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The Homogeneity of the Faecal Coliform Flora of Normal School-Girls, Characterized by Serological and Biochemical Properties

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Abstract. The homogeneity of fecal coliform flora in 52 schoolgirls was studied by serotyping and biotyping 10 randomly selected colonies in one fecal culture from each child. Ninety-eight clones were identified and of these 52 were dominant and 46 were minor strains. The probability of including at least one isolate of the dominant clone in a small random sample of colonies was calculated to be 86% for one colony, 94% for 2, 97% for 3, 99% for 4, and 99.3% for 5 randomly selected colonies.

Introduction

The ecology of *Escherichia coli* in the human gut has bearing on many important clinical problems. These include aspects of pathogenesis and treatment of urinary tract infection (UTI), as urinary bacteria commonly originate in the fecal flora [20] and re-infecting strains often reflect the impact of the treatment given for the original UTI on fecal flora [14].

Human fecal coliform flora is often highly complex. Usually one strain can be found in a greater number of colonies than any other, and is then characterized as the dominant strain [2,19]. Numerous minor strains may coexist, and it is virtually impossible to define with certainty the total content of *E. coli* strains in a fecal sample [2,19]. Most O groups present may, however, be identified by an examination of 5 colonies [19]. Selection of colonies on the grounds of morphology on a suitable medium such as bromothymol blue lactose agar is of great help in establishing serologic heterogeneity of the fecal flora [17]. For statistical evaluation, however, strict random selection of colonies is a prerequisite [18].

In studies of fecal coliform flora, information may be needed on the size of a sample of isolates from a specimen reasonably sure to include at least one isolate of a

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dominant strain [10]. For this purpose the homogeneity of the fecal coliform flora in a group of healthy schoolgirls was studied, using serological and biochemical markers.

Material and Methods

Probands. Fifty-two healthy, abacteriuric girls aged 7–16 years, from two schools in Gothenburg, each delivered one rectal swab specimen. The girls had not taken antibiotics during the previous year.

Bacteriological procedures have largely been described previously [11]. Four bromothymol blue lactose agar plates were used for ten separate inoculations with each swab. The last colony growing from each culture was subcultured and typed.

Biotyping of *E. coli* and coliforms was performed as described by Bettelheim and Taylor [1], classifying the pattern of fermentation of 16 carbohydrate substances. In a simplified *E. coli* O-grouping, antisera against 69 O groups were used. K-antigen typing was performed via serum agar technique [7] using rabbit antisera against 16 K groups. Active motility was demonstrated in motility indole tubes [4]. Haemolysis was assessed in agar plates with 5% washed horse erythrocytes. Antibiotic sensitivity was tested using a disc diffusion method [5].

Colonies identical in these characteristics and isolated from the same specimen were considered to be derived from the same original strain. They were thus grouped together as one clone, as that term has been used by Ørskov and Biering Sørensen [18]. In accordance with findings of Bettelheim and co-workers [1,3], we also considered closely related isolates from the same specimen to be derived from the same original strain and thus to belong to one clone displaying some degree of antigenic and/or biochemical variation [8]. Only probable loss variations were accepted.

Sensitivity to the bactericidal effect of normal human serum (SBS) was tested as described [6,16].

Statistics. Distributions were compared by means of Fisher's permutation test [15]. Two tailed significance tests were used and 2p less than 0.05 was considered significant.

The probability of a number of random isolates not including at least one colony belonging to the clone dominant in a certain specimen was calculated as follows: The proportion of the dominant clone is a random variable and is denoted u. The distribution of u in a population is f. If u were known for a certain specimen, the probability for the dominant clone to be missing among k independent isolates would be $(1-u)^k$. When a random specimen is considered, the probability is

$$\int_0^1 (1-u)^k f(u)\, du$$

The integral is approximated by

$$\Sigma\, (1-u)^k f^*(u)$$

where f^* is the observed frequency of u. This frequency was estimated in 52 specimens from each of which 10 isolates had been taken. Although founded on a rough estimate of the true distribution of u,

$$\sum_{i=1}^{10} (1 - \tfrac{i}{10})^k f^* (\tfrac{i}{10})$$

is an acceptable estimate of the probability of missing the dominant strain.

Results

Number of Clones. The 520 isolates were grouped into 98 clones altogether, as shown in Table 1. Ninety-one clones were *E. coli* and seven were other coliforms. In each specimen one clone made up a greater number of isolates than any other. The dominant clone was represented by eight or more isolates in 40 specimens, by six or seven in 5, and by five or four in 7 specimens.

Serotyping and Biotyping. Altogether 56 *E. coli* clones were 0 typable with the sera used, and 17 were K typable, including 14 K1 strains. Among the 59 clones typable as to 0 or K antigen or both, 31 serotypes and 33 biotypes were found. Each serotype was connected with up to three biotypes and each biotype was connected with up to four serotypes. Colonies of the same 0 type found in one specimen were always of the same biotype. In 6 cases, 0-typable and Sa (spontaneously agglutinable) colonies in the same specimen were of the same biotype and were classed as one 0-typable clone. In no case did ONa (not agglutinable in 0 sera used) and Sa colonies in the same specimen belong to the same biotype. In five specimens ONa colonies of two distinctly different biotypes (that is giving diverging reactions in 3 to 5 of the 16 carbohydrate substrates) were found. In one specimen one of the ONa clones was K-typable. One specimen was shown to contain three different ONa clones. In one specimen two clones were found to belong to the same biotype. They were one 03 and one ONa K non-typable clone.

Variation within Clones. Sixty-six *E. coli* clones were represented by two or more isolates (Table 1). Variation in one or more of the characteristics used for the definition of clones was found in 14 of them (21.2%). As recorded above, smooth to rough variation was found in six clones. K antigen loss variation was noted in one

Table 1. Number of isolates per clone in 52 rectal swab cultures (ten colonies were picked at random from each culture)

	Number of isolates per clone										
Bacterium	10	9	8	7	6	5	4	3	2	1	Total
Escherichia coli											
0-groupable	18	8	2	1		3	5	1	6	12	56
Not agglutinable	4	2	1	1	1	1	1	2	4	11	28
Spontaneously agglutinating	4			1						2	7
Coliforms			1		1			1		4	7
Total	26	10	4	3	2	4	6	4	10	29	98

Clones represented by 5 isolates dominated in their respective specimens, as did 3 of the clones represented by 4 isolates. Thus one dominant strain was found in each of the 52 specimens

018 K1 clone. Only six of the ten isolates of this clone were K1 positive at the first testing. At a second testing one year later, four of these isolates were K1-positive and, in addition, three others. Variability in motility was noted in two clones, including one 028—Sa clone. Variability in the fermentation of one carbohydrate was found in six clones, including variability in the fermentation of salicin in one 012—Sa clone. In one 06 clone only one out of nine isolates was hemolytic.

In 35 of the 66 clones (53%) the SBS-rating was the same for all the isolates. In another 25, 56—90% of the isolates had the same rating. In those 60 clones the dominant SBS-rating was resistant in 41, intermediate in 9, and sensitive in 10, including all the five Sa clones (Table 1).

An Analysis of the Probability of Including the Dominant Strain in a Small Sample of Isolates. On the basis of the proportion of dominant strain found in each specimen, an analysis was made of the probability of including at least one isolate of the strain dominant in a random specimen from the schoolgirls of the present study, when analysing an increasing number of isolates, each taken independently from the same specimen. The probability was found to be 0.86 when one colony was analysed, 0.94 for two, 0.97 for three, 0.99 for four, and 0.993 for five colonies.

Discussion

Variability of E. coli

As recently reviewed by Kauffman [8], *E. coli* comprises numerous groups of related serofermentative types. Biochemical and antigenic loss-variations in *E. coli* are well established phenomena, and may be selected during chronic infection of the urinary tract [1,12,13] and during unfavorable cultural conditions [8]. Also, a certain variability of antigenic and biochemical characteristics may sometimes occur among the descendants of one bacterial strain in the fecal flora, as shown by Bettelheim and coworkers [3]. In the present study, closely related but not completely identical isolates were sometimes found in the same specimen. In cases where one or more of the colonies showed loss of specific 0-agglutinability, K-typability, or motility, and/or fermentation of one carbohydrate, such isolates were regarded as more likely than not to be derived from the same original strain. They were thus classified as belonging to the same clone [18] Some of the variability displayed was probably due to slight variations in the techniques used and not to genetic heterogeneity.

With respect to resistance to the bactericidal effect of normal serum (SBS), we noted a greater tendency toward variation within the clones, only slightly more than half of them (35/66) being homogeneous. In another 25 clones more than half of the colonies could be referred to one of the three possible classes. Such findings raise some doubt as to the reproducibility of the bactericidal test. However, when analysing 231 fecal strains again after one year's storage as nutrient agar stab cultures under paraffin oil, Olling (unpublished) found that 76% of the isolates got concordant ratings at the first and the second testing. Eighty-six per cent of the isolates found to be resistant to the bactericidal effect at the first testing were resistant the second time, too. When analyzing different populations of bacterial strains, such as those from the urine of children with different clinical types of UTI, and from the feces of uninfected

controls, the SBS can be used as one of several parameters for comparing *E. coli* strains [12,13,16], those from chronic UTI tending to be sensitive.

Thus far as SBS is concerned, there seems to be a rather marked tendency towards variation within *E. coli* clones. Seroresistant variants seem to have a selective advantage in the gut and at the establishment of UTI, but their serosensitive counterparts may be more effective survivors in chronic UTI [12,13]. Similar findings have been reported from studies of *Pseudomonas* [6] and *Proteus* [9] infections.

Representativeness of Small Random Samples

Accepting variability within clones as discussed above, 520 enterobacterial isolates from 52 specimens of feces could be referred to 98 clones. In each specimen one clone was found in a greater number of isolates than any other and that clone was classified as dominant. In 40 specimens, at least 8 of the 10 isolates were referred to the same clone, but in three specimens, a clone represented by only 4 isolates had to be classified as dominant (Table 1). The coliform flora of the schoolgirls was found to be fairly homogeneous so often that one single, randomly selected colony would have represented the dominant clone in 86% of the specimens. In a sample of five randomized colonies, the probability of at least one colony of a dominant clone being included would be as great as 99.3%. Reducing the sample size to three colonies reduces the probability only to 97.0%.

Different intestinal milieu may exert varying selective pressures on the *E. coli* flora. Thus, the flora has been shown to be more homogeneous in breast-fed than in bottle-fed infants [18]. In the study by Ørskov and Biering Sørensen [18], 10 randomized colonies were obtained from bromothymol blue lactose agar by picking off colonies crossed by lines drawn on the back of the plates. The colonies were referred to clones on the grounds of full O typing of *E. coli* and biotyping of other coliforms. In the present study ten separate cultures were made in order to obtain independent random selection of ten colonies. A simplified *E. coli* serotyping was supplemented by biotyping.

Although not directly comparable as to the techniques used, the three groups of fecal specimens from bottle-fed infants [18], schoolgirls (present study), and breast-fed infants [18] can be used as examples of different degrees of homogeneity of the fecal coliform flora. A random sample of three colonies independently taken from a culture of infant stool would have included the dominant clone with a probability of 99% for a breast-fed infant. For a bottle-fed one, the probability would have been the same as for the schoolgirls, 97% (Wedel, unpublished).

In studies of *E. coli* ecology in normal fecal flora, a random sample of three colonies will thus be reasonably sure to include at least one isolate of a dominant clone. Three separate cultures can easily be made on one 9 cm diameter plate. The yield of different clones can be considerably extended if the same cultures are also used for selecting colonies with different morphology [17].

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