaceae*, mainly *E. coli*, which are resistant to one or more of the drugs used (18; P.A.M. Guinée, Ph.D. Thesis, Univ. of Utrecht, 1963; M.P. Starr and D.M. Reynolds, *Bacteriol. Proc.*, p. 15, 1951).

This resistance is often transferable due to the presence of functional resistance factors or R factors (2, 19, 20).

R factors may be exchanged between all genera of the *Enterobacteriaceae*. When R factor-carrying strains of animal origin are ingested by man, the resistance may be transferred to pathogenic microorganisms (*Salmonella*, *Shigella*). Antibiotic treatment of infections caused by such resistant bacteria becomes ineffectual (12, 21). Therefore, it is important to evaluate the significance of animal R<sup>+</sup> *E. coli* strains for human health. However, this evaluation is complicated by the fact that *E. coli* in general, though of fecal origin, has a ubiquitous prevalence. For the Netherlands, the following rough estimation might be explanatory.

Each member of the human population (13 × 10<sup>6</sup>) excretes daily an average of 200 g of feces, harboring 10<sup>5</sup> *E. coli* per gram, giving a total daily production of 26 × 10<sup>13</sup> *E. coli*.

The livestock population consists mainly of

4 × 10<sup>6</sup> cattle, 40 × 10<sup>6</sup> poultry, and 5 × 10<sup>6</sup> pigs (13). Assuming that the average daily feces production per animal for cattle is 200 g, for poultry 20 g, and for pigs 100 g, the total daily production by animals amounts to 21 × 10<sup>13</sup> *E. coli*. This estimation indicates that roughly equal numbers of *E. coli* are voided by the human and animal populations. These bacteria are partly collected in the sewage systems and led into the sewage plants where their number is reduced by a factor of 10 to 100 (Kampelmacher and Van Noorle-Jansen, *in press*). The effluent water is ultimately carried off into the surface waters. It is not known to what extent these bacteria are resistant and whether they originate from humans or animals. Occurrence of resistant bacteria depends on whether such bacteria are selected. Selection of resistant *E. coli* is a consequence not only of the nonmedical use but certainly also of the medical use of antibiotics, particularly in hospitals (4, 14, 16).

It has been suggested (1) that livestock is important for man as a source of bacteria with transferable drug resistance. The importance of this source cannot be estimated in retrospect. It only is possible to ascertain whether foods of animal origin (meat and meat products) represent an important source of infection with R<sup>+</sup> *E. coli*. During the process of slaughtering, fecal contamination of the carcasses cannot be prevented. It is, therefore, quite possible that *E. coli* is transmitted to the human consumer through meat and meat products (17), in the same way as *Salmonella* organisms.

In the present investigation, we tested whether

there is any difference in the prevalence of resistant, possibly R<sup>+</sup>, *E. coli* between meat-consuming individuals and those not consuming meat. The latter group consisted of vegetarians who do not consume meat but usually do consume other products of animal origin, such as milk, cheese, and eggs. Among them was a small group of vegans who abstain from all products of animal origin. Also, a number of babies born at home and below the age of 6 months were investigated since their food is pasteurized and intrinsically free from *Enterobacteriaceae*.

### MATERIALS AND METHODS

Fecal samples from persons belonging to one of the following classes were investigated: (i) adult non-vegetarians (military kitchen personnel with no personal details available and office employees who had no contacts with animals and had not been hospitalized or treated with antibiotics during the previous 3 months), and (ii) adult vegetarians and babies below the age of 6 months (mainly single vegetarians and only a few vegetarian families who had not been hospitalized or treated with antibiotics during the previous 3 months and babies below the age of 6 months who had been born at home and were living at home).

**Examination of feces.** Rectal swabs were examined by means of technique A. Fecal specimens were also examined with technique B. Technique A consisted of rubbing the cotton swab soiled with feces over the surface of two deoxycholate-lactose-agar plates (DC; P. A. M. Guinée, Ph.D. Thesis, Univ. of Utrecht, 1963) and a peptone-free nutrient agar plate. One of the DC plates was to show whether the specimen contained about normal numbers of *E. coli* bacteria; in all instances, confluent growth was obtained. Discs containing different drugs were placed on the surfaces of the two other plates. Amounts were: streptomycin, 200 µg; tetracycline, 50 µg; chloramphenicol, 50 µg; nitrofurantoin, 200 µg; kanamycin, 50 µg; and ampicillin, 50 µg.

The plates were read after overnight incubation at 37 C. Colonies growing within the inhibition zones were purified on DC plates supplemented with the drug concerned and were then identified. If these colonies (strains) belonged to the *Enterobacteriaceae* or *Pseudomonas* (as was nearly always the case), their sensitivity to the above-mentioned drugs was tested. If a strain was identified as *E. coli*, its O and K antigens were identified with the usual methods (5, 11). We used the standard O sera 1-149 and, in addition, the sera OX<sub>1</sub>-OX<sub>13</sub> (6), OX<sub>14</sub>-OX<sub>44</sub> (7), and O<sub>200</sub>-O<sub>212</sub> (P. A. M. Guinée, Ph.D. Thesis, Univ. of Utrecht, 1963).

Technique B was used to measure the proportion of tetracycline-resistant *E. coli* in fecal specimens. About 1 g of feces was diluted to 10<sup>-7</sup> with saline. A 0.1-ml amount of these dilutions was spread on DC with and without 30 µg of tetracycline per ml (DCT). After overnight incubation at 37 C, the number of colonies on suitable plates was counted, and the proportion of

tetracycline-resistant *E. coli* was determined. *E. coli* colonies, with a maximum of 10, and colonies belonging to other genera of the *Enterobacteriaceae*, with a maximum of 2, were picked from a DCT and further investigated as described above.

**Transfer of resistance.** Two strains of each *E. coli* serotype with identical antibiogram and, when present, two strains per genus of other *Enterobacteriaceae* were selected per specimen of feces. They were tested for resistance transfer to *E. coli* K-12 F<sup>-</sup>, since this strain is considered to be the best recipient for R factors (21). We used a nalidixic acid-resistant (Nal<sup>r</sup>) mutant of *E. coli* K-12 F<sup>-</sup>, strain W3110, to facilitate the selection of R<sup>+</sup> recipient cells from the conjugation mixture. In addition, each presumptive donor strain was crossed with a strain of *Salmonella typhimurium* (strain 12) and *S. panama* (strain S. p47; reference 8). To test the transfer of resistance, 5 ml of overnight broth culture of donor and recipient were mixed. The mixture was incubated at 37 C overnight. When *E. coli* K-12 Nal<sup>r</sup> was used as recipient, a loopful of the mating mixture was streaked on a series of DC plates containing 50 µg of nalidixic acid per ml as well as each separate drug to which the donor culture to be tested was resistant. When *Salmonella* was the recipient, we selected on similarly supplemented Brilliant Green-phenol red-agar prepared in this laboratory (3). Colonies growing on the selective plates were purified and identified, and their drug sensitivity was tested by the disc method; if the recipient was *S. panama*, they were phage-typed (9). In this way, we tested only for complete, transferable R factors and not for extra-chromosomal resistance genes without a transfer factor.

### RESULTS

**Adult nonvegetarians:military kitchen personnel.** Although we had no information regarding possible antibiotic treatment or hospitalization, material from military kitchen personnel was included in this investigation to make possible a comparison with results obtained in 1961 with material of a similar military group. One specimen per person from 400 persons was investigated with techniques A and B. The investigation was done with four groups of 100 each during the autumn of 1968, and during winter, spring, and summer of 1969. The numbers of specimens yielding resistant *E. coli* were 50, 32, 38, and 59, respectively. The total number of specimens yielding resistant *E. coli* was 179 (45%); those with R factor-carrying *E. coli* totalled 112 (28%). No enterobacterium other than *E. coli* transferred its resistance. The results, together with those obtained in other groups, are presented in Table 1. To enable comparison, Table 1 has been marked off to the number of specimens tested per person and the techniques employed, since these are different in the various groups. Also in Table 1 are presented the results of an in-

TABLE 1. Incidence of resistant and R<sup>+</sup> *Escherichia coli* in nonvegetarians, vegetarians, and babies below the age of 6 months

Subjects	No. of people investigated	Technique A, 1 specimen <sup>a</sup>		Technique A and B, 1 specimen <sup>a</sup>		Technique A, 2 specimens <sup>a</sup>		Technique A and B, 2 specimens <sup>a</sup>	
		Resistant <i>E. coli</i>	R factor	Resistant <i>E. coli</i>	R factor	Resistant <i>E. coli</i>	R factor	Resistant <i>E. coli</i>	R factor
Military kitchen personnel (1960-1961).....	100			19					
Military kitchen personnel (1968-1969).....	400	38	25(18) <sup>b</sup>	45	28(21)				
Office employees (1968-1969).....	86	23	9(8)	31	16(16)	34	13(12)	38	22(21)
Vegetarians (1968-1969)....	77	36	24(9)	44	26(10)	49	35(14)	52	36(16)
Babies below the age of 6 months living at home...	87	32	18(8)			49	31(16)		

<sup>a</sup> Values indicate per cent with resistant *E. coli* or R factors.

<sup>b</sup> Twenty five per cent yielded one or more *E. coli* strains which transferred their resistance to *E. coli* K-12 F<sup>-</sup> W 3110 NaI<sup>r</sup>; 18% yielded strains which transferred their resistance also to *S. typhimurium*, *S. panama*, or both.

vestigation carried out in 1961 of 100 military personnel, however, without resistance transfer experiments. We found 19 positive specimens in 1961; the proportion of positive samples in 1968-1969 was considerably higher.

**Nonvegetarians: office employees.** The 86 people examined possessed no pet animals and had no contact with animals whatsoever; they had not been treated with antibiotics nor had they been in the hospital during the previous 3 months. All volunteers sent two specimens each, with an interval of 3 to 8 weeks, which were examined with techniques A and B: 33 (38%) yielded resistant *E. coli*; 19 (22%) yielded one or more *E. coli* strains with transferable resistance (Table 1). No other enterobacterium with functional R factors was found. The investigation took place from September 1968 until May 1969; no seasonal influences could be observed.

**Vegetarians: adults.** The majority of them were lacto-vegetarians; a few were vegans, i.e., they abstained from all food of animal origin. Most of them were single; only a few vegetarian families were included. Seventy-seven persons sent two specimens each, which were tested with techniques A and B. Forty people (52%) yielded resistant *E. coli*; 27 persons harbored *E. coli*, and one person *Proteus mirabilis*, with a functional R factor. The percentage of people carrying transferable R factors was 36 (Table 1). The investigation was carried out from January to June 1969; no seasonal influences could be observed. The volunteers informed us that none of them had undergone hospitalization or antibiotic treatment during the 3 preceding months.

Some of them possessed pet animals; we found no association between the presence of pets on the one hand and the incidence of resistant *E. coli* in its owners on the other (Table 2).

**Vegetarians: babies under the age of 6 months.** Two rectal swabs per baby were obtained from 87 babies living at home, with an interval of 4 to 12 weeks between specimens, and tested with technique A; forty-three (49%) yielded resistant *E. coli*; 27 (31%) yielded *E. coli* carrying a functional R factor (Table 1). From many children, resistant enterobacteria other than *E. coli* were isolated; none of these strains transferred its resistance.

None of these babies had been living in a nursery or a hospital. Five were treated with ampicillin during the period of observation. Three of these yielded no resistant *E. coli*; two excreted ampicillin-resistant *E. coli*, but this resistance was not transferable. Ten of the 87 babies were breastfed; eight of them had no resistant *E. coli*, and two yielded resistant *E. coli* but the resistance was not transferable to *E. coli* K-12 F<sup>-</sup>.

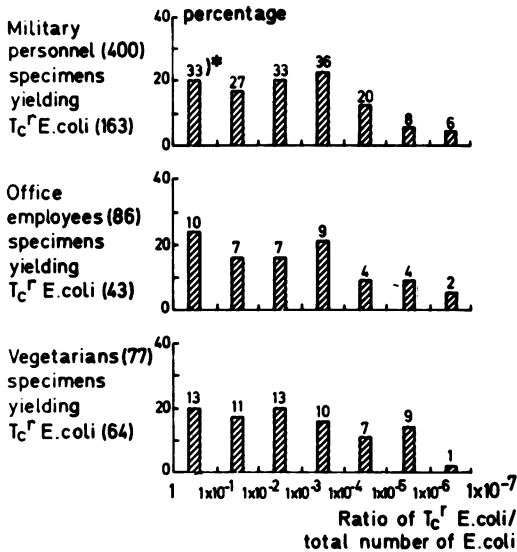
**Ratio of Tc<sup>r</sup> *E. coli* versus total number of *E. coli*.** Employment of technique B permitted only a semiquantitative estimation of the tetracycline-resistant (Tc<sup>r</sup>) *E. coli* versus the total number of *E. coli*. The results are shown in Fig. 1.

**Characteristics of the isolated R factors.** Many specimens, particularly those obtained from babies, yielded resistant enterobacteria other than *E. coli*. Only a few of these strains, even when resistant to four or more drugs, transferred

their resistance. Two R factor-carrying strains were considered identical if the R factors concerned were carried by *E. coli* strains which had the same O and K antigen and if R factor transfer to *S. panama* 47 resulted in the same changes in the phage pattern of the recipient strain (10).

TABLE 2. Incidence of resistant *Escherichia coli* in vegetarians in relation to the presence of pet animals

Determination	Pet animals present	Pet animals not present	Total
No resistant <i>E. coli</i> . . . . .	11	26	37
Resistant <i>E. coli</i> . . . . .	7	33	40
Total . . . . .	18	59	77



\* 33: actual number of specimens

FIG. 1. Number of specimens yielding a given ratio of Tc<sup>r</sup> *E. coli* per total number of *E. coli*.

Identical R<sup>+</sup> strains were encountered sporadically. Of all the resistant *E. coli* strains isolated, 62% transferred one or more of their resistance characters, depending on the method of selection. We found no transferable nitrofurantoin resistance, kanamycin resistance was sporadically transferable, and tetracycline resistance was most frequently transferable (Table 3).

DISCUSSION

If transport of resistant *E. coli* from animals to the human consumer through meat and meat products had been the most important mechanism in regard to the incidence of resistant *E. coli* in man, we would have found a lower percentage with resistant *E. coli* in vegetarians than in non-vegetarians. Table 1 indicates the contrary: the percentage with resistant *E. coli* in the group of vegetarians is larger than in the group of meat-eating office employees. The percentage with resistant *E. coli* in the group of babies was about equal to that of vegetarians and higher than in the group of nonvegetarian office personnel. The percentages of babies with resistant or R<sup>+</sup> *E. coli*, respectively, were lower than those reported by Moorhouse (15)–71 and 68%, but not all children in the latter study were below the age of 6 months. There was no association between the occurrence of resistant *E. coli* and antibacterial therapy. One must assume that these babies acquired the resistant organisms from their parents or other adults at home. The strains might still have originated from food of animal origin. However, the presence of resistant *E. coli* in vegetarians cannot be explained in this way. Foodstuffs of animal origin consumed by them (milk, cheese, eggs) are generally free from *Enterobacteriaceae*. Also, the presence of pet animals does not explain the presence of resistant *E. coli*.

The data presented here do not permit a conclusion about the importance of foods of

TABLE 3. Transferable resistance characters of R<sup>+</sup> strains isolated from nonvegetarians, vegetarians, and babies

Subjects	No. of R factors investigated	Resistance determinants transferable to <i>E. coli</i> K-12 F <sup>-</sup> W 3110 Nal <sup>r</sup>				
		Streptomycin	Tetracycline	Chloramphenicol	Kanamycin	Ampicillin
Military kitchen personnel (1968–1969) . . . . .	124	82	121	27	4	17
Office employees (1968–1969) . . . . .	20	8	19	2	0	0
Vegetarians (1968–1969) . . . . .	32	15	29	10	7	7
Babies below the age of 6 months . . . . .	28	22	9	7	6	7
Total . . . . .	204	127	178	46	17	31

animal origin as a source of infection with resistant *E. coli* in man. In our material, other factors apparently prevail over a possible effect of meat and meat products.

Further work should determine to what extent humans and animals are responsible for the environmental pollution and whether the ecology of resistant *E. coli* can be influenced by such measures as restriction of the use of antibiotics and improvement of sewage purification.

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