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ECOLOGICAL IMPACT OF NARROW SPECTRUM ANTIMICROBIAL AGENTS COMPARED TO BROAD SPECTRUM AGENTS ON THE HUMAN INTESTINAL MICROFLORA

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INTRODUCTION

The administration of antimicrobial agents may have a number of potentially adverse effects in relation to the human intestinal microflora (*Nord* et al., 1986). One is the overgrowth of already present microorganisms such as yeasts which may produce systemic infections in immunocompromised patients and of *Clostridium difficile* which may lead to diarrhoea and/or colitis. A second consequence is the development of antimicrobial resistance and the induction of beta-lactamases among bacteria in the normal microflora. A third effect is the reduction of colonization resistance, i.e. the resistance displayed by the host to implantation of new microorganisms in the normal microflora. Several factors influence the extent to which a given antimicrobial agent will decimate the normal microflora. Predominant among these is the incomplete absorption of orally administered drugs. Poorly absorbed agents can reach the intestine in active form where they destroy susceptible microorganisms and change the ecologic balance. Parenterally administered agents that are secreted in the bile or from the intestinal mucosa also tend to destroy the normal microbial population.

This investigation examined the

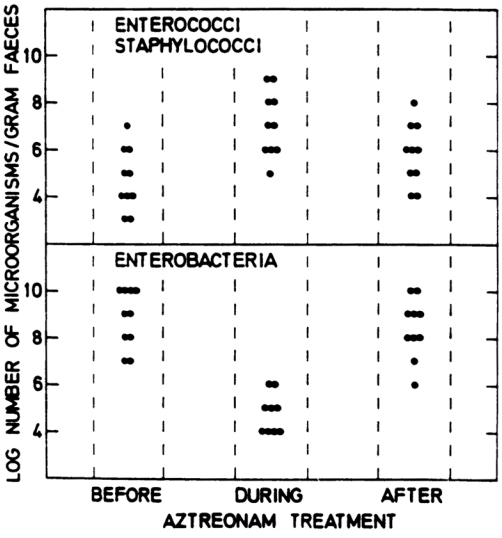

Figure 1: Impact of aztreonam on the aerobic intestinal microflora in 10 patients. The numbers of microorganisms are given in log numbers per gram faeces.

ecological impact of four narrow spectrum antimicrobial agents compared to two broad spectrum antimicrobial agents on the human intestinal microflora.

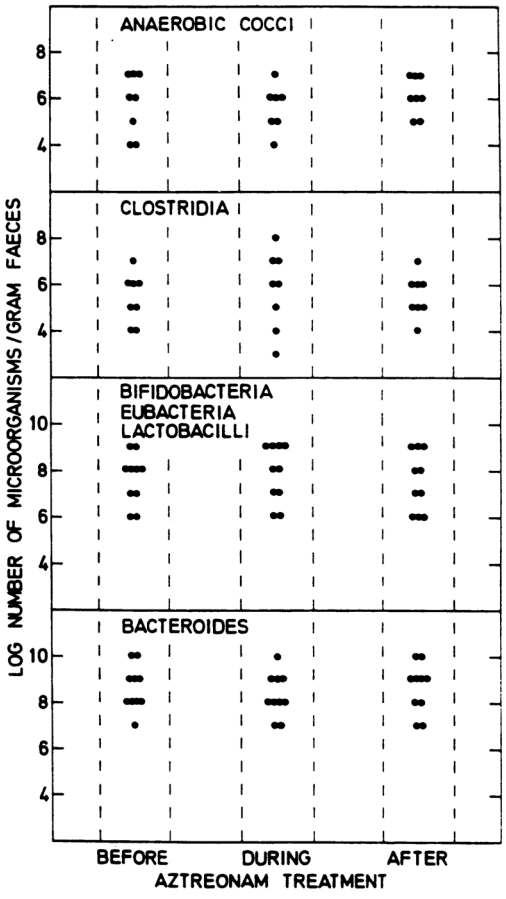

Figure 2: Impact of aztreonam on the anaerobic intestinal microflora in 10 patients. The numbers of microorganisms are given in log numbers per gram faeces.

MATERIAL AND METHODS

Patients

Seventy-nine patients, 42 men and 37 women between 21 and 75 years of age (medium age 51 years) with respiratory tract infections, intra-abdominal infections, or urinary tract infections, were included in the study. All patients gave their informed consent to participate in the study which had been approved by the ethical review committees.

Drug administration

Aztreonam

Aztreonam was given intravenously to ten patients in a dose of 1 g b.i.d for 6-12 days.

Cefoperazone

In this group, all patients (n=29) except one received 2 g cefoperazone b.i.d intravenously. One patient with elevated serum creatinine received 1 g b.i.d. The patients were treated for 7 to 14 days.

Clindamycin

Clindamycin was administered perorally to ten patients as 150 mg capsules q.i.d for 7 to 14 days.

Imipenem

Ten patients received 0.5 g imipenem combined with 0.5 g cilastatin q.i.d by intravenous infusion. The treatment period was between 6 and 11 days.

Metronidazole

Metronidazole was given to ten patients by mouth as tablets in a dose of 0.4 g t.i.d for 5-7 days.

Norfloxacin

Ten patients received 200 mg norfloxacin as tablets b.i.d. for 7-9 days.

Sampling procedures

Faecal specimens from all patients were taken before therapy, during therapy and one week to one month after end of therapy. The specimens were collected in sterile plastic containers, immediately frozen and stored at -70°C until they were assayed.

Assay of antimicrobial concentrations in faeces

The concentrations of antimicrobial agents in faeces were determined by the microbiological agar diffusion method; the specimens were processed as previously described by *Kager* et al. (1981).

Microbiological procedures

One gram of the faecal specimen was homogenized in 9 ml prereduced peptone-yeast extract medium. Ten-fold serial dilutions were made to 10^{-8}. Duplicate samples of 0.1 ml of the different dilutions were inoculated onto different non-selective and selective media (*Heimdahl* and *Nord*, 1979). All manipulations of the anaerobic media were carried out in an anaerobic chamber. After incubation, total counts were made on the aerobic and anaerobic blood agar plates and different colonies were isolated and identified as were the colonies found on the selective media.

The microorganisms were identified as described by *Heimdahl* and *Nord* (1979). Enterobacteria were identified biochemically with the API 20E test kit (Analytab Products, N.Y., USA), and oxidative-fermentative, Gram-negative rods with the Oxi-Ferm test kit (Hoffmann-La Roche, N.J., USA).

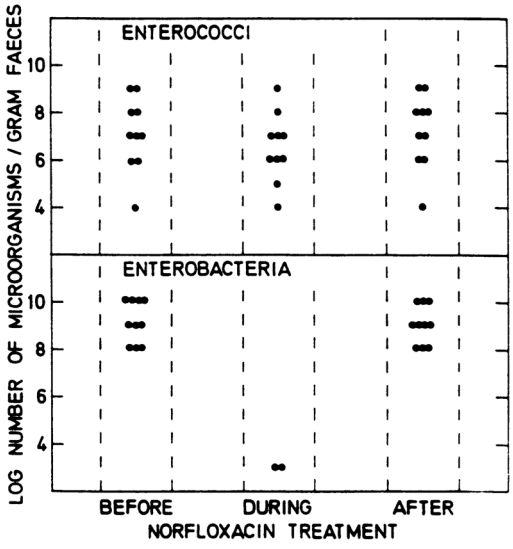

Figure 3: Impact of norfloxacin on the aerobic intestinal microflora in 10 patients. The numbers of microorganisms are given in log numbers per gram faeces.

Staphylococci were differentiated by oxidation-fermentation, coagulase and nuclease tests. Streptococci were identified by biochemical and serological tests, and anaerobic bacteria by biochemical tests and gas-liquid chromatography. Yeasts were typed by different cultural and biochemical characteristics.

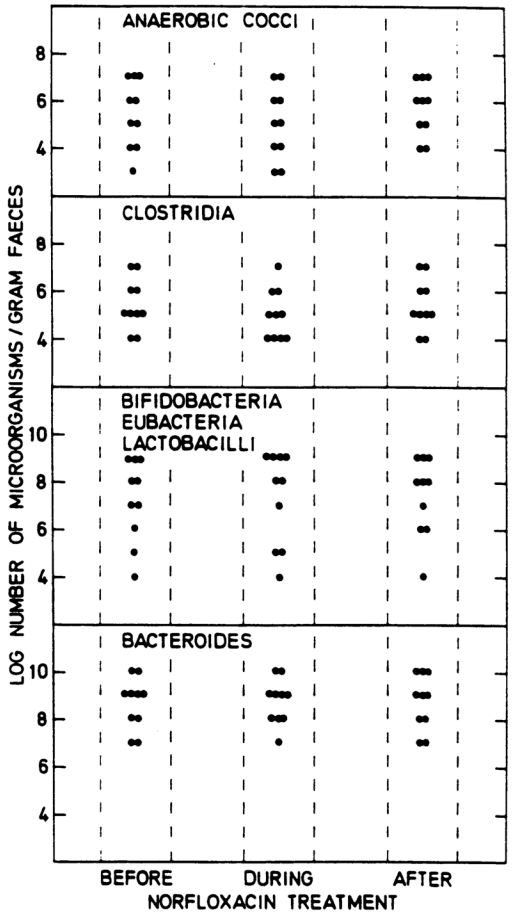

Figure 4: Impact of norfloxacin on the anaerobic intestinal microflora in 10 patients. The numbers of microorganisms are given in log numbers per gram faeces.

RESULTS

Impact of narrow spectrum antianaerobic agents on the intestinal microflora

Aztreonam

The impact of aztreonam on the aerobic intestinal microflora is shown in Figure 1. The numbers of enterobacteria were significantly decreased during the treatment while the numbers of Gram-positive cocci - enterococci and staphylococci - increased. At the same period, there were only minor changes in the anaerobic intestinal microflora (Figure 2). The microflora returned to pretreatment levels after the end of therapy.

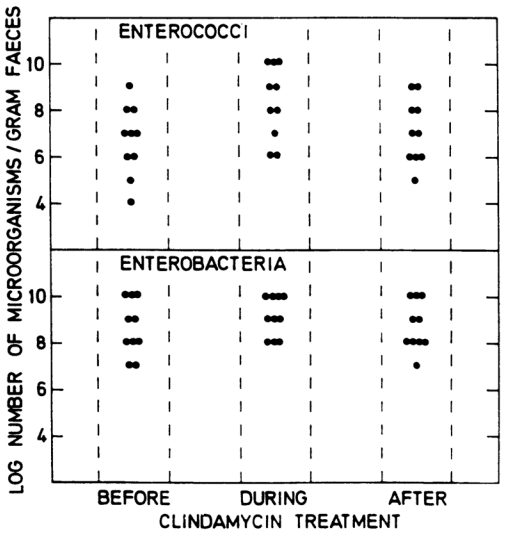

Figure 5: Impact of clindamycin on the aerobic intestinal microflora in 10 patients. The numbers of microorganisms are given in log numbers per gram faeces.

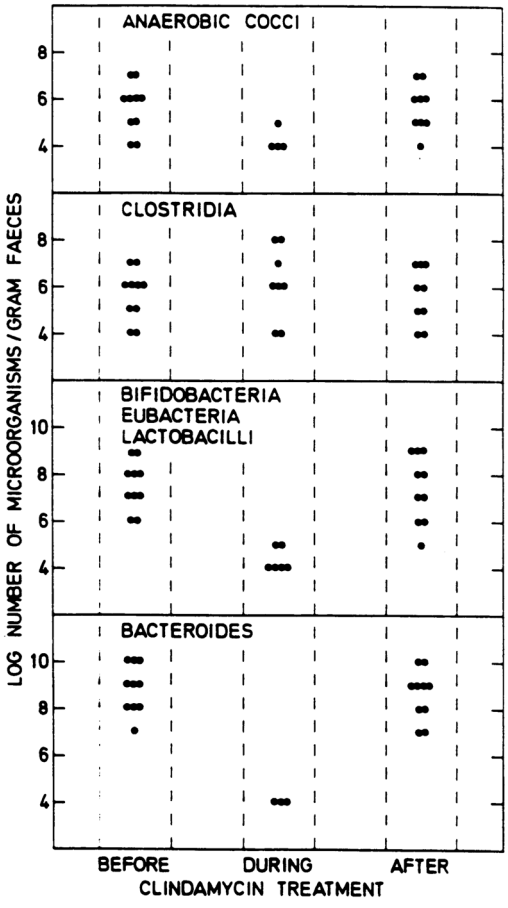

Figure 6: Impact of clindamycin on the anaerobic intestinal microflora in 10 patients. The numbers of microorganisms are given in log numbers per gram faeces.

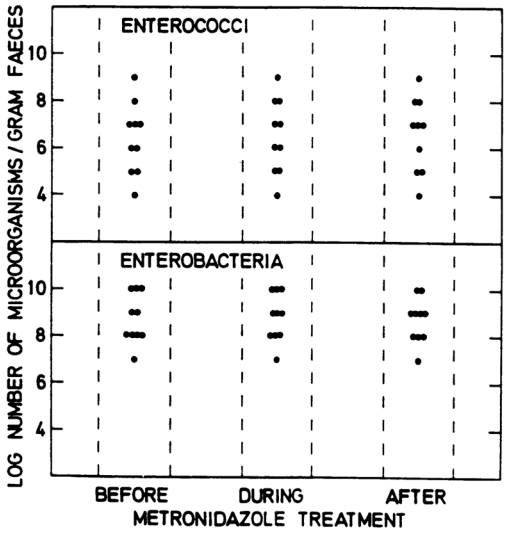

Figure 7: Impact of metronidazole on the aerobic intestinal microflora in 10 patients. The numbers of microorganisms are given in log numbers per gram faeces.

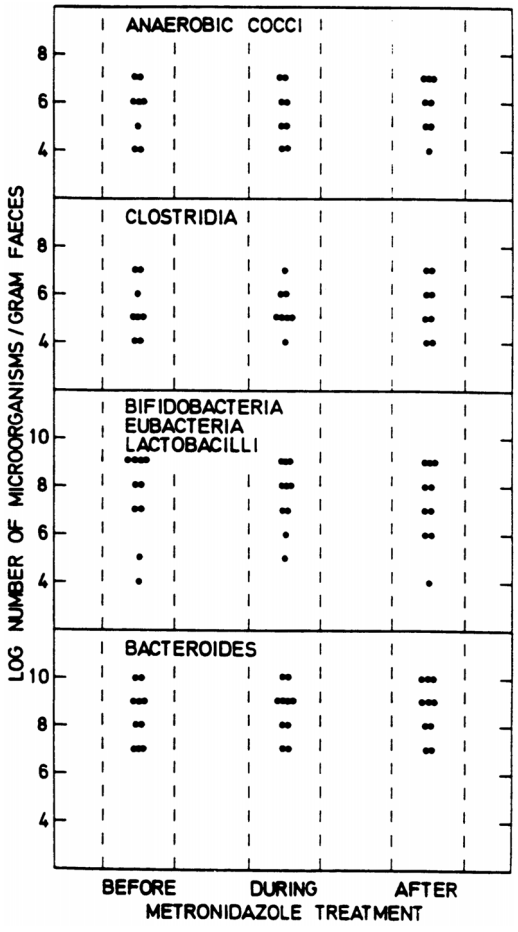

Figure 8: Impact of metronidazole on the anaerobic intestinal microflora in 10 patients. The numbers of microorganisms are given in log numbers per gram faeces.

Norfloxacin

The aerobic intestinal microflora was considerably affected by norfloxacin treatment (Figure 3). The numbers of enterobacteria were eliminated or strongly suppressed. Minor changes in the numbers of enterococci were noticed. The numbers of enterobacteria returned to normal within one month. Figure 4 shows the effect of norfloxacin on the anaerobic intestinal microflora. Bacteroides, bifidobacteria, lactobacilli, eubacteria, clostridia and Gram-positive cocci were not affected while the numbers of Gram-negative cocci decreased.

Impact of narrow spectrum anti-anaerobic agents on the intestinal microflora

Clindamycin

In the aerobic microflora, the numbers of enterococci slightly increased during the clindamycin treatment period but after one month the numbers were in the same range as before clindamycin treatment (Figure 5). No significant changes in the numbers of enterobacteria were observed during or after the administration of clindamycin. Figure 6 presents the effect of clindamycin on the anaerobic microflora. Pronounced changes occurred during clindamycin treatment. The numbers of anaerobic cocci, Gram-positive and Gram-negative rods decreased markedly and in five patients no anaerobic cocci and bacteroides could be isolated. The numbers of clostridia increased during the treatment period. After one month, the anaerobic microflora was normalized in all patients.

Metronidazole

The impact of metronidazole treatment on the aerobic intestinal microflora is shown in Figure 7. The aerobic microorganisms - enterococci and enterobacteria - were only slightly affected during and after treatment. Only minor changes in the anaerobic microflora occurred at the same period (Figure 8). The microflora normalized in all patients after treatment was terminated.

Impact of broad spectrum anti-aerobic/anti-anaerobic agents on the intestinal microflora

Cefoperazone

Figure 9 shows the effect of cefoperazone on the aerobic intestinal microflora. There was a general decrease in the numbers of aerobic microorganisms during the cefoperazone treatment period. In all patients except one, the numbers of enterobacteria were suppressed to undetectable levels during treatment. The enterococci increased in most patients during and after cefoperazone therapy. In many patients, staphylococci and streptococci decreased to undetectable levels during and after treatment. The numbers of Gram-positive rods were also markedly depressed. The numbers of anaerobic microorganisms were significantly changed (Figure 10). The anaerobic cocci, bacteroides, fusobacteria, bifidobacteria, eubacteria and lactobacilli decreased in many patients to undetectable levels. The numbers of clostridia were not so strongly influenced by cefoperazone therapy as the other anaerobic bacterial groups. In most patients, the intestinal microflora returned to pretreatment levels after one month.

Imipenem

The impact of imipenem therapy on the aerobic intestinal microflora is presented in Figure 11. The numbers of enterobacteria decreased slightly during the treatment period and also the numbers of enterococci were affected to a minor extent. The aerobic flora normalized in all patients after the termination of therapy. The anaerobic intestinal microflora was also slightly affected (Figure 12). There was a minor decrease in the numbers of anaerobic cocci

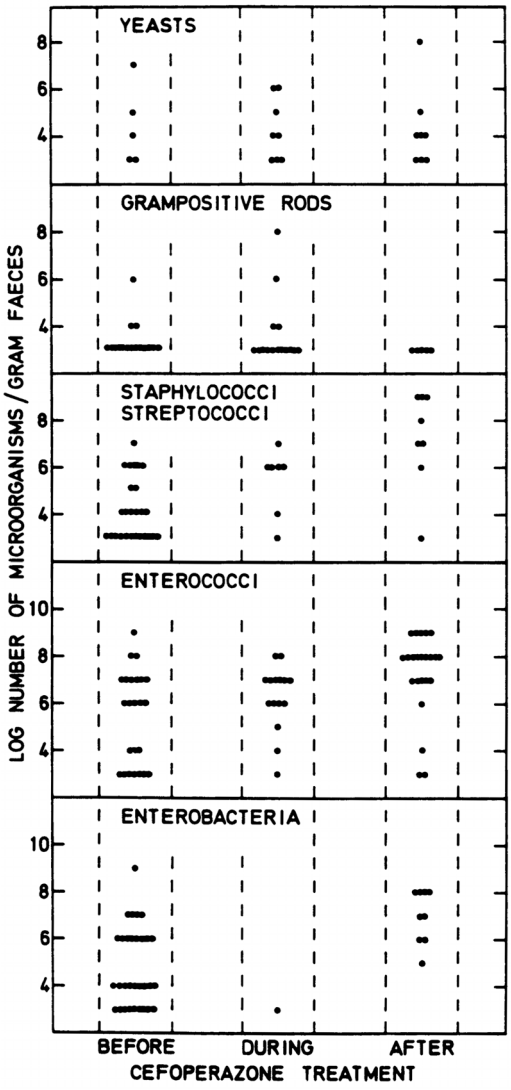

Figure 9: Impact of cefoperazone on the aerobic intestinal microflora in 29 patients. The numbers of microorganisms are given in log numbers per gram faeces.

and bacteroides during the treatment period, while the numbers of Gram-positive rods were not influenced by the imipenem therapy. After treatment the anaerobic microflora returned to normal in all patients.

Concentration of antimicrobial agents in faeces

Table 1 shows the faecal concentrations of aztreonam, norfloxacin, clindamycin, metronidazole, cefoperazone and imipenem before, during and after treatment with respective agent. As can be seen from the table, very high concentrations of cefoperazone were obtained while high norfloxacin concentrations were noticed. Moderate concentrations of aztreonam and clindamycin were demonstrated in faeces. Imipenem and metronidazole could not be detected by the microbiological test.

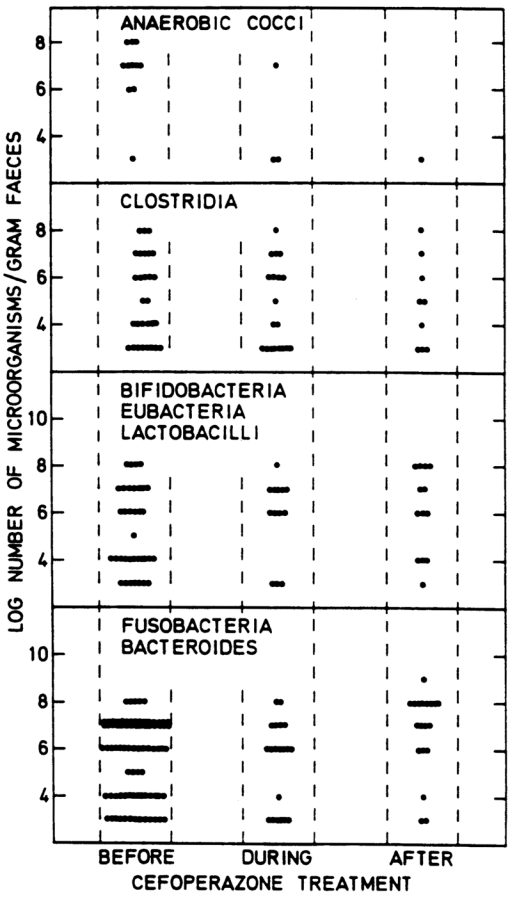

Figure 10: Impact of cefoperazone on the anaerobic intestinal microflora in 29 patients. The numbers of microorganisms are given in log numbers per gram faeces.

DISCUSSION

It has become evident with the introduction of broad spectrum antimicrobial agents that their suppressive activities are directed not only against invading pathogenic microorganisms but also against the host's normal microflora (*Nord* et al., 1986). The changes in the intestinal microflora may result in overgrowth of bacteria and yeasts, proliferation of antimicrobial resistant organisms and increased susceptibility to colonization by new microorganisms (*van der Waaij*, 1982). The knowledge of antimicrobial impacts on the intestinal microflora is especially important in neutropenic and intensive care unit patients in whom the concept of colonization resistance has become a major issue (*Young*, 1989).

In the patients treated with aztreonam, the numbers of enterococci and staphylococci increased. These findings can have clinical implications since enterococcal superinfections during aztreonam treatment have been reported (*Chandrasekar* et al., 1984). The other narrow spectrum antiaerobic agent - norfloxacin - did not cause any significant changes in the aerobic Gram-positive microflora despite high faecal con-

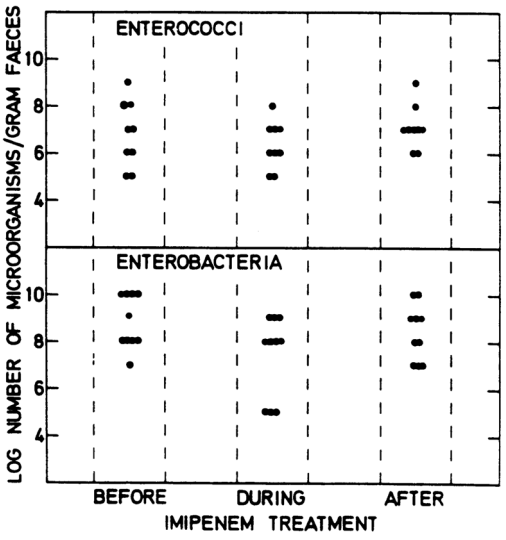

Figure 11: Impact of imipenem on the aerobic intestinal microflora in 10 patients. The numbers of microorganisms are given in log numbers per gram faeces.

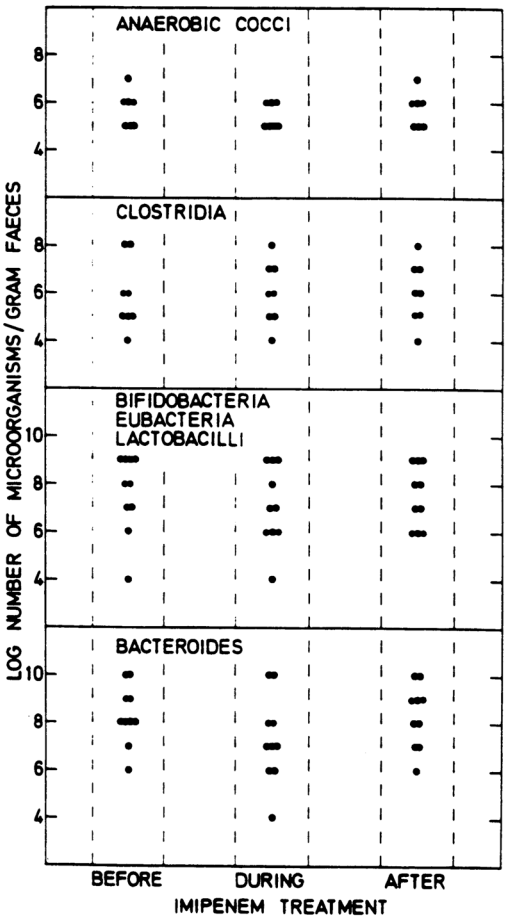

Figure 12: Impact of imipenem on the anaerobic intestinal microflora in 10 patients. The numbers of microorganisms are given in log numbers per gram faeces.

Table 1: Concentrations of aztreonam, norfloxacin, clindamycin, metronidazole, cefoperazone and imipenem, respectively, in faeces in 79 patients (The regimens and dosages are given in Material and Methods)

antimicrobial agent	before treatment		during treatment		after treatment	
	mean value (mg/kg faeces)	range (mg/kg faeces)	mean value (mg/kg faeces)	range (mg/kg faeces)	mean value (mg/kg faeces)	range (mg/kg faeces)
Aztreonam	ND[1]	ND	73	21-88	ND	ND
Norfloxacin	ND	ND	915	305-1900	ND	ND
Clindamycin	ND	ND	110	64-140	ND	ND
Metronidazole	ND	ND	ND	ND	ND	ND
Cefoperazone	ND	ND	4300	2100-7800	ND	ND
Imipenem	ND	ND	ND	ND	ND	ND

[1]ND = not detected

centrations. It has recently been shown that norfloxacin binds to faeces which may explain together with an inoculum effect, the paradox of high faecal concentrations of norfloxacin versus the effect on the intestinal microflora (*Edlund* et al., 1988). Thus different antimicrobial agents with narrow antiaerobic spectra can have different ecological impacts on the intestinal microflora.

Clindamycin caused considerable changes in the intestinal microflora due to the high concentration of the agent in the lower intestinal tract. The clinical implication of this finding is well known: *Clostridium difficile* diarrhoea/colitis. Only minor changes in the intestinal microflora occurred in those patients treated with metronidazole. No measurable concentrations of metronidazole could be demonstrated which explains the actual impact on the intestinal microflora. Thus two agents with similar narrow anti-anaerobic spectra can induce different ecological changes in the intestinal microflora.

Cefoperazone and imipenem have broad antimicrobial spectra including both aerobic and anaerobic intestinal microorganisms. However, only cefoperazone treatment was associated with major changes in the intestinal microflora. Cefoperazone is to a large extent excreted unchanged through the bile to the intestine while less than 1% of imipenem is found in the faeces. Thus two broad spectrum antimicrobial agents can have different ecological impacts on the intestinal microflora.

It has often been stated that narrow antimicrobial agents should always be used in preference to broad spectrum antimicrobial agents in order to avoid these ecological problems. This statement is an oversimplification and other factors such as mode of excretion, activity, inactivation and development of resistance must also be considered. These ecological impacts are often difficult to predict when antimicrobial agents are developed, and the clinical studies of new agents should always include an investigation of their effects on the intestinal microflora.

LITERATURE

Chandrasekar, P.H., Smith, B.R., Le Frock, J.L., and Carr, B.: Enterococcal superinfection and colonization with aztreonam therapy. Antimicrob. Agents Chemother. 26, 280-282 (1984).

Edlund, C., Lindqvist, L., and Nord, C.E.: Norfloxacin binds to human fecal material. Antimicrob. Agents Chemother. 32, 1869-1874 (1988).

Heimdahl, A., and Nord, C.E.: Effect of phenoxymethylpenicillin and clindamycin on the oral, throat and faecal microflora of man. Scand. J. Infect. Dis. 11, 233-242 (1979).

Kager, L., Ljungdahl, I., Malmborg, A.S., Nord, C.E., Pieper, R., and Dahlgren, P.: Antibiotic prophylaxis with cefoxitin in colorectal surgery. Ann. Surg. 193, 277-282 (1981).

Nord, C.E., Heimdahl, A., and Kager, L.: Antimicrobial induced alterations of the human oropharyngeal and intestinal microflora. Scand. J. Infect. Dis. 49, 64-72 (1986).

van der Waaij, D.: Colonization resistance of the digestive tract: clinical consequences and implications. J. Antimicrob. Chemother. 10, 263-270 (1982).

Young, L.S.: Clinical trials of broad-spectrum antibiotics. J. Infect. Dis. 160, 430-432 (1989).