Colonization of resistant faecal aerobic Gram-negative bacilli among geriatric patients in hospital and the community

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Among the elderly most infections are caused by organisms of faecal origin. The study of the resistance of such Gram-negative bacilli should therefore be a priority. In this study, we determine the occurrence of resistance to five antimicrobials commonly used in geriatric outpatient care, and compare it with long-term and short-term hospitalized geriatric patients treated and not treated with antimicrobials.

Introduction
Because of their vulnerability to infection, the elderly are likely to be in hospital and to be at greater risk from hospital acquired infection (Davey et al., 1994). The majority of hospital infections in elderly people are urinary tract infections caused by organisms of faecal origin (Wingard et al., 1993). Thus, the question of antimicrobial resistance of faecal aerobic Gram-negative bacilli to antimicrobials is of major interest. Colonization of patients by resistant bacteria, has been related to the length of hospitalization and their antimicrobial treatment (Shaw et al., 1973; Laufs et al., 1979). However, studies on faecal aerobic Gram-negative bacilli among the elderly and the effect of duration of hospitalization on antimicrobial resistance are scarce, though many reports emphasize the value of a better knowledge of antibiotic resistance in hospital populations not treated with antibiotics (Levy et al., 1988) and also in the outpatient population (Bonten et al., 1990).

The purpose of this cross-sectional study was to evaluate the occurrence of resistance to five antimicrobials commonly used in geriatric care among outpatients with a stable gut flora, and to compare it to long-term and short-term hospitalized geriatric patients treated and untreated with antimicrobials.

Materials and methods

Patients
The sample consisted of 145 hospital patients in five wards of the Department of Medicine and Geriatrics of the Turku City Hospital, and of 61 outpatients from the geriatric outpatient service of the same hospital in 1993.

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Of the untreated hospital patients, i.e., patients who had not received antimicrobials within the 3 months before sampling \((n = 76)\), 56 patients had a hospitalization time of less than 15 days (short-term hospitalization; on average 7 days). The remaining 20 patients had been hospitalized 15 days or more (long-term hospitalization; on average 41 days).

Of the treated patients, i.e., patients who had received antimicrobials \((n = 69)\) during hospitalization before sampling, 34 patients had been hospitalized for less than 15 days (on average 8 days) and 35 patients 15 days or more (on average 44 days). The short-term hospitalized patients had used 3.2 defined daily doses (DDD) of antimicrobials per patient and those long-term hospitalized 13.3 DDD/patient. The majority (87%) of the treated inpatients had received the treatment within three weeks before sampling. To our knowledge none of the 61 outpatients had received any antimicrobials or been hospitalized during the three months before the study. In addition, as far as we know, there were no outbreaks of infection in the hospital during the study period.

**Sample collection and processing**

Faecal samples were collected from each inpatient in the morning. The samples were taken from the rectum with sterile dacron swabs. Outpatients brought fresh stool samples when visiting the outpatient service. All samples were transferred to the laboratory within 2 h and were cultured the same day.

Several dilutions of the faecal sample were made in physiological saline and cultured on to MacConkey plates (Oxoid Ltd, Basingstoke, Hampshire, England) and incubated aerobically overnight at 35°C. Bacterial colonies were counted with a counterpen. Those MacConkey plates with a range of 100-1000 discrete colonies (on average 405) were used for further studies.

After counting the enteric bacilli, the plate was replicated using a velvet replica-plating method (Lederberg & Lederberg, 1952; Levy et al., 1988) on to a series of IsoSensitest agar plates (Oxoid Ltd., Basingstoke, Hampshire, England) with fixed amounts of the specified antimicrobials: ampicillin 32 \(\mu\)g/mL, cefuroxime 16 \(\mu\)g/mL, trimethoprim 8 \(\mu\)g/ml, sulphamethoxazole 512 \(\mu\)g/mL, and tetracycline 4 \(\mu\)g/mL. Control cultures were made on pure IsoSensitest agar plates. The number of colonies on the antibiotic plates was counted after overnight incubation at 35°C.

If there was no bacterial growth (or less than 1% of colonies showing resistance) on an antibiotic plate the sample was deemed susceptible to that antimicrobial agent.

**Results and discussion**

The frequency of susceptible organisms among outpatients was close to that of short-term hospitalized untreated patients (Figure). The only significant differences found between outpatients and short-term hospitalized patients were in cefuroxime \((P = 0.015)\) and trimethoprim resistance levels \((P = 0.036)\). However, there was a decreasing trend, although not statistically significant, in the frequency of susceptible organisms the longer the patients had been hospitalized and the more extensive the use of antimicrobials had been. Despite this expected trend, there was no significant difference between long-term hospitalized untreated patients and outpatients. This may
Figure. Per cent of susceptible samples (less than 1% of colonies resistant) among geriatric outpatients who had not received antimicrobial therapy (1); among patients without preceding antimicrobial therapy and hospitalized for less than 15 days (2) or for more than 15 days (3); and among patients with prior antimicrobial therapy and hospitalized for less than 15 days (4) or for more than 15 days (5). P-values for the differences between outpatient and inpatient groups were calculated by using the Chi-square test and SAS library. (a) Ampicillin; (b) Cefuroxime, (c) Trimethoprim; (d) Sulphamethoxazole; (e) Tetracycline
be explained by the fact that the number of patients in the long-term care group might be too low for valid conclusions to be drawn.

The use of antimicrobial agents caused a predictable selection pressure for resistant Gram-negative bacilli (GNB). In addition, the longer the hospitalization the more antimicrobials were used, which probably explains why the occurrence of susceptible organisms was significantly lower among the long-term hospitalized treated patients, than among other hospital patient groups.

When the four groups of hospitalized patients were compared, the treated long-term hospitalized patients carried fewer cefuroxime-susceptible bacteria than both the short-term and long-term untreated patients ($P = 0.006$). This phenomenon can be explained by the extensive use of cephalosporins, especially cefuroxime, in this hospital. Cefuroxime therapy has been shown to increase both the risk of individual colonization by Enterobacter spp. and the emergence of cefuroxime resistance in such strains (Tullus & Burman, 1989). In this study we did not identify the GNB to the species level.

The velvet replica-plating method has been used to study faecal aerobic GNB. In the study by Levy et al. (1988), the authors divided the faecal samples into two groups: $\geq 10\%$ and $\geq 50\%$ of resistant colonies. In this study a detection limit of $1\%$ resistance was used, but with a limit of $\geq 50\%$, our results can be compared to those of Levy et al. (1988) as follows: $24\%$ of hospitalized patients without a history of antimicrobial treatment had $\geq 50\%$ of colonies resistant to ampicillin, compared with $27\%$ in Levy et al. (1988). They used an ampicillin concentration of $30 \mu g/mL$; $32 \mu g/mL$ was used in this study. The corresponding frequencies for outpatients were $10\%$ and $19\%$. For tetracycline, resistance occurred among $18\%$ of the hospitalized patients in this study and among $23\%$ in the study of Levy et al. (1988) (at a concentration of $10 \mu g/mL$ compared with $4 \mu g/mL$ in this study). In outpatients, the frequencies were $16\%$ and $25\%$, respectively. However, the mean age of the subjects in the study of Levy et al. (1988) was 43 years, whereas our patients were geriatric patients with a mean age of 76 years (range 60–95 years) in the whole sample.

Interestingly, there were no significant differences in the frequency of sulphonamide resistance between outpatients and hospital patients. This probably reflects the universal occurrence of sulphonamide resistance determinants in faecal GNB, a theory also supported by the overall low occurrence of sulphonamide-susceptible samples—$49–65\%$ in our study groups (Figure). Sulphonamides have not been extensively used in this hospital, which may explain why there were no such significant differences between outpatients and hospital patients. Moreover, the genetic determinants for sulphonamide resistance are linked with trimethoprim resistance genes (Huovinen et al., 1995). In addition, although trimethoprim has been little used in this hospital since before the study, trimethoprim resistance remained fairly common (Figure). This may be a delayed reflection of the wide use of trimethoprim in this hospital in the past (Huovinen et al., 1986; Heikkilä, Sundström & Huovinen, 1990). This also supports our earlier hypothesis that the removal of trimethoprim may not have an immediate impact on trimethoprim-resistance levels (Huovinen et al., 1995).

The velvet replica-plating method allows the frequency of resistant bacteria in clinical samples such as faeces to be studied. However, unless large numbers of plates are used, the method does not provide reliable data on samples with less than $1\%$ of resistant colonies. Colonization by small numbers of resistant bacteria may therefore escape detection. Technical improvements are necessary to overcome this problem.
In conclusion, use of antimicrobial agents caused a remarkable selection pressure on Gram-negative bacteria. However, the dynamics of long-term hospitalization and the importance of hospital hygiene during nursing procedures on resistance levels need further investigation. More effort should be devoted to the study of the response of bacterial populations of different origin both in the community, and in hospital, because no areas are free of resistant organisms. Thus, antimicrobial resistance should be studied not only in clinical samples but also in normal bacterial flora, and resistance factors in the community are as important as those in the hospital when determining antimicrobial therapy.

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