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# The Effect of Antimicrobial Agents on Fecal Flora of Children

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**Influences of antibiotics on the fecal flora in children were studied for oral ampicillin, penicillin V, erythromycin, cefaclor, and gentamicin and for intravenous ampicillin, methicillin, cefpiramide, and ceftazidime. All antibiotics affected the normal flora, although the quality and quantity of the changes were variable. No substantial differences were noted between the oral and intravenous use of ampicillin with regard to its effect on the flora. Three penicillins, ampicillin, penicillin V, and methicillin, caused remarkable changes. The characteristic pattern observed was the considerable suppression of *Bifidobacterium*, *Streptococcus*, and *Lactobacillus* species. Although enterobacteria did not significantly change in number, *Klebsiella* spp. frequently replaced *Escherichia coli*. In patients given erythromycin and cefaclor, the reduction in the number of *Bifidobacterium* spp. was 1 $\log_{10}$ and that of members of the family *Enterobacteriaceae* was 3 $\log_{10}$. Gentamicin administered orally caused a drastic change, including a remarkable decline of *E. coli* to less than 2 $\log_{10}$/g of feces. Cefpiramide, a parenteral expanded-spectrum cephalosporin, suppressed normal flora so markedly that almost all species of organisms were eradicated, and the active growth of yeasts was promoted (2.6 $\log_{10}$ increase). Ceftazidime caused similar changes as cefpiramide, but the changes were less extensive. Yeasts increased after treatment with most antibiotic groups. This increase was particularly prominent in patients given oral penicillins and expanded-spectrum cephalosporins.**

The normal intestinal flora consists mainly of anaerobes ($10^{10}$ to $10^{12}$ organisms per g of feces) and is the largest bacterial reservoir in humans. Although the role of the normal flora is not fully understood, there is evidence that alterations in the flora may have important consequences, as illustrated by the case of *Clostridium difficile* colitis (4). It is therefore an essential requirement of the body to continuously maintain a balanced flora. Quality of food, drug ingestion, and the general state of health are all factors which influence the intestinal bacteriology. Of these factors, antibiotic use is known to be among the most significant. The purpose of this study is to delineate changes in the intestinal bacteriology of children caused by the use of a variety of antibiotics.

## MATERIALS AND METHODS

Fifty-four children, who were hospitalized in the Asahikawa Medical College Hospital for antibiotic treatment of pneumonia, pharyngitis, otitis media, or urinary tract infection, were the subject of this study. The age of the patients ranged from 10 months to 12 years. They did not receive any antimicrobial agents before hospitalization. Patients suffering diarrhea or showing other symptoms of gastroenteritis were excluded from the study. The antibiotics used, the routes of administration, and dosages are listed in Table 1.

For oral use the daily dose was divided into three to four doses and given every 6 to 8 h. For intravenous use the daily dose was given in four doses, one every 6 h, except cefpiramide, which was given in three doses, one every 8 h. Antibiotic use was continued for 5 to 14 days. Patients were prescribed symptomatic drugs according to their clinical condition, but they were not given any other antimicrobial agents concomitantly. Fifteen patients, who were staying in the Asahikawa Medical College Hospital for endocrinologic or developmental evaluation, ranging in age from 1 to 13 years (mean, 5.6 ± 3.3 years), served as controls.

Stool specimens were obtained before the treatment, on days 4 to 6 of antibiotic use, and 3 to 6 days after the cessation of drug administration. Quantitative aerobic and anaerobic culture methods were based on those described by Mitsuoka et al. (13, 14). The culture media used are listed in Table 2. Stool specimens were immediately put into a transport medium (13) and stored at 4°C until cultured. The transport medium consisted of brain heart infusion broth (Difco Laboratories, Detroit, Mich.) containing 0.5 g of L-cysteine · HCl · $H_2O$, 4 g of $Na_2CO_3$, 0.5 mg of resazurin, and 1 g of Bacto-Agar (Difco) per 1,000 ml (13). The transport medium was prereduced and anaerobically sterilized (9), as were all other media used in this study. Air in the medium was driven off by boiling. Oxygen-free $CO_2$ gas was then bubbled through the medium with a cannula, and the medium was stored in tightly stoppered tubes. When the stopper was removed, a cannula carrying an oxygen-free

TABLE 1. Antibiotics, routes of administration, and dosages in individual patient groups

| Route | Antibiotic | Age of patients (yr)[a] | No. of patients[b] | Daily dose |
|---|---|---|---|---|
| Oral | Ampicillin | 6.2 ± 2.3 | 6/6/6 | 40–50 mg/kg |
| | Penicillin V | 8.4 ± 2.4 | 5/5/5 | 50,000 U/kg |
| | Cefaclor | 5.8 ± 2.3 | 6/6/6 | 40–50 mg/kg |
| | Erythromycin | 6.2 ± 2.9 | 6/6/5 | 40–50 mg/kg |
| | Gentamicin | 6.3 ± 3.7 | 3/3/3 | 60 mg/kg |
| Intravenous | Ampicillin | 5.8 ± 3.1 | 6/6/5 | 150–200 mg/kg |
| | Methicillin | 3.2 ± 3.0 | 8/8/8 | 150–200 mg/kg |
| | Cefpiramide | 4.9 ± 3.6 | 7/7/5 | 60 mg/kg |
| | Ceftazidime | 4.8 ± 1.8 | 7/7/6 | 80 mg/kg |

[a] Mean ± standard deviation.
[b] Number of patients before/during/after antibiotic treatment.

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TABLE 2. Culture media used for quantitative isolation of fecal microorganisms

| Culture media | Reference | Main organisms counted | Manufacturer[a] |
|---|---|---|---|
| Anaerobic culture | | | |
| Medium 10 | 2, 13 | Fastidious anaerobes | |
| Glucose-blood-liver agar | 14 | Anaerobes | Nissui |
| Modified Eggert-Gagnon alkaline blood agar | 14 | Anaerobes | Nissui |
| BS agar[b] | 14 | *Bifidobacterium* spp. | |
| ES agar[c] | | *Eubacterium* spp. | |
| NBGT blood agar[d] | 14 | *Bacteroides* spp. | |
| Neomycin Nagler agar | 12, 13 | *Clostridium* spp. | |
| Modified VS agar (*Veillonella* agar Rogosa) | 13, 14 | *Veillonella* and *Megasphaerae* spp. | |
| Modified LBS agar | 14 | *Lactobacillus* spp. | BBL |
| Aerobic culture | | | |
| Trypticase soy blood agar | | Aerobes | Difco |
| DHL agar | | *Enterobacteriaceae* | Eiken |
| TATAC agar | 14 | *Streptococcus* spp. | |
| PEES agar | 14 | *Staphylococcus* spp. | |
| Potato dextrose agar | | Yeasts and molds | Difco |
| Nalidixic acid-cetrimide agar | 8 | *Pseudomonas* spp. | Eiken |

[a] Nissui, Nissui-Seiyaku Co., Tokyo, Japan; BBL, BBL Microbiology Systems, Cockeysville, Md.; Difco, Difco Laboratories, Detroit, Mich.; Eiken, Eiken-Kagaku Co., Tokyo, Japan.
[b] BS agar, Glucose-blood-liver agar containing 200 μg of neomycin per ml, 50 μg of paromomycin per ml, 3 mg of lithium chloride per ml, and 15 mg of sodium propionate per ml.
[c] ES agar, Eggert-Gagnon alkaline blood agar containing 10 μg of colistin per ml, 200 μg of neomycin per ml, 500 μg of streptomycin per ml, and 15 mg of sodium propionate per ml.
[d] NBGT blood agar, Eggert-Gagnon alkaline blood agar containing 500 μg of neomycin per ml, 2.5 μg of brilliant green (Merck & Co., Inc., Rahway, N.J.) and 2.5 mg of sodium taurocholate per ml.

$CO_2$ gas stream was inserted into the tube to prevent oxygen from entering.

Approximately 1 g of stool specimen was weighed, homogenized, and serially diluted 10-fold in buffer solution, which was prereduced (13). In the course of homogenization and dilution, a cannula with $CO_2$ gas flow was applied to exclude air. This was done in all culture and transfer manipulations. From the series of dilutions, 0.05-ml portions were taken and inoculated onto the media with a glass rod. The lowest detectable number of microorganisms was 2 $\log_{10}$/g of feces. The inoculated media were incubated at 37°C in room air for aerobic cultures. Medium 10 was incubated anaerobically by the plate-in-bottle method (13). Other media for anaerobic organisms were cultured in an anaerobic jar by the steel wool method. The incubation time was 5 days for medium 10, 3 days for other anaerobic media, overnight for DHL agar (Eiken-Kagaku Co., Tokyo, Japan), and 2 days for all remaining media. Aerobic and facultative isolates were identified by standard methods (11). The criteria used for the identification of anaerobic isolates have been outlined previously (9). The number of bacteria was expressed in terms of CFU per gram (wet weight of feces).

The Newman-Keuls test (17), based on the distribution of the range by Student's *t* test, was used for comparison of the differences in the number of organisms among the specimens taken before, during, and after antibiotic use. Student's *t* test was performed to compare numbers in the controls and the experimental groups before antibiotic use.

TABLE 3. Counts of fecal microrganisms in control children and in patients receiving cefpiramide intravenously

| Organism | Organism counts ($\log_{10}$/g of feces) in control children[a] ($n$ = 15) | Organism counts a ($\log_{10}$/g of feces) at the following times in patients receiving cefpiramide intravenously[a]: | | |
|---|---|---|---|---|
| | | Before (n = 7) | During (n = 7) | After (n = 5) |
| *Bacteroidaceae* | 10.6 ± 0.8 (13) | 10.5 ± 0.2 (7) | 9.0 ± 1.4[b] (5) | 10.0 ± 0.5 (4) |
| *Eubacterium* spp. | 9.3 ± 1.0 (5) | 9.3 (1) | ND[c] | ND |
| *Peptococcaceae* | 9.0 ± 1.3 (6) | 8.7 ± 0.3 (4) | ND[d] | 7.9 ± 0.5 (2) |
| *Bifidobacterium* spp. | 10.5 ± 0.5 (15) | 10.2 ± 0.3 (7) | 6.0 ± 1.6[d] (4) | 9.4 ± 0.9[b] (4) |
| *Veillonella* spp. | 6.7 ± 1.8 (12) | 6.3 ± 1.6 (5) | ND[d] | ND[d] |
| *Magasphaerae* spp. | 5.8 ± 0.4 (3) | ND | ND | ND |
| *Clostridium* spp. | 7.0 ± 1.4 (14) | 8.1 ± 1.1 (7) | 7.8 ± 0.8[d] (2) | 8.1 ± 0.9 (4) |
| *Lactobacillus* spp. | 6.5 ± 1.5 (13) | 4.1 ± 1.3 (5) | ND[b] | ND[b] |
| *Enterobacteriaceae* | 8.8 ± 0.8 (15) | 8.8 ± 0.4 (7) | 3.8 ± 0.6[b] (3) | 6.9 ± 2.5[b] (5) |
| *Streptococcus* spp. | 8.3 ± 0.6 (15) | 8.7 ± 0.5 (7) | 7.9 ± 0.9[b] (3) | 8.7 ± 0.3 (5) |
| *Staphylococcus* spp. | 4.5 ± 1.8 (9) | 3.6 ± 0.4 (2) | ND | ND |
| *Bacillus* spp. | 7.5 ± 1.9 (3) | ND | ND | ND |
| Yeasts | 4.3 ± 1.0 (9) | 4.1 ± 0.7 (3) | 6.5 ± 0.8[b] (4) | 5.3 ± 1.5 (2) |
| *Pseudomonas* spp. | 3.0 ± 2.2 (4) | 4.0 (1) | ND | 3.1 (1) |

[a] Values are mean ± standard deviation (when organisms are present). Values in parentheses are the number of patients that tested positive for the indicated organisms.
[b] $P < 0.05$.
[c] ND, None detected (counts less than 2 $\log_{10}$/g of feces).
[d] $P < 0.01$.

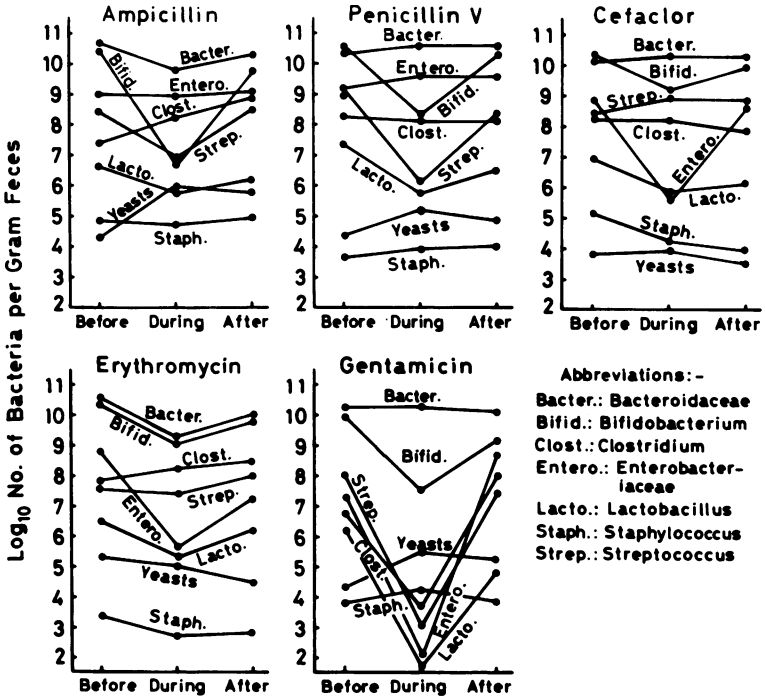


FIG. 1. Changes in the count of the eight main constituents of fecal flora before, during, and after oral antibiotic administration.

## RESULTS

**Stool flora in control patients.** The bacterial counts of the stool flora in the 15 control patients are listed in Table 3. *Bacteroidaceae* spp. and *Bifidobacterium* spp. were the predominant bacterial species, and their incidence was high. *Eubacterium* spp. and *Peptococcaceae* spp. were isolated less frequently, although the organism number was high when they were present. Among aerobic organisms, members of the family *Enterobacteriaceae* and *Streptococcus* spp. grew from all stool specimens at a constant number of almost 8 $\log_{10}$/g of feces.

The organism numbers of bacterial species in individual patients before antibiotic use were compared with those in control patients, and it was confirmed that there were no significant differences in flora bacteriology between control and antibiotic groups ($P > 0.05$).

**Changes in flora bacteriology after oral antibiotic use.** Among 14 bacterial species studied, the incidence of *Eubacterium*, *Peptococcaceae*, *Veillonella*, *Megasphaerae*, *Bacillus*, and *Pseudomonas* was generally low, and changes caused by the use of antibiotics were not remarkable. Therefore, only changes in the remaining eight main constit-

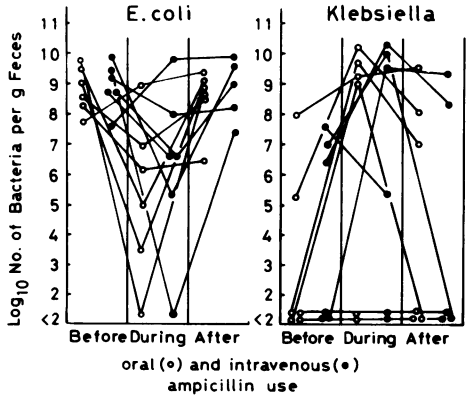


FIG. 2. Changes in the count of *Escherichia coli* and *Klebsiella* spp. in patients given ampicillin orally and intravenously.

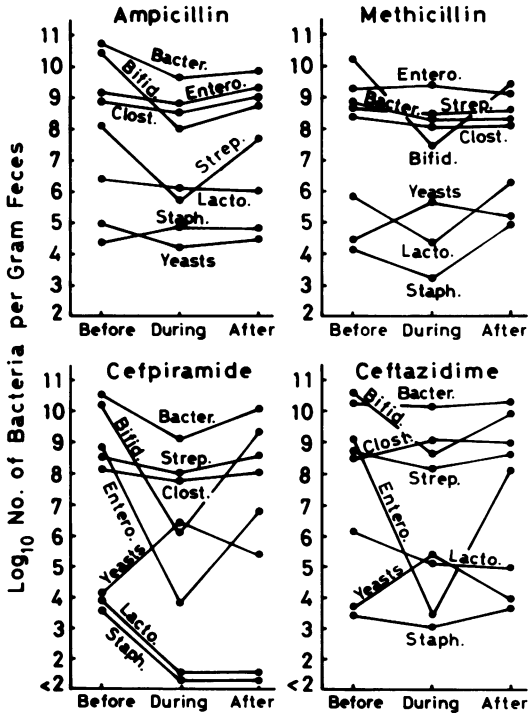


FIG. 3. Changes in the count of the eight main constituents of fecal flora before, during, and after intravenous antibiotic administration (see Fig. 1 for abbreviations).

uents of *Bacteroidaceae*, *Bifidobacterium*, *Clostridium*, *Enterobacteriaceae*, *Lactobacillus*, *Staphylococcus*, *Streptococcus*, and yeasts were investigated. The profile of changes in organism number of these species observed after oral antibiotic use is shown in Fig. 1. With ampicillin and penicillin V, the decline of *Bifidobacterium* and *Streptococcus* was remarkable (reduction of 2 to 4 $\log_{10}$). The decrease in the number of *Lactobacillus* was 1 $\log_{10}$ or more; the numbers of *Enterobacteriaceae*, *Clostridium*, and *Streptococcus* did not undergo notable changes. Although the total numbers of *Enterobacteriaceae* did not change in the group receiving ampicillin *Escherichia coli*, which was the prevailing organism, was replaced by *Klebsiella* spp. in the course of antibiotic treatment (Fig. 2). With cefaclor and erythromycin, the decline of *Enterobacteriaceae* was remarkable (3 $\log_{10}$ reduction, on average), but inhibition of *Bifidobacterium* spp. by these drugs was much less significant than was the case with penicillins.

Changes associated with oral gentamicin therapy were striking. *Bifidobacterium* spp. showed a reduction of nearly 3 $\log_{10}$. *Streptococcus*, *Clostridium*, *Enterobacteriaceae*, and *Lactobacillus* species were also strongly suppressed (reduction of 4 $\log_{10}$ or more). Counts of *Bacteroidaceae* and *Staphylococcus* did not change significantly.

**Changes in flora bacteriology in patients receiving antibiotics intravenously.** *Bifidobacterium*, *Streptococcus*, and *Lactobacillus* species were moderately suppressed by ampicillin and methicillin treatment (reduction of up to 2.5 $\log_{10}$) (Fig. 3). These effects were in the same direction as those observed with ampicillin and penicillin V administered orally. Suppression of the flora caused by cefpiramide, an expanded-spectrum cephalosporin, was strong. The count and occurrence of almost all species studied showed a sharp fall, and their numbers did not recover completely until 3 to 6 days after termination of antibiotic treatment (Table 3 and Fig. 3). The count of yeasts increased appreciably (4 $\log_{10}$). Changes related to ceftazidime use were less drastic, except for the effects on *Enterobacteriaceae*.

An increase in yeasts was a general finding in all antibiotic treatment groups, except for erythromycin and ampicillin administered intravenously.

## DISCUSSION

Finegold et al. (6) reported that ampicillin administered orally evoked significant suppression of normal aerobic and anaerobic flora and caused overgrowth of *Klebsiella*, *Proteus*, and *Candida* species and that these changes were less intensive with the tetracyclines. Their findings with ampicillin were in good agreement with the results of our study of the oral use of penicillins. An overgrowth of *Klebsiella* spp. was also demonstrated. Sutter and Finegold (18) noted that cephalexin caused relatively little change in the normal flora. In our study, suppression of normal flora by cefaclor, an oral cephalosporin, was rather insignificant, except for a reduction in enterobacteria. Changes caused by normal doses of erythromycin are reported to be less marked than those caused by penicillins (5). We noted, however, a significant decrease in the count of enterobacteria.

Aminoglycoside antibiotics, when given orally, usually exert a striking effect on the stool flora (3). The effects of gentamicin administration in this study confirmed this result.

Older parenteral cephalosporins, including cephalothin and cefazolin, are reported to cause only slight changes (1), whereas newer ones have been known to produce an extensive suppression of the normal flora. Some researchers (7, 15) have observed a heavy growth of yeasts by intravenous administration of cefoperazone and cefpiramide, while other species of organisms were eradicated. The effect on the intestinal flora varies according to the antibiotic given, and the differences are related to the antibacterial spectra of the individual drugs. Another factor that affects the results is the concentration of the drug in the intestine. Among the antibiotics given parenterally, gentamicin, cephalothin, or cefotaxime, which are excreted in the bile at lower concentrations, alter the flora only slightly (1, 10). In contrast, cefoperazone or cefpiramide, which are known to be excreted in the bile at high concentrations, usually cause significant changes in the stool flora (7, 15). The intense effects of cefpiramide on the stool flora were confirmed in the present study.

A further point to be mentioned is the rather rapid return of the disturbed flora to normal levels within 3 to 6 days after therapy. Although some researchers (16) believe that recovery of the flora requires a longer period of time (up to 2 weeks or more), we do not necessarily agree with this view, since antibiotics are known to be eliminated from the body rather rapidly.

Suppression of the normal flora results in lowered colonization resistance and promotes overgrowth of resistant organisms (19). This situation might lead to serious secondary infections, which is particularly hazardous in immunocompromised hosts. Although the advantages of antibiotics are often emphasized, one must be fully aware of their effects on the normal flora when prescribing these drugs.

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