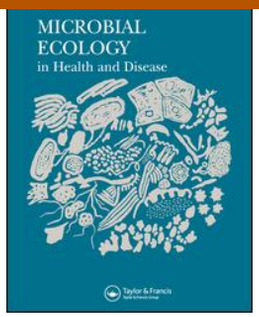

Microbial Ecology in Health and Disease

ISSN: (Print) 1651-2235 (Online) Journal homepage: http://www.tandfonline.com/loi/zmeh20

Effects of Six Antimicrobial Drugs on Rat Intestinal Flora: Is the Rat Model of Predictive Value in Clinical Practice?

E. Bertazzoni Minelli & A. Benini

To cite this article: E. Bertazzoni Minelli & A. Benini (1993) Effects of Six Antimicrobial Drugs on Rat Intestinal Flora: Is the Rat Model of Predictive Value in Clinical Practice?, Microbial Ecology in Health and Disease, 6:2, 53-66

To link to this article: http://dx.doi.org/10.3109/08910609309141562

MICROBIAL ECOLOGY IN HEALTH AND DISEASE VOL. 6: 53–66 (1993)

Effects of Six Antimicrobial Drugs on Rat Intestinal Flora: Is the Rat Model of Predictive Value in Clinical Practice?

E. BERTAZZONI MINELLI* and A. BENINI

Institute of Pharmacology, University of Verona, Policlinico Borgo Roma, 37134 Verona, Italy

Received 1 September 1992; revised 10 February 1993

The effects of six antimicrobial agents on rat intestinal microflora after oral and/or parenteral administration were studied in parallel. Antimicrobial drugs were administered by the oral (norfloxacin, pefloxacin, ciprofloxacin) and parenteral routes (pefloxacin, imipenem, aztreonam, teicoplanin) at the doses used in clinical practice. Faecal specimens were collected before and after two, four, seven or 11 treatments. Qualitative and quantitative determination of flora composition was performed using a modified version of standard methods. Fluoroquinolones reduced the levels of enterobacteria, while gram-positive bacteria (enterococci, staphylococci, lactobacilli) were little affected. Comparable effects were observed after intraperitoneal and oral administration of pefloxacin. The changes induced by fluoroquinolones on intestinal flora showed a uniform trend: certain differences may be ascribed to different pharmacokinetic properties such as bioavailability and metabolism. Imipenem caused a significant decrease in mean concentrations of *E. coli*, clostridia and fungi. Aztreonam induced a prompt and marked inhibition of *E. coli* and *Proteus* spp., while prolonged treatment induced an overgrowth of fungi and bacteroides. Teicoplanin caused a significant decrease in the levels of clostridia and anaerobic lactobacilli. Irregular concentrations of all drugs, with great intersubject variability, were detected at different times. These results are comparable to those observed in humans. The potential of an antimicrobial agent to change the intestinal microflora is related to its antibacterial activity, route of administration and pharmacokinetic properties. The parenteral route induced changes in the intestinal ecosystem, as did oral administration. The rat appears to be a useful experimental model for studying the effects of antimicrobial drugs on intestinal flora.

KEY WORDS—Intestinal microflora; Antibiotics; Fluoroquinolones; Imipenem; Teicoplanin; Aztreonam; Rat.

INTRODUCTION

There is increasing recognition of the importance of the microbial flora as a reservoir of potential pathogens, and iatrogenic changes in intestinal microecology have been considered as being of clinical importance. Although interpersonal variations exist, the intestinal ecosystem has a stable composition.[11]

The faecal flora can be altered by various factors, but pathological conditions and antimicrobial chemotherapy[22,26] induce profound and rapid changes. The imbalance of the ecosystem following the administration of antimicrobial agents may entail other consequences such as new colonisation or overgrowth of naturally non-sensitive microorganisms (Enterobacteriaceae, fungi, staphylococci) or the development of resistance among constituents of the normal flora: in brief, the phenomena that occur in impaired colonisation resistance.[10]

The extent of these changes depends on the antimicrobial properties, pharmacokinetic characteristics and route of administration of the drug.[10,22] Parenteral administration is considered more sparing of the intestinal microflora than oral administration.[21] Following oral administration, a large amount of drug may remain in the gut depending on its bioavailability, while, after parenteral administration, antimicrobial agents may reach the intestinal tract by hepatobiliary excretion or transintestinal elimination.[24,29]

We studied the effects of different antimicrobial drugs in parallel on rat intestinal microflora after oral and/or parenteral administration. Six antibiotics were chosen according to their antimicrobial properties and pharmacokinetic characteristics:

*Author to whom correspondence should be addressed.

0891–060X/93/020053–14 $12.00

aztreonam and teicoplanin were included in the study owing to their selective narrow antimicrobial spectrum against aerobic gram-negative microorganisms and gram-positive cocci, respectively; imipenem was studied because of its broad spectrum, high activity and parenteral route of administration; fluoroquinolones (norfloxacin, ciprofloxacin and pefloxacin) were studied as chemically related compounds with similar antimicrobial activity and different pharmacokinetic characteristics.[27] Pefloxacin was administered both orally and parenterally for the purposes of directly comparing the influence of the route of administration.

The study was conducted in the rat, whose intestinal flora composition may be partly considered similar to that of man. The aim was to assess whether the rat model may be of predictive value in human studies.

METHODS

Animals and treatments

Thirty-five outbred female Wistar rats, 5–6 wk old and weighing 140–160 g, were obtained from Morini (Parma, Italy). The rats were housed under conventional conditions in a temperature ($21 \pm 2°C$) and humidity (55 ± 5 per cent) controlled room with 12 h light/dark cycle (light on from 06.00 to 18.00 h) and kept there for 1 wk before use. The animals were fed with commercial rat pellets (MIL, Morini, Italy) and water *ad libitum*. All rats were housed in groups of five in macrolon cages on sawdust. No special measures were taken to prevent coprophagia.

Rats were divided into seven groups and treated with the following drugs at the dosages indicated: (1) norfloxacin, 10 mg/kg/d, p.o. (Merck-Sharpe & Dohme, Rome); (2) ciprofloxacin, 8 mg/kg/d, p.o. (Bayer, Milan, Italy); (3) perfloxacin, 10 mg/kg/d, p.o.; (4) pefloxacin, 10 mg/kg/d, i.p. administration (Rhône-Poulenc, Milan, Italy); (5) imipenem-cilastatin, 10–10 mg/kg/d, i.m. injection, (Sigma-Tau, Pomezia, Italy); (6) aztreonam, 100 mg/kg/d, i.m. (Menarini, Florence, Italy); (7) teicoplanin, 8 mg/kg/d, i.m. (Lepetit, Milan, Italy).

Solutions were prepared fresh each day by dissolving the drugs in saline, according to the manufacturer's instructions.

All drugs were administered once daily in the morning by gastric intubation (gastric needle-tube) (10 ml/kg body weight) or by parenteral injection (1 ml/kg body weight) for a period of 8–12 d (except Sundays). The doses of antimicrobial drugs corresponded, on a body weight basis, to the doses administered to patients with mild infections in normal clinical practice.

Samples

Four to five fresh faecal pellets were taken aseptically at emission from each rat in the morning before drug administration. Faecal samples were placed in sterile tightly capped vials and processed immediately. Specimens were collected before (control) and after two, four, seven and 11 treatments as well as 18 d after the end of drug administration: each rat was therefore used as its own control.

Culture methods

Quantitative and qualitative analysis of faecal flora was carried out using a modified version[3] of standard techniques.[15,28]

Sterile virology plexiglas plates with 80 wells (8×10, diameter 16 mm, 1 ml volume) were used instead of Petri dishes. Each well was filled with 0·5 ml of the medium. This rapid, accurate technique, which is a simplified micromethod previously described in detail,[2] allows a saving of time and materials, and yields qualitative and quantitative results comparable to those obtained with the more expensive standard techniques (Petri dishes, etc.).

One gram of faeces from each rat was suspended by manual homogenisation in 9 ml prereduced sterile saline and filtered through gauze, whereafter serial ten-fold dilutions were made from 10^{-2} through 10^{-12} in the same fluid. Samples (10 µl) from each dilution were seeded on the surface of selective and non-selective culture media prepared in the plexiglas plates; the same sample was assayed simultaneously on the following 15 media (from Difco Laboratories, Detroit, USA unless otherwise specified): blood agar for total aerobes and anaerobes; Schaedler agar (total anaerobes); kanamycin (12 mg/l)–vancomycin (20 mg/l)–Schaedler agar (bacteroides); Reinforced Clostridial agar (clostridia); *Clostridium difficile* selective agar (Oxoid); Phenylethyl alcohol agar (PEA, Oxoid, gram-positive anaerobic cocci); Mitis Salivarius agar (aerobic and anaerobic cocci); Tomato juice agar and Rogosa SL agar (bifidobacteria, aerobic and anaerobic lactobacilli); Bile esculine azide agar (enterococci); Mannitol salt agar (staphylococci); McConkey agar and SS agar (Enterobacteriaceae); Littman oxgall agar and Sabouraud dextrose agar (yeasts). Duplicate samples of 0·1 ml of the original 1/10 suspension of faeces were seeded on blood agar

solidified in Petri dishes. All steps of the microbiological assay were carried out in an anaerobic box (95 per cent CO_2–5 per cent H_2 atmosphere). The aerobic plates were incubated at 37°C for 24 h and anaerobic plates for 48–72 h in an anaerobic chamber (Heraeus) in an 85 per cent N_2–10 per cent CO_2–5 per cent H_2 atmosphere, at negative pressure (400 Torr). After incubation, different colony types were counted, from both aerobic and anaerobic plates.

We considered as positive the last well presenting growth of at least three colonies. The number of wells showing the growth of bacteria indicated the logarithm of the concentration of microorganisms per gram of faeces (quantitative determination). Thereafter, the morphologically distinct colonies were isolated in pure culture and identified by standard methods on the basis of Gram-stain and morphological and biochemical analysis (API System S.A., La Balme Les Grottes, France). Enterobacteriaceae were identified using an API 20 E test-kit system; streptococci by API 20 Strep; staphylococci by API Staph; fungi by API C AUX, and anaerobes by the Rap ID ANA System. The lowest detectable number of organisms is 2·0 $\log_{10}$ of the number of bacteria per gram of faeces. Results are expressed as mean $\log_{10}$ viable organisms per gram sample ± standard deviation of the mean.

Faecal samples were tested for the presence of *C. perfringens* enterotoxins by the reversed passive latex agglutination test (Oxoid PET RPLA).[1] *C. difficile* cytotoxin was assayed by inoculating fixed dilutions (in PBS) of stool filtrates into 96-well microdilution plates seeded with HeLa cells, incubated overnight and read after 24 h. Positive samples were confirmed by neutralisation with a specific antitoxin.[9]

Student's *t*-test was used for comparison of mean values. A probability level of $P<0·05$ was considered significant.

Antibiotic concentration in faeces

The concentrations of antibiotics in faeces were determined on the first dilution of the samples used for the microbiological determination.

Samples of faeces of rats treated with imipenem were immediately stabilised by addition of an equal volume of MES (morpholinethan-sulphonate buffer, pH 6) plus ethylene glycol (1:1 mixture)[2,3] in order to reduce possible degradation of the drug. The samples were centrifuged and the assay carried out on supernatant using the agar well diffusion microbiological method. The media, test microorganisms and final concentration of inoculum are listed in Table 1. The assay plates (24 × 24 cm, Bioassay, Nunc, Denmark) were overlaid with different agar media. After solidification, 8 mm diameter wells were punched out of the agar medium and filled with 50 µl of supernatant. Appropriate standard concentrations of each drug were prepared in sterile phosphate-buffered saline (pH = 7). Imipenem dilutions were obtained in MES-ethylene glycol. All samples were assayed in triplicate. After overnight incubation at 37°C, the inhibition zones were measured. The antibiotic concentrations in the faeces were determined by comparison with results obtained with the antibiotic standards and were expressed as µg of antibiotic per g faeces.

RESULTS

Effects of drugs on intestinal microflora of rats

The quantitative effects of different antibiotics on rat faecal flora composition are summarised in Tables 2–5, while qualitative changes are shown in Table 6. In the figures, the results are expressed as the difference in mean values vs controls, where each column represents the positive or negative effects of antibiotic treatments on colonic microflora in comparison to the mean count values determined before drug administration (baseline = time 0). Each animal is its own control, i.e. the composition of the flora was followed up in each animal before, during and after the end of treatment.

We noted a number of variations in the faecal flora of rats in pretreatment samples, fungi being predominant and the levels of bacteroides and bifidobacteria very low. This composition was different from that of animals utilised in other experiments, obtained from the same breeders and of the same species, sex, age, weight, etc., and maintained in the same standard housing conditions. Each rat was assigned to different groups on the basis of the faecal composition analysis done before drug administration, and therefore each group showed the same rate of variability. We decided to perform these experiments because this condition may reflect the sample variability in human practice.

All treatments induced the disappearance of gram-positive non-spore-forming rods, namely bifidobacteria.

Table 1. Microbiological assay of antimicrobial drugs: media, microorganisms, and inoculum concentrations

Drugs	Medium (agar)	Test microorganisms	Inoculum* (ml/100 ml agar)
Aztreonam	Antibiotic Medium 1 (Difco)	*E. coli* SC 12155	0·15
Ciprofloxacin	Isosensitest (Oxoid)	*E. coli* ATCC 25922	0·50
Pefloxacin	Isosensitest (Oxoid)	*E. coli* Kp 05124	0·15
Norfloxacin	Isosensitest (Oxoid)	*E. coli* Kp 05124	0·10
Teicoplanin	Isosensitest (Oxoid)	*B. subtilis* ATCC 6633 (Spore suspension; Difco)	0·02
Imipenem	Brain–Heart Agar (Difco)	*B. subtilis* ATCC 6633 (Spore suspension; Difco)	0·02

*Inoculum from 18 h culture.

Fluoroquinolones

The changes induced by fluoroquinolones on intestinal flora showed a uniform trend.

Norfloxacin (Table 2). Norfloxacin caused significant inhibition of *Escherichia coli*, yeasts, total anaerobes, clostridia and anaerobic lactobacilli after two and four treatments. After 1 wk of treatment *E. coli* and anaerobic lactobacilli continued to be inhibited. It is interesting to note the substantial decrease in levels of fungi (5 log) after two and four administrations of norfloxacin. Bacteroides, enterococci and anaerobic cocci were not affected by norfloxacin treatment (Figure 1).

Ciprofloxacin (Table 2). Four treatments with ciprofloxacin caused significant inhibition of *E. coli* (4–5 log), yeasts, bacteroides, clostridia, anaerobic lactobacilli and cocci. After 1 wk of treatment these effects were still observable for Enterobacteriaceae, while yeasts and most components returned to normal values (Figure 1). Prolonged administration (11 treatments) caused an increase in yeasts, bacteroides and clostridia compared to mean values determined before drug administration.

Group D streptococci were little affected by treatment with ciprofloxacin at any of the times considered.

Pefloxacin. The oral administration of pefloxacin caused strong inhibition of *E. coli* and yeasts at all times; enterococci increased (Table 3). These effects were comparable to those observed after intraperitoneal administration of the drug. Pefloxacin, administered by the parenteral route, induced marked changes at the beginning of treatment (2 days). The modifications corresponded to high concentrations of drug in stools. Staphylococci were also inhibited. After 1 wk of treatment the behaviour was similar in both groups of rats treated via the different routes (Figure 1).

Pefloxacin, ciprofloxacin and norfloxacin induced comparable quantitative changes (Figure 1) as well as changes in the bacterial species. The administration of all fluoroquinolones caused the disappearance of *Bifidobacterium* spp. and *E. coli* 2 (a biotype frequently isolated), as well as other changes shown in Table 6. Among the enterococci, we frequently found *E. faecalis* instead of *E. faecium*.

Imipenem (Table 4)

Imipenem confirmed its broad spectrum of activity against intestinal flora. After two treatments, imipenem caused a significant decrease in mean counts of *E. coli* (4 log), clostridia (6 log), total anaerobic count and fungi. Enterococci were also

Table 2. Effect of oral administration of norfloxacin (10 mg/kg/d) and ciprofloxacin (8 mg/kg/d) on faecal flora during treatment and 18 d after end of treatment. Results are expressed as $\log_{10}$ no. bacteria/g fresh faeces (mean ± SD, $n = 5$). Antibiotic concentrations in stool (μg/g) are included

Micro organisms	Norfloxacin					Ciprofloxacin			
	0†	2†	4†	7†	18 d after treatment	0†	4†	7†	11†
E. coli	9·6 ± 2·1	4·0 ± 1·2**	4·4 ± 0·5**	7·4 ± 1·1	6·0 ± 0·0**	7·6 ± 2·1	2·3 ± 0·7**	4·5 ± 3·8**	2·4 ± 1·3**
P. mirabilis	2·8 ± 1·1	2·4 ± 0·9	< 2·0	< 2·0	< 2·0	2·8 ± 1·3	< 2·0	2·2 ± 0·4	2·4 ± 0·9
Enterococci	4·6 ± 0·5	5·0 ± 0·7	4·6 ± 0·5	4·0 ± 1·2	6·2 ± 1·2*	4·1 ± 0·9	4·2 ± 0·8	5·3 ± 0·8**	5·9 ± 1·9**
Staphylococci	3·0 ± 0·0	3·2 ± 0·4	3·2 ± 0·4	2·6 ± 0·5	2·7 ± 0·5	2·8 ± 0·4	3·0 ± 1·0	2·7 ± 0·7	2·4 ± 0·5
Lactobacilli	5·4 ± 0·5	4·0 ± 0·7	4·6 ± 1·1	6·8 ± 0·4**	4·0 ± 0·8	5·9 ± 1·5	6·2 ± 0·8	3·4 ± 1·3**	4·2 ± 1·3
Fungi	9·2 ± 1·8	4·0 ± 1·0**	4·2 ± 0·4**	8·0 ± 0·7	5·5 ± 0·5**	7·5 ± 1·6	2·6 ± 0·5**	7·0 ± 4·1	10·0 ± 0**
Total anaerobes	9·2 ± 1·6	6·4 ± 2·4*	6·4 ± 2·3*	7·4 ± 2·1	4·3 ± 0·5**	7·5 ± 2·8	3·8 ± 0·4	7·6 ± 2·9	7·4 ± 1·9
Bacteroides	4·2 ± 0·8	3·2 ± 1·1	3·6 ± 0·5	5·8 ± 1·5	3·8 ± 0·9	4·6 ± 0·9	3·6 ± 0·9*	3·6 ± 0·9*	5·6 ± 2·1
Clostridia	7·2 ± 2·8	3·6 ± 0·4*	4·0 ± 0·7*	5·6 ± 0·9	3·2 ± 0·9*	5·9 ± 1·6	4·0 ± 0·7*	4·4 ± 1·5	7·8 ± 0·5*
AnO_2 lactobacilli	9·4 ± 1·3	6·4 ± 2·3*	5·3 ± 2·1**	4·2 ± 0·4**	7·2 ± 1·0*	7·0 ± 1·6	4·6 ± 0·5**	8·4 ± 2·3	6·4 ± 4·1
AnO_2 cocci	3·2 ± 1·1	4·2 ± 0·8	5·6 ± 1·9*	4·8 ± 0·4**	5·2 ± 0·9*	5·7 ± 1·9	3·8 ± 0·4*	6·6 ± 2·3	5·4 ± 1·1
Drug conc. (μg/g)		0 (5/5)	0·9 ± 0·1 (3/5)	3·2 ± 0·2 (4/5)			0	1·7 ± 0·7 (5/5)	11·9 ± 6·7 (4/5)

†Number of treatments.
Significance: *$P \leq 0{\cdot}05$; **$P \leq 0{\cdot}01$ versus control. Student's t test.
Figures in parentheses are no. of positive samples/total no. of rats.

Table 3. Effect of oral and intraperitoneal administration of pefloxacin (10 mg/kg/d), on faecal flora during treatment and 18 d after end of treatment. Results are expressed as $\log_{10}$ no. bacteria/g fresh faeces (mean ± SD, $n = 5$). Antibiotic concentrations in stool (μg/g) are included

	Oral pefloxacin					Intraperitoneal pefloxacin				
Micro organisms	0†	2†	4†	7†	18 d after treatment	0†	2†	4†	7†	18 d after treatment
E. coli	6·2 ± 1·7	5·5 ± 0·5	4·2 ± 0·5	4·5 ± 0·6	6·0 ± 1·1	6·2 ± 1·7	2·5 ± 0·6**	4·0 ± 0·8*	4·2 ± 0·5	7·3 ± 1·1
P. mirabilis	3·2 ± 0·9	3·5 ± 1·3	< 2·0	2·5 ± 0·6	3·2 ± 0·5	3·2 ± 0·9	< 2·0	2·5 ± 0·6	3·0 ± 0·0	2·3 ± 0·5
Enterococci	4·0 ± 0·0	7·2 ± 1·5*	6·0 ± 0·8*	5·2 ± 1·2	4·7 ± 0·5	4·0 ± 0·0	4·7 ± 0·9	4·2 ± 0·5	5·5 ± 0·5*	5·0 ± 0·0
Staphylococci	4·0 ± 0·0	3·4 ± 0·9	3·0 ± 0·0	3·9 ± 0·3	2·6 ± 0·5	4·0 ± 0·0	2·5 ± 0·5**	2·7 ± 0·5**	3·8 ± 0·5	3·0 ± 0·0
Lactobacilli	3·5 ± 1·1	3·5 ± 0·6	5·5 ± 0·7	6·0 ± 0·8	3·7 ± 0·5	3·5 ± 2·1	2·7 ± 0·9	6·2 ± 1·7	6·2 ± 0·5	3·6 ± 0·6
Fungi	9·5 ± 0·7	3·0 ± 0·0**	4·0 ± 0·8**	4·0 ± 0·0**	5·7 ± 0·9**	9·5 ± 0·7	3·2 ± 0·9**	3·7 ± 0·9**	3·7 ± 0·5**	7·0 ± 1·7*
Total anaerobes	7·7 ± 2·6	6·5 ± 1·7	5·7 ± 1·2	4·2 ± 0·5*	7·2 ± 1·5	7·7 ± 2·6	7·7 ± 2·1	4·2 ± 1·7	4·0 ± 0·0*	8·0 ± 1·0
Bacteroides	4·5 ± 2·4	3·7 ± 0·7	2·7 ± 0·5	3·0 ± 0·0	5·2 ± 1·3	4·5 ± 2·4	3·5 ± 1·0	2·5 ± 0·6	3·2 ± 0·5	5·0 ± 1·7
Clostridia	5·2 ± 2·5	4·0 ± 1·6	5·5 ± 1·7	6·5 ± 2·5	7·5 ± 1·7	5·2 ± 2·5	3·0 ± 0·8	4·7 ± 0·5	7·5 ± 2·6	6·6 ± 0·6
AnO_2 lactobacilli	5·2 ± 1·7	7·0 ± 3·5	5·5 ± 1·3	6·5 ± 3·7	5·2 ± 0·5	5·7 ± 1·7	9·7 ± 0·5**	6·7 ± 2·1	4·5 ± 0·6	5·3 ± 0·6
AnO_2 cocci	6·0 ± 2·1	4·2 ± 0·5	8·7 ± 1·5	4·0 ± 0·0	4·2 ± 0·5	6·0 ± 2·2	3·7 ± 1·2	7·2 ± 1·7	4·0 ± 0·0	5·0 ± 0·0
Drug conc. (μg/g)		0	1·6 ± 0·1 (2/4)	1·8 ± 0·4 (5/5)			4·6 ± 2·3 (5/5)	1·5 (1/5)	1·9 ± 0·4 (4/5)	

†Number of treatments.
Significance: *$P \leq 0{\cdot}05$; **$P \leq 0{\cdot}01$ versus control. Student's t test.
Figures in parentheses are no. of positive samples/total no. of rats.

Table 4. Effect of intramuscular administration of imipenem (10 mg/kg/d) and aztreonam (100 mg/kg/d) on faecal flora during treatment and 18 d after end of treatment. Results are expressed as $\log_{10}$ no. bacteria/g fresh faeces (mean ± SD, $n = 5$). Antibiotic concentrations in stool (μg/g) are included

	Imipenem					Aztreonam			
Micro organisms	0†	2†	4†	7†	18 d after treatment	0†	4†	7†	11†
E. coli	9·0 ± 3·4	4·8 ± 1·1*	6·2 ± 2·2	7·4 ± 0·5	6·3 ± 0·9	6·8 ± 1·4	3·2 ± 1·2**	2·8 ± 0·9**	2·2 ± 0·4**
P. mirabilis	3·0 ± 1·1	2·6 ± 0·9	2·4 ± 0·9	2·6 ± 1·3	2·5 ± 1·0	3·7 ± 1·2	3·2 ± 1·1	3·4 ± 0·5	2·6 ± 0·9
Enterococci	6·5 ± 0·5	5·4 ± 0·5*	4·8 ± 0·8*	5·2 ± 0·4**	5·3 ± 1·2	6·0 ± 1·1	4·5 ± 0·7**	5·8 ± 0·8	4·2 ± 0·4**
Staphylococci	3·0 ± 0·0	3·0 ± 0·6	3·0 ± 0·0	2·8 ± 0·8	2·7 ± 0·5	4·1 ± 1·0	2·6 ± 0·9**	3·0 ± 1·2	2·8 ± 0·8**
Lactobacilli	4·5 ± 0·5	3·4 ± 0·9	6·4 ± 2·8	3·0 ± 0·0**	3·5 ± 1·0	6·5 ± 2·6	5·2 ± 0·4	3·8 ± 1·0*	3·8 ± 1·1*
Fungi	9·5 ± 2·8	5·6 ± 1·5*	5·8 ± 1·9*	8·8 ± 0·8	6·3 ± 0·9*	7·0 ± 1·3	4·6 ± 1·1**	4·2 ± 0·4**	10·0 ± 0·0**
Total anaerobes	12·0 ± 0·0	7·4 ± 2·4**	5·2 ± 0·4**	4·6 ± 0·5**	4·8 ± 0·9**	4·9 ± 0·8	4·0 ± 0·7	5·2 ± 1·1	7·6 ± 2·3**
Bacteroides	2·5 ± 0·6	3·4 ± 0·5*	3·4 ± 0·5*	3·8 ± 0·4**	3·7 ± 0·5**	2·8 ± 0·8	3·6 ± 0·5	3·2 ± 0·4	8·6 ± 2·6**
Clostridia	10·5 ± 1·7	3·8 ± 0·4**	4·4 ± 0·5	3·8 ± 0·4**	4·2 ± 1·5**	6·6 ± 2·9	5·0 ± 1·2	7·2 ± 1·3	7·4 ± 1·3
AnO_2 lactobacilli	5·0 ± 0·0	5·4 ± 1·5	4·6 ± 0·5	3·8 ± 0·8**	5·0 ± 0·7	5·1 ± 1·0	4·4 ± 0·5	7·8 ± 1·5**	6·8 ± 2·1*
AnO_2 cocci	4·0 ± 0·0	4·6 ± 0·9	4·6 ± 0·5	4·4 ± 0·9	5·7 ± 1·2	7·1 ± 2·9	4·0 ± 1·0*	6·8 ± 1·1	5·0 ± 1·0
Drug conc. (μg/g)		0	1·1 ± 0·2 (4/5)	0·9 ± 0·2 (3/5)			0	0	121·5 ± 27·9 (3/5)

†Number of treatments.
Significance: *$P \leq 0{\cdot}05$; **$P \leq 0{\cdot}01$ versus control. Student's t test.
Figures in parentheses are no. of positive samples/total no. of rats.

Table 5. Effect of i.m. administration of teicoplanin (8 mg/kg/d) on faecal flora during treatment and 18 d after end of treatment. Results are expressed as $\log_{10}$ no. bacteria/g fresh faeces (mean ± SD, $n = 5$). Antibiotic concentrations in stool (μg/g) are included

Micro organisms	Treatments (no.) 0	2	4	7	18 d after treatment
E. coli	8·0±3·0	6·0±1·2	5·8±0·8	9·0±1·0	5·6±0·5
P. mirabilis	2·0±0·0	2·0±0·0	2·0±0·0	2·6±0·9	2·6±0·9
Enterococci	5·4±0·5	5·8±0·8	5·4±0·5	5·2±0·4	6·2±0·4
Staphylococci	3·0±0·0	2·3±0·5**	3·0±0·0	3·0±0·0	2·8±0·4
Lactobacilli	4·0±0·0	3·6±0·9	5·4±2·3	3·0±0·0	4·2±0·4
Fungi	8·0±3·0	7·0±2·8	5·8±1·1	9·4±0·9	5·6±1·1
Total anaerobes	11·8±0·4	7·8±2·1**	6·0±2·3**	9·4±1·3**	4·7±0·9**
Bacteroides	3·4±0·5	3·6±1·1	4·0±0·7	4·8±0·4	3·6±0·5
Clostridia	12·0±0·0	4·2±0·4**	4·2±0·8**	5·4±0·5**	5·0±0·7**
AnO_2 lactobacilli	7·8±1·0	5·0±1·9*	4·4±0·9**	4·4±0·5**	7·2±2·2
AnO_2 cocci	4·2±0·4	4·6±0·4	6·0±1·2*	4·8±0·8	5·2±1·1
Drug conc. (μg/g)		0	0	1·3±0·2 (3/5)	

Significance: *$P \leq 0{\cdot}05$; **$P \leq 0{\cdot}01$ versus control. Student's t test.
Figures in parentheses are no. of positive samples/total no. of rats.

inhibited, while bacteroides increased. These effects were maintained throughout the treatment (Figure 2). We observed new colonisation by *Proteus penneri* instead of *P. mirabilis*, which is a constant and normal component of rat intestinal flora. Imipenem induced no qualitative changes in the clostridia. At the end of treatment, when yeasts were present in normal levels, we isolated ascospores of the *Candida* genus.

Aztreonam (Table 4)

The administration of aztreonam, a selective agent against gram-negative aerobic bacteria, caused a marked, prompt inhibition of *E. coli* (2–3 log) and *Proteus* spp. This effect was maintained throughout the treatment and 18 d after the end of drug administration. We also observed an initial decrease in the number of anaerobes which returned to normal values in the course of treatment.

After 11 treatments there was a significant increase ($P \leq 0{\cdot}01$) in fungi levels (3 log) and overgrowth of bacteroides (6 log) (Figure 2).

Aztreonam caused the disappearance of all the most common Enterobacteriaceae including *E. coli* 1 and *E. coli* 2, but selected potentially pathogenic microorganisms such as *Klebsiella pneumoniae*, *K. oxytoca*, *Morganella morganii* and *Shigella sonnei*.

Teicoplanin

Teicoplanin showed marked inhibition of clostridia and anaerobic lactobacilli as reported in Table 5. This significant inhibitory effect was maintained in the course of seven treatments. Staphylococci were slightly affected after two treatments only, while the mean enterococci count was unaltered (Figure 2). However, among the enterococci, we observed a number of qualitative changes: *E. faecalis*, a major component of normal rat flora, was inhibited by teicoplanin, and *E. faecium* was isolated in the course of treatment. Moreover, teicoplanin caused disappearance of the gram-positive anaerobic non-spore-forming bacilli.

Long-term effects

Eighteen days after the end of treatment the concentrations of intestinal microflora were normalised in pefloxacin-treated rats, while in other groups the total anaerobe levels remained lower, including the concentrations of clostridia. The concentrations of fungi 18 d after the end of treatment were lower than those observed before drug

Table 6. Qualitative effects of antimicrobial treatments on the composition of rat faecal flora expressed as inhibition of the prevalent species and growth of new species isolated during treatments

	Microorganisms	
Antibiotic	Inhibition	Growth
Teicoplanin	*Bifidobacterium* spp.	*E. coli* 2*
	Escherichia coli 2	*E. faecium*†
	Enterococcus faecalis	*Actinomyces odontholyticus*
	Eubacterium limosum	*C. limosum*
	Clostridium perfringens	*C. cadaveris*
	Veillonella spp.	*C. histolyticum*
	Bacteroides levii	
Imipenem	*Bifidobacterium* spp.	*E. coli* 2*
	E. coli 2	*E. faecium*†
		Proteus penneri
		Candida spp.
Aztreonam	*Bifidobacterium* spp.	*Klebsiella oxytoca*
	E. coli 2	*K. pneumoniae*
	E. coli 1	*Morganella morganii*
	Propionibacterium granulosum	*Shigella sonnei*
	C. tyrobutyricum	*C. butyricum*
	C. fallax	*C. innocuum*
Fluoroquinolones norfloxacin, pefloxacin	*Bifidobacterium* spp.	*B. capillosus*
	E. coli 2	*E. lentum*
	E. faecium	*P. acnes*
	P. granulosum	*Veillonella* spp.
	B. buccae	*C. ramosum*
	C. fallax	*C. beijerinkii*
	C. tyrobutyricum	*C. butyricum*
		C. limosum
		C. histolyticum
		In addition in pefloxacin groups:
		K. pneumoniae
		Fusobacterium necrogenes
		B. intermedius
		Streptococcus intermedius
		S. constellatus
		S. morbillorum
		E. limosum
		A. israelii
Ciprofloxacin	*Bifidobacterium* spp.	*P. acnes*
	E. coli 2	*C. ramosum*
	P. granulosum	*C. beijkerinkii*
	C. fallax	*C. butyricum*
	C. tyrobutyricum	

*Isolated at the end of drug administration (seven treatments).
†High frequency.

administration, but these values correspond to normal levels in rats analysed in other experiments. Moreover, the qualitative changes shown in Table 6 were maintained. Neither *C. perfringens* enterotoxins nor *C. difficile* and its cytotoxins were found.

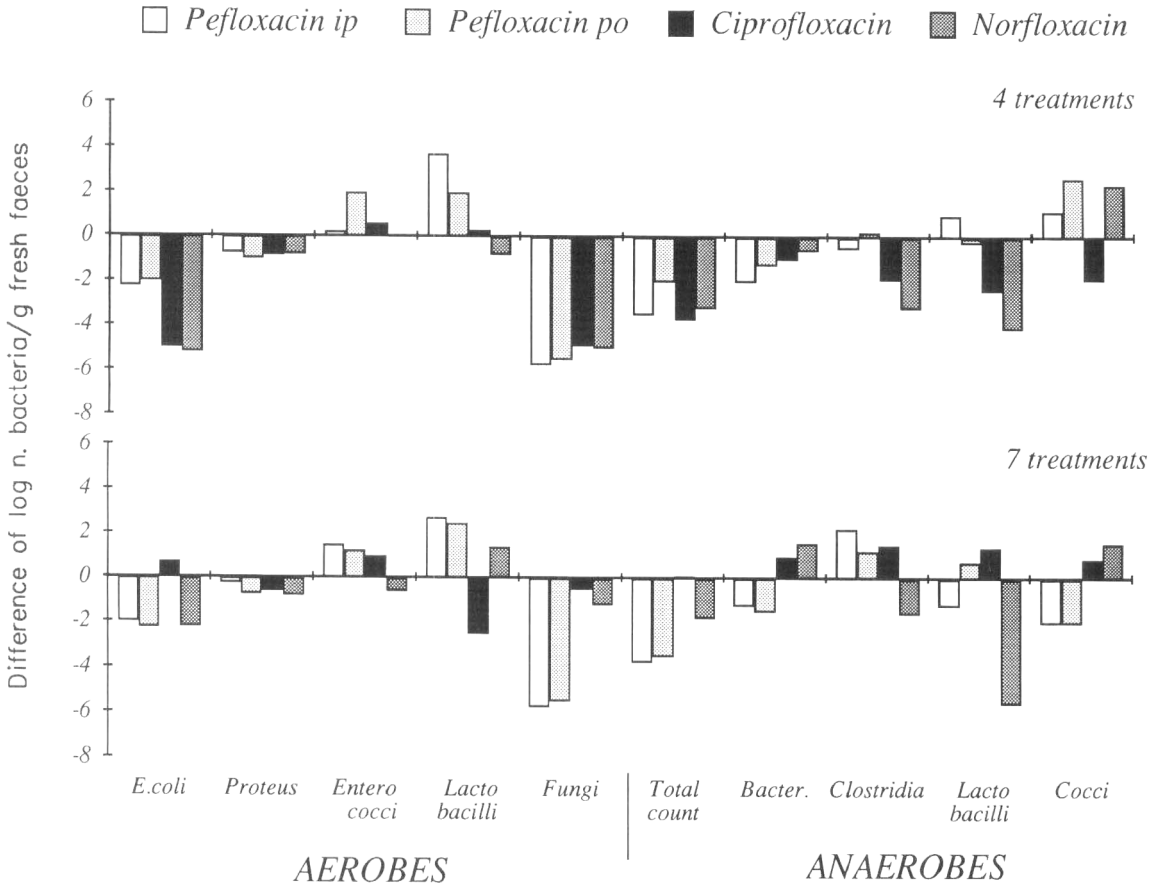

Figure 1. Changes in faecal microflora composition of rats treated with pefloxacin (10 mg/kg/d, oral and i.p.), ciprofloxacin (8 mg/kg/d, oral) and norfloxacin (10 mg/kg/d, oral) after four and seven treatments. Results are expressed as the difference in mean $\log_{10}$ count values obtained during treatments vs mean count determined in the same rat before starting treatment (baseline = time 0)

Antibiotic concentrations in faeces

Following two treatments pefloxacin administered by the intraperitoneal route was present in faeces in appreciable concentrations (Table 3). After 1 wk of treatment, all antibiotics were eliminated in faeces with the exception of aztreonam. Prolonged administration (11 days) also induced substantial faecal elimination of aztreonam ($121{\cdot}5 \pm 27{\cdot}9$ μg/g of faeces) and ciprofloxacin ($11{\cdot}9 \pm 6{\cdot}7$ μg/g).

The presence of different antibiotics was irregular, with considerable intersubject variability, as shown by the numbers of positive samples.

DISCUSSION

The present work confirms that antimicrobial drugs may influence the intestinal microbial flora to different extents.

The microflora changes were evident at the beginning of drug treatment: two administrations once daily induce early significant modifications which may progressively become more marked (imipenem and teicoplanin) or may revert to normal values in the course of treatment (ciprofloxacin).

The effects induced by fluoroquinolones are representative for this class of antimicrobial drugs. Quantitative changes, such as a marked suppression of Enterobacteriaceae, were observed alongside qualitative modifications of other components of the intestinal microflora. *E. coli* 2, *E. faecium*, bifidobacteria and *C. tyrobutyricum* disappeared and were replaced by new species, such as the potential pathogens *A. israelii* and *B. intermedius*. Certain differences in the intestinal effects of quinolones may be ascribed more to their pharmacokinetic properties than to their antimicrobial activity. For example, ciprofloxacin, a drug with good

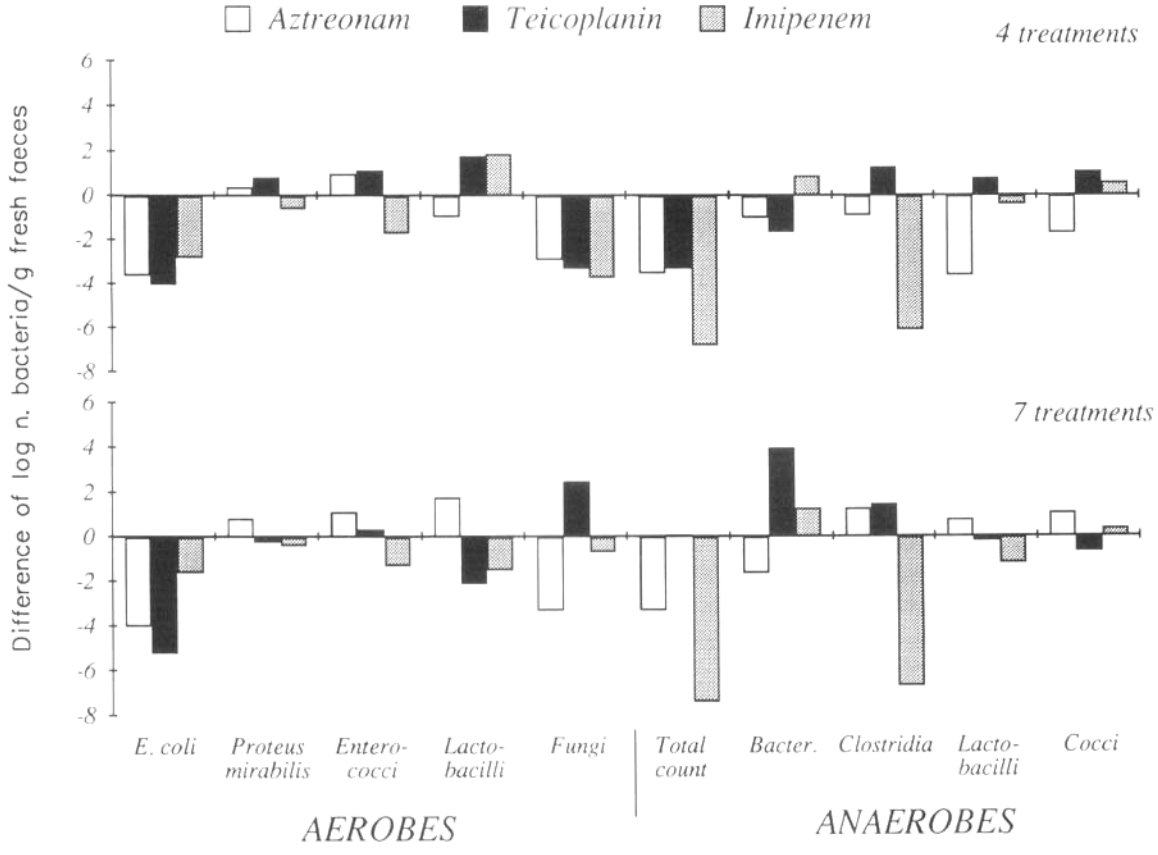

Figure 2. Changes in faecal microflora composition of rats treated with aztreonam (100 mg/kg/d, i.m.), teicoplanin (8 mg/kg/d, i.m.) and imipenem (10 mg/kg/d, i.m.). Results are expressed as the difference in mean $\log_{10}$ count values obtained during treatments vs mean count determined in the same rat before starting treatment (baseline = time 0)

bioavailability and low hepatobiliary excretion,[6,27] showed good activity against gram-negative bacilli and did not cause any significant changes in the faecal concentration of enterococci or yeasts. Moreover, ciprofloxacin induced slight modifications in anaerobic flora. Among the quinolones, ciprofloxacin seems to be more sparing of the intestinal ecosystem. Norfloxacin induced more evident changes than those observed with ciprofloxacin; we considered this effect to be a consequence of the lower bioavailability of the drug. The effects of pefloxacin were of the same order as those caused by norfloxacin. However, pefloxacin, which is a drug possessing good bioavailability, is partly secreted into the bile as parent drug and as metabolites, norfloxacin being the most active (30%).[13] Therefore, from the point of view of the impact on the intestinal ecosystem, the different pharmacokinetic properties and metabolic pathways of norfloxacin and pefloxacin are of little relevance. No significant differences were observed in antimicrobial activity against intestinal microflora between oral and parenteral administration of pefloxacin. The effects on faecal flora and its recovery in faeces are closely related to those described in humans after oral[16] and parenteral administration.[31] The faecal concentrations of quinolones were all in the same range after seven days of treatment.

These results are in agreement with those observed in humans, as reported in a recent review by Nord and Edlund,[22] who concluded that 'the administration of all quinolones caused a marked suppression or elimination of Enterobacteriaceae in humans, while enterococci and anaerobic micro-organisms were little affected'.

Aztreonam and teicoplanin administered parenterally induced changes in specific bacterial species, according to their respective antimicrobial activities.[5,7] The aerobic gram-negative flora was

suppressed during treatment with aztreonam; the impact on anaerobic bacteria was variable depending on the period of administration. Prolonged administration of aztreonam induced an overgrowth of bacteroides and yeasts and corresponding high levels of the drug in the faeces. Aztreonam treatment in mice did not lead to complete eradication of aerobic gram-negative rods in faeces.[30] In our experiments most Enterobacteriaceae were inhibited, while *M. morganii* and *Klebsiella* spp. appeared; complete eradication of these microorganisms from the faeces could not be achieved in our rats. These data are similar, but not identical to those observed in patients.[17] Cancer patients receiving 2 g of aztreonam every 8 h for 7–9 d showed persistence of isolates of bacteroides and yeasts, but no overgrowth of these microorganisms.

Teicoplanin induced a significant decrease in the number of clostridia; the concentrations obtained in faeces were lower than the minimum inhibitory concentrations for most cocci. These effects were comparable to those observed in humans treated with teicoplanin as perioperative prophylaxis in orthopaedic surgery.[4] Imipenem is highly active against gram-positive and gram-negative bacteria[8] and covers both aerobic and anaerobic components of the intestinal microflora. Although the faecal elimination is very low, imipenem induced constant minor changes in aerobic and anaerobic rat flora. These changes corresponded in terms of quality and intensity to those observed in patients treated with imipenem for surgical prophylaxis.[18]

The imbalance of the intestinal flora observed during this study was evident in the course of the treatments but not severe, and *C. difficile* was not isolated: 18 d after the end of drug administration the intestinal microflora became fairly normalised.

These results provide further evidence that the parenteral route of administration, whether intraperitoneal or intramuscular, can also induce significant disturbances in the intestinal microflora. In fact, the antimicrobial drugs may reach the intestinal contents via transintestinal secretion,[24] blood diffusion, bile[21] or lymphatic fluid.[12,14,20] Even parenteral administration of antimicrobial drugs with a narrow spectrum and poor secretion into bile[19] may induce modifications. Moreover, on increasing the duration of treatment these effects become more evident. The increase in faecal drug concentrations corresponded to a proportional inhibitory effect on the mean microbial concentrations. Our method also allows discriminatory evaluation of the effects of chemically related drugs, as shown by comparative analysis of the fluoroquinolones. We are also able to note changes in species of microorganisms whose mean concentrations are not affected, as occurs after administration of aztreonam against gram-positive non-spore-forming bacilli and teicoplanin against enterococci and bacteroides.

Although the flora composition was unusual before treatments, these results obtained in rats are comparable to those reported in studies carried out in humans, as recently reviewed by Nord and Edlund.[22] Therefore, the experimental data may partly reflect what occurs in humans, namely the effects on the numbers and genera of microorganisms. Among the different animal species, the rat may be considered the one with the intestinal flora closest to that of man in terms of composition, though not in terms of metabolic capacity.[25] The latter may be a factor influencing the antimicrobial activity of orally administered antibiotics, as well as the toxicological risk and/or inactivation of drugs, but the effects of antimicrobial drugs on the balance of the intestinal microflora are more important. We regard the rat as a useful experimental model for studying the effects of antimicrobial drugs on intestinal flora. New antimicrobial agents may be evaluated in the comparative and preliminary screening of efficacy, safety and intestinal side-effects. Basic antimicrobial screening models should include the effects on the intestinal ecosystem. These results render the rat model suitable for obtaining a rough estimate of new antibiotics and for making parallel comparisons between different drugs. Such studies may be of predictive value in clinical practice.

In conclusion:

(1) Changes in the intestinal microflora of the rat are: (i) similar for quinolones; (ii) selective for aztreonam and teicoplanin; (iii) also induced by parenteral administration.
(2) Faecal concentrations of antibiotics increase with treatments.
(3) Results in rats are comparable to those observed in humans.

ACKNOWLEDGEMENTS

This investigation was supported by the Ministero dell'Università e della Ricerca Scientifica e Tecnologica (MURST 60%).

REFERENCES

1. Berry PR, Sringer MF, Uemura T. (1986). Comparison of latex agglutination and ELISA for

detection of *Clostridium perfringens* type A enterotoxin in faeces. *Letters in Applied Microbiology* **2,** 101–102.
2. Bertazzoni Minelli E, Benoni G, Berti T, Deganello A, Zoppi G, Gaburro D. (1977). A simplified method for evaluation of human faecal flora in clinical practice. *Helvetica Paediatrica Acta* **32,** 471–478.
3. Bertazzoni Minelli E, Beghini AM, Vesentini S, Marchiori L, Mortani E, Nardo G, Cerruti R. (1990). Intestinal microflora as an alternative metabolic source of estrogens in women with uterine leiomyoma and breast cancer. In: Steroid Formation, Degradation, and Action in Peripheral Tissues. *Annals of the New York Academy of Sciences* **595,** 473–479.
4. Bertazzoni Minelli E, Benini A, Barzoi E, De Bastiani G, Periti P. (1992). Effects on intestinal microflora during systemic antimicrobial prophylaxis in orthopaedic patients: Teicoplanin (TC) versus Cefazolin (CZ). In: Adam D, Lode H, Rubinstein E (eds) *Recent Advances in Chemotherapy. Proceedings of 17th International Congress of Chemotherapy, Berlin, 1991*, Vol 1. Futuramed Publ., Munich, pp. 1230–1231.
5. Brogden RN, Heel RC. (1986). Aztreonam. A review of its antibacterial activity, pharmacokinetic properties and therapeutic use. *Drugs* **31,** 96–130.
6. Campoli-Richards DM, Monk JP, Price A, Benfield P, Todd PA, Ward A. (1988). Ciprofloxacin. A review of its antibacterial activity, pharmacokinetic properties and therapeutic use. *Drugs* **35,** 373–447.
7. Campoli-Richards DM, Brogden EN, Faulds D. (1990). Teicoplanin. A review of its antibacterial activity, pharmacokinetic properties and therapeutic use. *Drugs* **40,** 449–486.
8. Clissod SP, Todd PA, Campoli-Richards DM. (1987). Imipenem. A review of its antibacterial activity, pharmacokinetic properties and therapeutic use. *Drugs* **33,** 183–241.
9. de Lalla F, Privitera G, Rinaldi E, Ortisi G, Santoro D, Rizzardini G. (1989). Treatment of *Clostridium difficile*-associated disease with teicoplanin. *Antimicrobial Agents and Chemotherapy* **33,** 1125–1127.
10. Finegold SM, Mathisen GE, George LW. (1983). Changes on human intestinal flora related to the administration of antimicrobial agents. In: Hentges DJ (ed) *Human Intestinal Microflora in Health and Disease*. Academic Press, London, pp. 356–438.
11. Finegold SM, Sutter VL, Mathisen GE. (1983). Normal indigenous intestinal flora. In: Hentges DJ (ed) *Human Intestinal Microflora in Health and Disease*. Academic Press, London, pp. 3–31.
12. Glass GBT. (1968). *Introduction to Gastrointestinal Physiology*. Prentice Hall, Englewood Cliff, NJ, Chap 8.
13. Gonzales JP, Henwood JM. (1989). Pefloxacin. A review of its antibacterial activity, pharmacokinetic properties and therapeutic use. *Drugs* **37,** 628–668.
14. Gutschow K, Weissenbacher ER, Wachter I, Deininger FD, Muth P, Sorgel F. (1991). Pharmacokinetics of ceftriaxone in lymphatic fluid and serum following radical hysterectomy. *17th International Congress of Chemotherapy, Berlin*, 23–28 June, abstr 961.
15. Holdeman LV, Cato EP, Moore WEC (eds). (1977). *Anaerobe Laboratory Manual*, 4th edn. Virginia Polytechnic Institute and State University, Blacksburg, Virginia.
16. Janin N, Meugnier H, Desnottes JF, Woehrle R, Fleurette J. (1987). Recovery of pefloxacin in saliva and feces and its action on oral and faecal flora of healthy volunteers. *Antimicrobial Agents and Chemotherapy* **31,** 1665–1668.
17. Jones PG, Bodey GP, Swabb EA, Rosenbaum B. (1984). Effect of aztreonam on throat and stool flora of cancer patients. *Antimicrobial Agents and Chemotherapy* **26,** 941–943.
18. Kager L, Brismar B, Malmborg AS, Nord CE. (1989). Imipenem concentrations in colorectal surgery and impact on the colonic microflora. *Antimicrobial Agents and Chemotherapy* **33,** 204–208.
19. Martinez OV, Levi JU, Devlin RJ. (1984). Biliary excretion of aztreonam in patients with biliary tract disease. *Antimicrobial Agents and Chemotherapy* **25,** 358–361.
20. Naber KG. (1987). Renal lymph concentration of antibiotics. *Scandinavian Journal of Infectious Disease* **14,** 164–165.
21. Nord CE. (1990). Studies on the ecological impact of antibiotics. *European Journal of Clinical Microbiology and Infectious Disease* **9,** 517–518.
22. Nord CE, Edlund C. (1990). Impact of antimicrobial agents on human intestinal flora. *Journal of Chemotherapy* **2,** 218–237.
23. Norrby SR, Alestig K, Bjonegard B, Burman LA, Ferber F, Huber JL, Jones KH, Kahan FM, Kahan JS, Krops H, Meisinger MAP, Sundelof J. (1983). Urinary recovery of *N*-formimidoyl thienamycin (MK 0787) as affected by coadministration of *N*-formimidoyl thienamycin dehydropeptidase inhibitors. *Antimicrobial Agents and Chemotherapy* **23,** 300–307.
24. Rohwedder R, Bergan T, Thorsteinsson SB, Scholl H. (1990). Transintestinal elimination of ciprofloxacin. *Chemotherapy* **36,** 77–84.
25. Rowland R, Mallet AK, Wise A. (1986). The effect of the diet in the mammalian gut flora and its metabolic activities. *Critical Reviews in Toxicology* **16**(1), 31–103.
26. Simon GL, Gorbach SL. (1984). Intestinal flora in health and disease. *Gastroenterology* **86,** 174–193.
27. Sorgel F, Jaede U, Naber K, Stephan U. (1990). Pharmacokinetic disposition of quinolones in

human body fluids and tissues. *Clinical Pharmacokinetics* **16,** (suppl 1), 5–24.
28. Sutter VL, Citron DM, Edelstein MAC, Finegold SM. (1986). *Wadsworth Anaerobic Bacteriology Manual*, 4th edn. Star Publishing Company.
29. van Ogtrop ML, Guiot HFL, Mattie H, van Furth R. (1991). Modulation of the intestinal flora of mice by parenteral treatment with broad-spectrum cephalosporins. *Antimicrobial Agents and Chemotherapy* **35,** 976–982.
30. Van Ogtrop ML, Guiot HFL, Mattie H, van Strijen E, Razab Sekh B, van Furth H. (1991). Modulation of the intestinal flora of mice by treatment with aztreonam and tigemonan. *Antimicrobial Agents and Chemotherapy* **35,** 983–985.
31. Vollaard EJ, Clasener HAL, Janssen JHM. (1990). Decontamination of the bowel by intravenous administration of pefloxacin. *Journal of Antimicrobial Chemotherapy* **26,** 847–852.