Antimicrobial Resistance of Fecal Aerobic Gram-Negative Bacilli in Different Age Groups in a Community

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We measured the occurrence of antimicrobial resistance in fecal aerobic gram-negative bacilli by age in community subjects. For none of the eight antimicrobial agents studied were there any statistically significant differences in the carriage rates of resistance in different age groups. Bacterial resistance was common in all age groups, including the children, and occurred for all antimicrobial agents tested.

The normal intestinal microflora is the major source from which common hospital- and community-acquired infections such as urinary tract infections originate (4, 16). Most of these infections are caused by fecal aerobic gram-negative bacilli (20). As the occurrence of bacterial resistance has increased, there has been a growing interest in the identification of the specific reservoirs of bacterial resistance (19).

Studies made in the 1980s highlighted the role of the normal intestinal flora as a reservoir of genes carrying resistance into human pathogens (4, 12). The origins of drug-resistant human fecal flora still remained obscure. However, some studies have suggested that a person’s age and environmental and social networks are related to bacterial resistance and may encourage the survival of bacteria (11, 18).

The current study was designed to determine whether age is an important factor for colonization with resistant fecal aerobic gram-negative bacilli, to determine whether the elderly are colonized by resistant bacilli more often than are children, and to define the present level of resistant fecal flora in different age groups in a community. In addition to analyzing possible connections in bacterial resistance, this study presents data on the distribution of different aerobic gram-negative bacterial species in the samples.

This study was approved by the ethics committees of the Turku City Health Service and the Tampere University Hospital.

Study population. We studied the fecal carriage of antimicrobial resistance in aerobic gram-negative bacilli in a total of 334 urban inhabitants from the cities of Turku and Tampere of southern Finland in 1993 to 1995. The study population consisted of normal ambulatory people. This urban population was composed of students from the Students Health Care Center of Turku (outpatient clinic), persons from the private sector and a geriatric outpatient clinic from the health-care center of Turku, and children from the health-care center of Tampere (outpatient clinic) and the pediatric outpatient clinic from the Tampere University Hospital. For each subject a questionnaire was filled in, specifying the following items: sex, age, residence, employment, and history of antimicrobial therapy at least within the previous 3 months. Inclusion criteria were that none of the subjects had been hospitalized during the 3-month period preceding the sampling or been institutionalized, none of the under-3-month-old infants had been hospitalized except at birth, and none of the subjects had taken antimicrobial agents for a 3-month period before the sampling. The subjects were divided into eight age groups: 0 to 15 (mean, 4.00), 16 to 25 (21.90), 26 to 35 (30.60), 36 to 45 (40.20), 46 to 55 (50.00), 56 to 65 (61.30), 66 to 75 (69.60), and ≥76 (79.60) years.

Data collection and methods. One fecal sample was collected from each subject by swabbing a freshly passed stool with a sterile Dacron swab. The swabs were stored in Transportcult’s transport medium (Orion Diagnostica, Espoo, Finland) for a maximum of 48 h. Four serial 10-fold dilutions of the fecal sample were made in physiological saline and cultured onto MacConkey plates (Oxoid Ltd., Basingstoke, Hampshire, England) with a sterile cotton swab. MacConkey medium selects for growth of aerobic gram-negative enteric bacilli. The plates were incubated aerobically overnight at 35°C. A plate with 100 to 1,000 colonies (preferably about 200) was used for replica plating. Replica plating was used because we have found it a fast and practical method which also provides results essentially the same as those obtained by a standard method (14).

Bacterial colonies (gram-negative enteric bacilli) on the selected plate were counted. The best of each pair of MacConkey plates (i.e., clearly isolated colonies) was replicated by a velvet replica-plating method (8, 14) onto a series of antibiotic-containing Iso-Sensitest agar plates (Oxoid). We used eight antimicrobial agents for replica plating: ampicillin (32 μg/ml), ceftoxime (16 μg/ml), cephalotaxime (16 μg/ml), nalidixic acid (32 μg/ml), trimethoprim (8 μg/ml), sulfamethoxazole (512 μg/ml), tetracycline (4 μg/ml), and ciprofloxacin (1 μg/ml). One plate without any antibiotic was used as a control. After incubation aerobically overnight at 35°C, colonies on the antibiotic plates and on the control plate were counted. Colonies of ambiguous appearance were Gram stained and disregarded if they proved not to be gram-negative bacilli. If 1% or more of the original colonies grew on the antimicrobial plate, the sample was regarded as resistant.

From the antimicrobial-agent-free medium (MacConkey) two colonies of each morphologically distinct type were selected for the identification of gram-negative bacteria from 45 samples. The colonies were identified by standard methods. In
brief, only oxidase-negative colonies (not *Pseudomonas*-like spp.) were tested. Colonies were checked for β-glucuronidase activity with a PGUA disk (A/S Rosco, Taastrup, Denmark) and if positive were regarded as *Escherichia coli*. The rest were tested by the API 20E identification system (bioMérieux, Lyon, France).

The percentages of samples resistant to the eight antimicrobial agents tested and their distribution in different age groups are shown in Fig. 1. Resistance was found to all antimicrobial agents tested and was common in all age groups, including the infants (data not shown separately in Fig. 1). Because there were no significant differences in the frequency of bacterial resistance between the children ≥1 years old and the infants <1 years old (*n* = 20), we did not form two separate groups of children. The on average most frequently occurring resistance factors were to ampicillin (38%), sulfamethoxazole (24%), tetracycline (21%), and trimethoprim (14%). There were no significant differences between the distributions of samples resistant to the eight antimicrobial agents tested in the different age groups. Also, there were no statistically significant
TABLE 1. Occurrence of *E. coli* and other aerobic gram-negative isolates in fecal samples of children (≤15 years) and adults (>15 years)

| Isolate type | No. of isolates (% of total) in samples from: |  |
|--------------|-----------------------------------------------|--|
|               | Children (*n* = 21; mean age, 4.00 yr)         | Adults (*n* = 24; mean age, 43.00 yr) |
| *E. coli*     | 58 (74)                                       | 56 (76)                              |
| Other         | 20 (26)                                       | 18 (24)                              |
| Total         | 78 (100)                                      | 74 (100)                             |

*Includes Citrobacter freundii, Hafnia alvei, Enterobacter spp., Klebsiella spp., Pseudomonas-like spp., and Rahnella aquatilis.*

differences between the carriage rates of bacterial resistance and age when age was treated as an unclassified variable using logistic model. Thus, statistically significant differences could not be found with either method. Nor were any significant differences found when the two oldest age groups (>65 years; *n* = 55) were combined and compared with the child group (≤15 years; *n* = 62). However, by the regression analysis model a *P* value close to significance was obtained for trimethoprim; resistance tended to decrease with increasing age (*P* = 0.052). In addition, the distribution of bacterial species in the adult flora was close to that of the child flora (Table 1).

The reasons for the similarities of the different age groups can only be speculated upon. Elderly people can be assumed to have had a higher cumulative exposure to antimicrobial agents during their lifetime than children yet have had. This would imply higher resistance frequencies. On the other hand, in their youth much fewer antimicrobial agents were available (13) and presumably less frequently prescribed. The resistance we found may therefore have developed fairly recently; indeed, the proportion of antimicrobial agent users has increased in recent years among the elderly (7), putting their fecal flora under a selection pressure that could lead to a development of resistance. For example, sulfamethoxazole-resistant agents are the most commonly used anti-infective drugs in Finland among the elderly and, as can be seen in Fig. 1, are also the most prevalent resistance factor in persons over 75 years old.

In addition, the acquisition of resistant bacterial strains in hospitals is very common; a hospital stay can profoundly affect the normal flora (9). As elderly people on average are hospitalized more often than the rest of the population, and for longer times (21), the hospitalized person colonized by resistant bacteria may more often than not be elderly.

Antimicrobial agents are widely used in child populations (5). In our study, 80% of the children had been prescribed antimicrobial agents during their lifetimes, although no antimicrobial therapy had been given during the 3 months preceding sampling. To our knowledge, among the infant group (*n* = 20) 16% had been prescribed antimicrobial agents during their lifetimes. In England and Wales, already in the 1970s children up to the age of 6 received a quarter of antimicrobial prescriptions for respiratory illness although they represented only 10% of the population (2). In Sweden in 1992, every child less than 4 years old got on average 2.3 antimicrobial prescriptions each (5). In 1994, Finnish children used twice as many antimicrobial agents as the population on average (17). From this point of view, it is not surprising that isolates resistant to antimicrobial agents are common in children.

In newborns, a typical adult-type flora becomes established within 1 month (6). The frequent antimicrobial courses prescribed to children could theoretically be expected to affect their fecal flora and favor colonization by intrinsically resistant enterobacteria, such as *Klebsiella* and *Enterobacter* species (3, 4). A higher rate of colonization by these bacteria in children under the age of 6 was indeed found by Degener et al. (4). The findings in the study of Bennet (1) indicated that when the colonization resistance provided by anaerobic intestinal flora was decreased, high counts of *Klebsiella* organisms were found in infants. Our results do not confirm these studies; equal numbers of *E. coli* were found in the feces of adults and children (Table 1). However, the number of subjects might be too low for valid conclusions to be drawn. In addition, food may be a more significant source of stable colonization by resistant strains than recognized (10) and possibly the most important factor creating the overall similarity found by us. Environmental factors (like food) may be especially important factors in the infant group, since they usually have not been treated with antimicrobial agents and are cared for at home. This means that infants could be the resistance indicators of our environment.

In conclusion, there were no statistically significant differences between carriage rates of bacterial resistance and age. Antimicrobial resistance was common among fecal aerobic gram-negative bacilli in all age groups. There is a need for further investigations in the form of prospective studies to examine individually the development of resistance and its origins from childhood to adulthood in community populations so that our knowledge about the occurrence of antimicrobial-agent-resistant bacteria in humans would be less fragmentary.

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