Effects of treatment with antimicrobial agents on the human colonic microflora

Fatemeh Rafii
John B Sutherland
Carl E Cerniglia

Division of Microbiology, National Center for Toxicological Research, FDA, Jefferson, AR, USA

Abstract: Antimicrobial agents are the most valuable means available for treating bacterial infections. However, the administration of therapeutic doses of antimicrobial agents to patients is a leading cause of disturbance of the normal gastrointestinal microflora. This disturbance results in diminishing the natural defense mechanisms provided by the colonic microbial ecosystem, making the host vulnerable to infection by commensal microorganisms or nosocomial pathogens. In this minireview, the impacts of antimicrobials, individually and in combinations, on the human colonic microflora are discussed.

Keywords: antibiotics, intestinal bacteria

Introduction

The human colonic microflora ecosystem, its metabolic functions, and its colonization resistance are vital for the well-being of the host, production of vital metabolites, and prevention of infection (Edlund and Nord 1999b; Sullivan et al 2001a, 2001b). Various enzymes in the microbial ecosystem are implicated in deconjugation, reduction, and other biochemical activities, which result in altered bioavailability, activation, or detoxification of different molecules, including those drug metabolites released by the liver via bile ducts (Rowland 1995). *In vitro* models have shown that change in population composition of the human intestinal microflora is concurrent with reduced colonization resistance (Wagner et al 2008). Furthermore, the microbial community may have an unknown influence on the immune system (van der Waaij and Nord 2000). The colonic microflora appears to stimulate the host immune system to respond rapidly to pathogen challenges (Berg 1996). Although the cells of the intestinal tract coexist with the normal commensal microflora, they recognize and clear invading pathogens before returning to homeostasis with the commensal bacteria. The roles and influences of different commensal bacteria vary. Host responses to the effects of commensal bacteria are genetically determined (Prantera et al 1997). In patients with inflammatory bowel syndrome, the mucosal immune system shows an abnormal reaction to commensal bacteria in genetically susceptible individuals. The use of therapeutic agents to affect the bacterial population may result in microbial imbalances that affect health and cause disease. Based on clinical and epidemiological studies, it has been hypothesized that both the therapeutic use of antibiotics and the typical diets in industrialized countries may disrupt normal microbiota-mediated mechanisms of immunological tolerance in the mucosa, which also may result in an increase in allergic airway disease (Noverr and Huffnagle 2005). In the following sections, other effects of the clinical usage of antimicrobial agents on intestinal microflora will be discussed.

Disturbance of the ecosystem

Although the colonic ecosystem is generally stable, it can be disturbed by the administration of antimicrobial agents for treatment or prophylaxis (Peck et al 1984). The colonic

Correspondence: Fatemeh Rafii
Division of Microbiology, National Center for Toxicological Research, FDA, Jefferson, AR 71602, USA
Tel +1 870 543 7342
Fax +1 870 543 7307
Email fatemeh.rafii@fda.hhs.gov

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bacteria are exposed to antimicrobial agents whether they are administered orally or through injection via the circulation (Edlund and Nord 1999b). This exposure may occur through incomplete absorption of antimicrobial agents administered orally, through exposure to antimicrobial agents in secretions by the salivary glands or intestinal mucosa or in bile from the liver (Nord et al 1984a, 1984b; Arvidsson et al 1988).

The extent of changes that occur in the intestinal microflora community as the result of administration of antimicrobial agents depends on the antibacterial spectrum and its concentration in the luminal content (Nord et al 1984a, 1984b). Antibiotics given orally that are absorbed in the upper part of the small intestine differ in their effects from those that are poorly absorbed. Parenteral antimicrobial agents, whether secreted in the bile or from mucosa, also affect susceptible bacterial populations (Nord and Edlund 1990; Edlund and Nord 1999b). Exposure of the colonic ecosystem to antimicrobial agents may result in a shift in population of different components as the result of suppression or elimination of some microorganisms, overgrowth by microorganisms not susceptible to these agents, and establishment of antimicrobial-resistant members that normally are excluded (Nord 1993).

Effect of commensal microorganisms on colonization

Several factors contribute to the prevention of colonization by non-commensal microorganisms, among which are competition for nutrients, competition for attachment, and production of volatile fatty acids (Nord et al 1984b). Volatile fatty acids produced by anaerobic bacteria are toxic to some other species, especially enterobacteria. Production of bacteriocins by enterobacteria, streptococci, and anaerobic bacteria also limits bacterial overgrowth.

Development of antimicrobial-resistant bacteria

Another consequence of exposure to antibiotics that may have lasting effects is the emergence of antimicrobial-resistant bacteria that may cause problems in the long run (Nord and Edlund 1990). These organisms may be transmitted to different sites within the host, where they may cause infection, and to other hosts (Nord et al 1984b; Edlund and Nord 2000). An example is urinary tract infection caused by antimicrobial-resistant *Escherichia coli* (Winberg et al 1993; Edlund and Nord 2000).

A multicenter susceptibility survey (Aldridge et al 2001) has shown changes in the patterns of resistance among anaerobic bacteria to different antibiotics. Antibiotic resistance in anaerobic bacteria now is known for β-lactams, clindamycin, macrolides, tetracyclines, fluoroquinolones, and nitroimidazole (Nord 2008). Methicillin-resistant *Staphylococcus aureus* (MRSA) strains have been isolated from children in an outpatient clinic who had microbial imbalances of the large intestine resulting from antibiotic use (Nikolaeva et al 2001).

Induction of enzymes in the colonic bacteria that make antimicrobial agents ineffective is another side effect of antimicrobial agents (Finegold 1986). Administration of β-lactams has resulted in increased levels of β-lactamases in the normal intestinal microflora (Edlund et al 1994). Induction of β-lactamases in some aerobic and anaerobic bacteria by cefoxitin has been reported. However, since feces appear to inhibit the induction of β-lactamases, Stark and colleagues (1995) attribute increases in β-lactamases to the selection of "stably derepressed mutants" rather than to β-lactamase induction.

Fungal infections

Another problem that may arise as the result of antibiotic treatment is fungal acquisition (Louie et al 1985). Fungal overgrowth has been observed, specifically after treatment with antibiotics that reduce the population of anaerobic bacteria, in experimental animals, healthy individuals, and neutropenic patients (Kennedy and Voltz 1985; Louie et al 1985; Pletz et al 2004). Louie and colleagues (1985) conclude, "Preservation of the anaerobic flora appears critical in the prevention of fungal acquisition in neutropenic patients." Fungal infections may cause serious problems in immunocompromised patients, producing disseminated infections in various sites that could result in severe complications (Gauthier and Klein 2008).

Kennedy and colleagues (1987) used *Candida albicans* to challenge antibiotic-treated and untreated mice and found an association of the fungus with the intestinal epithelia of antibiotic-treated mice but not with those of the control mice that had indigenous bacterial flora. In the treated mice, some of the fungal cells penetrated deep into the mucosa of the intestinal tract, but most of them were associated with the cecal mucosa.

Effects on bacterial pathogenicity

Antimicrobial agents, in addition to limiting the effectiveness of the colonization barrier by disturbing the normal intestinal microbiota, may also affect the pathogenicity of certain bacteria. Fluoroquinolones have been shown to

induce bacteriophages and enhance Shiga toxin production in *E. coli* O157:H7, which may increase the virulence of this strain (Zhang et al 2000). In our laboratory, one out of five gatifloxacin-resistant *Clostridium perfringens* strains also had enhanced production of phospholipase C and perfringolysin O (Rafii et al 2008). Subinhibitory concentrations of ampicillin and clindamycin may directly affect adherence of *Clostridium difficile* by increasing the expression of genes necessary for colonization (Deneve et al 2008). In an *in vitro* animal study, it was shown that subcutaneous administration of antibiotics that disrupt anaerobic flora facilitated the growth and toxin production of *C. difficile* in mouse cecal contents. Antibiotics that did not have major effects on anaerobic populations did not affect growth or toxin production (Pultz and Donskey 2005). These findings justify the concerns of government regulatory agencies, the World Health Organization, and health care professionals about the use of antimicrobial agents in farm animals (Cerniglia and Kotarski 1999).

Other side effects

The use of antimicrobial agents usually is accompanied by gastrointestinal upsets, especially antibiotic-associated diarrhea, which frequently is resolved shortly after discontinuance of the antimicrobial agent and the return of balance to the ecosystem (Nord and Edlund 1990). It occurs in 15%–25% of patients treated with antibiotics and its incidence differs with various antibiotics. It is caused by mild to severe disturbance of gut microflora; the mild cases may result from disturbances in carbohydrate or bile acid metabolism. Proliferation of pathogens as the result of this disturbance may cause severe cases of antibiotic-associated diarrhea (Schröder et al 2006).

In an immunocompromised host, or those weakened by surgery or advanced age, severe infections by opportunistic pathogens can occur. Potential pathogens, like *C. difficile* residing in the colon, may cause *C. difficile*-related antibiotic-associated diarrhea and/or pseudomembranous colitis (Aronsson et al 1981, 1985; Ambrose et al 1985; Finegold 1986; Edlund and Nord 2000). Finegold (1986) noted that, with the exception of vancomycin and parenterally administered aminoglycosides, most other antimicrobial agents have been implicated in *C. difficile* infections.

Systemic infections in immunocompromised patients, caused by normally innocuous microorganisms, have also been observed following antibiotic treatment. Superinfection, in which all major types of anaerobes have been involved, also has been associated with the use of antimicrobial agents. In general, antimicrobial-related complications are the result of suppression of the indigenous flora that prevent colonization and overgrowth by potential pathogens residing in the colon, which may cause diarrhea, colitis or other septic conditions (Finegold 1986).

Evaluation of the effect of antimicrobial agents on intestinal microflora

The apparent consequences of exposure of the colonic microflora to antimicrobial agents have been measured by enumeration of bacterial species following clinical treatment of the patient or experimental exposure of volunteers to different antimicrobial agents (Nord and Edlund 1990). Evaluation of resistance patterns of anaerobic bacteria in saliva and feces of outpatients and hospitalized patients, regardless of treatment with antibiotics, has shown an association among the relative numbers of antibiotic-resistant anaerobic bacteria, hospitalization, and antibiotic treatment. The number of resistant strains is correlated with the duration of antibiotic treatment (Stark et al 1993).

In numerous studies, scientists have evaluated the effects of antimicrobial agents on colonic bacteria by administering the antibiotics to their subjects and measuring their effects on the populations of various bacterial genera. All major classes of antimicrobial agents have been studied. Most of this work has been performed by Carl Nord's laboratory at Karolinska University Hospital in Stockholm, Sweden, and several reviews have been written (Nord et al 1984a, 1984b, 1988; Nord and Edlund 1990; Nord 1993; Edlund and Nord 1999b; Sullivan et al 2001a). Fecal specimens, which have generally been accepted as representative of the colonic microbiota, were cultured on non-selective and selective media. Different colony types were counted, isolated in pure culture, and identified to genus level. Their numbers were compared with the numbers of bacteria present from each genus before treatment to measure the effect of the antibiotic in decreasing the number of bacteria or allowing the increase, overgrowth or proliferation of others. All new colonizing bacteria were tested for susceptibility to the antibiotics that had been administered (Nord et al 2006a, 2006b). The bacteria that were eliminated and those that overgrew other species were reported. The development of resistance among bacteria in the normal flora and overgrowth of microorganisms like yeasts, which are controlled by the normal microflora, were also detected. In most cases, the populations of certain bacterial genera decreased while the number of bacteria from other genera increased. This increase was accompanied either by an increase in resistant

strains of bacteria or by overgrowth of fungi (Louie et al 1985; Nilsson-Ehle et al 1985).

Rapid methods using DNA analysis also have been used to show the effects of antimicrobial agents on the human intestinal microflora. De La Cochetiere and colleagues (2005) isolated fecal DNA from volunteers after antibiotic treatment and amplified bacterial 16S rRNA genes by PCR with general primers, which were analyzed by temporal temperature-gradient gel electrophoresis. By comparing the patterns of dominant species with the patterns generated before treatment, they determined the effects of treatments on alteration of the microbiota.

As the widespread emergence of various antimicrobial-resistant bacteria has been problematic, and fatalities have resulted from the overgrowth of otherwise innocuous commensal microorganisms that do not respond to available antimicrobial agents, it appears timely to review the effects of these compounds on colonic microorganisms, with an emphasis on those that have not been covered in previous reviews.

β-Lactams

β-Lactams are the oldest and the most widely prescribed class of antibiotics; the class includes penicillins, cephalosporins, monobactams, carbapenems, and β-lactamase inhibitors.

Penicillins

The members of this group include phenoxymethylpenicillin, ampicillin, amoxicillin, piperacillin, azlocillin, temocillin, ticarcillin, pivampicillin, talampicillin, and bacampicillin.

Cephalosporins

Most of these are semisynthetic compounds and are not susceptible to cleavage of the β-lactam ring by many β-lactamases. They include cefaloridine, cefazolin, cefaclor, cephrocile, cefbuperazone, cefuroxime, cefoxitin, cefotiam, cefotaxime, cefoperazone, ceftriaxone, ceftazidime, ceftiazoxime, cefmenoxime, cefotetan, cephradine, and ceftibutan.

Monobactams

The β-lactam ring in monobactams is alone and not fused to another ring. Aztreonam is an example; it usually is used in combination with other drugs.

Carbapenems

As more and more bacteria develop resistance to β-lactam antimicrobial agents by producing various β-lactamases, efforts have been made to find drugs that are not affected by β-lactamases. The carbapenems includes imipenem, meropenem, ertapenem, faropenem, doripenem, panipenem, and betamipron. Imipenem is inactivated in the kidney by a dehydropeptidase; to prevent this, it is used in combination with cilastatin, an inhibitor of kidney dehydropeptidase (Birnbaum et al 1985; Nord et al 1985). In addition to being resistant to β-lactamases, the carbapenems have broad antibacterial activity.

β-Lactamase inhibitors

These compounds contain a β-lactam ring but have negligible antimicrobial activity. Because they bind to β-lactamases and prevent their attack on other β-lactams, they are co-administered with antimicrobial β-lactams.

Impact of treatment with β-lactams on the human intestinal microflora

The impacts of different β-lactam antibiotics have been evaluated both in human volunteers and in patients undergoing treatment. Various studies have considered the effects of the penicillin group: ampicillin (Leigh 1979), ampicillin plus sulbactam (Kager et al 1982), piperacillin (Kager et al 1983), azlocillin (Nord et al 1986a), temocillin (van der Waaij 1985), and ticarcillin and clavulanic acid (Nord et al 1989) (Table 1). All of them decreased the numbers of enterobacteria, enterococci, and anaerobic bacteria. Treatment with amoxicillin (Leigh and Nash 1979; Gipponi et al 1985) resulted in suppression of the numbers of enterobacteria in some trials but increased their numbers in others (Brismar et al 1993b). In some trials, overgrowth of *C. difficile* was observed after treatment with amoxicillin, with or without clavulanic acid (Brismar et al 1993b; Lode et al 2001). Overgrowth by bacteria or *Candida* after the administration of the above drugs was observed in some trials. Ticarcillin/clavulanic acid administration resulted in increases in enterococci. Phenoxymethylpenicillin did not have much effect (Adamsson et al 1997).

Since β-lactams were the first antibiotics to be discovered, they have been frequently prescribed for treatment of various infections and many bacteria have developed resistance to them. The presence of β-lactamases in various bacteria has resulted in the frequent ineffectiveness of these drugs. To avoid development of resistance and to enhance potency of the drugs, other β-lactams have been developed and used in combinations with β-lactamase inhibitors or other drugs (Table 2).

Pivmecillinam

Sullivan and colleagues (2001b) studied the impact of treatment with pivmecillinam on the intestinal microfloras

Table 1 Effects of penicillins, alone or in combination with other drugs, on intestinal microflora

Compound	Bacteria suppressed	Bacteria proliferated	Overgrowth	Reference
Ampicillin	Enterobacteria, enterococci, and anaerobic bacteria		*Candida* in some	Leigh (1979)
Ampicillin and sulbactam	Enterobacteria, enterococci, and anaerobic bacteria		*Candida* or *C. difficile* in some	Kager and colleagues (1982)
Piperacillin	Enterobacteria, enterococci, and anaerobic bacteria		*Candida* or *C. difficile* in some	Kager and colleagues (1983)
Amoxicillin	Enterobacteria	Resistant enterobacteria	*Candida* or *C. difficile* in some	Gipponi and colleagues (1985)
Ticarcillin and clavulanic acid	Enterococci		*Candida* or *C. difficile* in some	Nord and colleagues (1989)
Phenoxymethylpenicillin	Little effect			Adamsson and colleagues (1997)

of 15 individuals. They were treated for 7 days with 400 mg of pivmecillinam twice daily and then again 14 and 21 days after the start of administration. There was a decrease in the numbers of *E. coli* but no changes occurred in the anaerobic microflora.

Piperacillin/tazobactam

The effect of piperacillin on the colonic bacteria has been reviewed (Nord and Edlund 1990). The impact on colonic ecology of its use in combination with a β-lactamase inhibitor, tazobactam, has also been investigated (Nord et al 1992, 1993; Nord and Lahnborg 1994). The effect of a piperacillin/tazobactam combination (4/0.5 g) on patients with intraabdominal infections who were treated three times a day for 8 days was evaluated; there were decreases in the mean numbers of enterobacteria, enterococci, and anaerobes (bifidobacteria, eubacteria, lactobacilli, clostridia, and Gram-positive cocci). Anaerobic Gram-negative cocci and *Bacteroides* spp. were unaffected (Table 2). The number of bacteria returned to normal after treatment was stopped (Nord et al 1992, 1993).

Omeprazole and amoxicillin

A combination of omeprazole (20 mg, a proton pump inhibitor for eradication of *Helicobacter pylori*) and amoxicillin (1000 mg) twice daily for 14 days resulted in changes in the microflora of the intestinal tract, increases in the numbers of resistant enterobacteria, and an increase in β-lactamase production detected in fecal samples (Stark et al 1995; Adamsson et al 1999).

Omeprazole/amoxicillin/ metronidazole combinations

The effects of amoxicillin in combination with antimicrobial agents effective against anaerobes also have been studied. Metronidazole, which is used for the treatment of anaerobic and parasitic infections, is also used in the treatment of *H. pylori* in combination with other drugs. Treatment of patients with a combination of 20 mg omeprazole, 1 g amoxicillin, and 40 mg metronidazole for 7 days resulted in alteration of normal microflora and pronounced suppression of the anaerobic microflora. During treatments, some patients also were colonized by yeasts. The MIC values of those drugs for *Enterococcus* spp. and the Enterobacteriaceae increased (Adamsson et al 1999).

Amoxicillin/clavulanic acid

Administration of 1000 mg of amoxicillin/clavulanic acid for 7 days to 12 healthy individuals (the same persons who had been treated with 600 mg linezolid for 7 days, with a washout period of 4 weeks between treatments) resulted in increases in the numbers of enterococci and *E. coli* and significant decreases in the numbers of anaerobes, including bifidobacteria, lactobacilli, and clostridia. *C. difficile* was isolated from three volunteers (Lode et al 2001). The authors concluded that unlike linezolid, amoxicillin/ clavulanic acid does not eliminate Gram-positive bacteria.

Ten volunteers who received 500 mg amoxicillin three times a day for 7 days were colonized with amoxicillin-resistant bacteria, mostly *E. coli*, *Klebsiella*, and *Enterobacter* (Brismar et al 1993b). β-Lactamase activity was detected in the microfloras of six of the subjects.

Imipenem

Kager and colleagues (1988) evaluated the effects of imipenem, both when used for treatment and for prophylaxis, on the intestinal microflora. Use of 0.5 or 1 g of imipenem four times daily for 4–11 days resulted in a decrease in numbers of enterobacteria, anaerobic cocci, and *Bacteroides* spp. during treatment. The microflora was restored to normal afterward.

Table 2 Effects of selected β-lactams other than cephalosporins, including those in combination with other drugs, on intestinal microflora

Compound	Treatment (mg per day)	Number of days and number of treatments per day	Number of subjects	Bacteria suppressed	Bacteria proliferated	Resistant strains developed or other results	Days to normal after treatment discontinued	Reference
Piperacillin/tazobactam	4000/500	4–8	20	Enterobacteria Enterococci Bifidobacteria Eubacteria Lactobacilli Clostridia Gram-positive cocci				Nord and colleagues (1993)
Omeprazole/amoxicillin	20/1000	14/2	14	Alteration in microflora		Enterobacterial β-lactamase production		Stark and colleagues (1996)
Amoxicillin/clavulanic acid	1000	7	12	Bifidobacteria Lactobacilli Clostridia	Enterococci *Escherichia coli*	*Clostridium difficile* isolated from three volunteers		Lode and colleagues (2001)
Amoxicillin	500	7/3	10			Amoxicillin-resistant bacteria, including *E. coli*, *Klebsiella* and *Enterobacter*		Brismar and colleagues (1993a, 1993b)
Omeprazole/amoxicillin/ metronidazole	20/1000/40	7/2	14	Marked changes	Total anaerobic microflora	*Streptococcus* spp. *Enterococcus* spp. Enterobacteria Yeast colonization in nine subjects	35 days	Adamsson and colleagues (1999)
Imipenem (surgical prophylaxis)	500/1000	2/4	20	Staphylococci Streptococci Enterococci Enterobacteria Anaerobic cocci Bifidobacteria Eubacteria Lactobacilli Clostridia Fusobacteria *Bacteroides*			No colonization; normal after 14 days	Kager and colleagues (1988)
Pivmecillinam	400	14–41	15		*E. coli*			Sullivan and colleagues (2001b)
Ertapenem	1000	7	10	Lactobacilli Bifidobacteria Clostridia *Bacteroides* *E. coli*	Enterococci	Overgrowth of yeast	21–35 days	Pletz and colleagues (2004)

Aztreonam plus tobramycin		15 febrile neutropenic patients	Enteric Gram-negative bacilli Fecal anaerobes	Fungal contamination		Louie and colleagues (1985)
Aztreonam plus cloxacillin		14 febrile neutropenic patients	Enteric Gram-negative bacilli Fecal anaerobes	Fungal contamination		Louie and colleagues (1985)
Moxalactam plus tobramycin			Enteric Gram-negative bacilli Fecal anaerobes	Fungal contamination		Louie and colleagues (1985)
Imipenem	500–1000	4–11/4	10	Enterobacteria Anaerobic cocci *Bacteroides*		Kager and colleagues (1988)
Meropenem	500	7/3	10	Enterobacteria Streptococci Clostridia *Bacteroides* Gram-negative cocci	Enterococci	14 days	Bergan and colleagues (1991)

Patients receiving 0.5 or 1 g of imipenem for surgical prophylaxis, every 6 hours for 48 hours, had suppressed levels of staphylococci, streptococci, enterococci, enterobacteria, anaerobic bacteria, cocci, bifidobacteria, eubacteria, lactobacilli, clostridia, fusobacteria, and *Bacteroides* spp. during treatment; the levels were normal afterward. Colonization by imipenem-resistant bacteria did not occur (Kager et al 1988, 1989).

Aztreonam with either tobramycin or cloxacillin

Louie and colleagues (1985) used different combinations of drugs to treat febrile neutropenic patients and compared the impacts on the microbial ecology. Combinations of aztreonam with either tobramycin (15 subjects) or cloxacillin (14 subjects) resulted in the eradication of enteric Gram-negative bacilli. These combinations also caused reduction of the numbers of fecal anaerobes, accompanied by a reduced concentration of short-chain fatty acids in fecal supernatants (Louie et al 1985). Fungal proliferation occurred in 27% of subjects receiving aztreonam/tobramycin and 43% of subjects receiving aztreonam/cloxacillin. Moxalactam/tobramycin combination treatments of 15 patients had similar effects on reduction of anaerobes and fungal acquisition (81%) as the previous two combinations. All of the subjects were febrile neutropenic patients, so the authors concluded that maintaining the anaerobic flora was essential for the prevention of fungal acquisition in febrile neutropenic patients.

Meropenem

Intravenous injection of 500 mg meropenem three times daily for 7 days in 10 patients resulted in decreases in the numbers of enterobacteria and streptococci and increases in the numbers of enterococci, clostridia, *Bacteroides* spp., and Gram-negative cocci. Other Gram-positive cocci and rods were not affected. Two weeks after termination of therapy, the intestinal microflora had returned to normal (Bergan et al 1991).

Ertapenem and ceftriaxone

Administration of either ertapenem (1 g per day) or ceftriaxone (2 g per day) to 10 healthy individuals for 7 days via injection resulted in a 4-log reduction in *E. coli* as well as a reduction of the anaerobic microflora, including lactobacilli, bifidobacteria, clostridia, and *Bacteroides* spp. There was a 4-log increase in the number of enterococci. Overgrowth of yeast was observed in both treatments, but in both cases the microflora returned to normal within 21–35 days after treatment had concluded (Pletz et al 2004).

Cephalosporins

Various cephalosporins have major effects on the colonic microflora (Nord and Edlund, 1990). Cefbuperazone (Kager et al 1986), cefoxitin (Kager et al 1982), cefotaxime (Lambert-Zechovsky et al 1985), cefoperazone (Alestig et al 1983), ceftriaxone (Arvidsson et al 1982), cefaclor (Nord et al 1987), and cefotetan (Ambrose et al 1985) all decreased the numbers of anaerobic bacteria and enterobacteria. All of these drugs, except cefoperazone and ceftriaxone, also decreased the number of enterococci in most treatments. Cefotaxime decreased both enterobacteria and enterococci in one trial. Cephaloridine (Ambrose et al 1985) and cephradine (Brumfitt et al 1986) had little effect. However, resistant bacterial strains developed as the result of administration of all of these drugs except for cephradine, ceftazidime and cefbuperazone. *C. difficile* emerged in some trials with cefepime, ceftriaxone, and cefixime. Anaerobic Gram-positive cocci increased after treatment with both ceftriaxone and cefixime (Nilsson-Ehle et al 1985, Sullivan et al 2001a). The effects of other cephalosporins (cefprozil, cefpodoxime proxetil, ceftibuten, and cefadroxil) on the intestinal flora are shown in Table 3.

Cefprozil

Administration of cefprozil resulted in changes in the populations of certain genera. Lode and colleagues (1992) studied the effects of cefprozil in eight individuals. They administered 500 mg of cefprozil twice daily for 8 days and found that it resulted in moderate decreases in enterobacteria and slight increases in enterococci, staphylococci, and *Bacteroides* spp. The microbial populations were normal 4 days after treatment. The major microbiologically related consequence was soft stools. In some cases, *C. difficile* overgrowth was observed after treatment with cefprozil (Lode et al 1992).

Cefpodoxime proxetil

Cefpodoxime proxetil has major effects on the colonic bacteria. Brismar and colleagues (1993b) evaluated the effects of this compound on volunteers receiving 200 mg twice a day for 7 days. The numbers of streptococci, enterobacteria, and clostridia decreased substantially in the fecal flora, and overgrowth of enterococci, yeasts and *Clostridium difficile* was observed (Brismar et al 1993b).

Cefadroxil

Administration of cefadroxil to healthy individuals does not cause measurable disturbance to the colonic ecology. Adamsson and colleagues (1997) evaluated the effect of 500

Table 3 Effects of cephalosporins on intestinal microflora

Compound	Treatment (mg per day)	Number of days and number of treatments per day	Number of subjects	Bacteria suppressed	Bacteria proliferated	Resistant strains developed or other side effects	Days to normal after treatment discontinued	Reference
Cefprozil	500	8/2	8	Enterobacteria	Enterococci Staphylococci *Bacteroides*	Soft stools	4 days	Lode and colleagues (1992)
Cefadroxil	500	10	20	Not much change	Not much change			Adamsson and colleagues (1997)
Cefpodoxime proxetil	200	7/2	10	Streptococci Enterobacteria Clostridia	Enterococci *Clostridium difficile*	β-Lactamase activity in the flora of some subjects		Brismar and colleagues (1993b)
Ceftriaxone	2000	7	10	Lactobacilli Bifidobacteria Clostridia *Bacteroides* *E. coli*	Enterococci	Overgrowth of yeast	1 to 35 days	Pletz and colleagues (2004)
Ceftibutan	400	10	14	Enterococci	*E. coli* and anaerobic cocci	Increased β-lactamase *C. difficile* colonization in six individuals		Brismar and colleagues (1993a)

mg cefadroxil taken for 10 days by 20 healthy volunteers. The effect on the intestinal microflora was slight and the microflora was normal two weeks after withdrawal of the drug.

Ceftibuten

Brismar and colleagues (1993a) administered ceftibuten to 14 healthy individuals. Each received a daily dose of 400 mg ceftibuten orally, but only two individuals had detectable levels of ceftibuten in the feces. During the administration period, the number of enterococci increased and the numbers of *E. coli* and anaerobic cocci decreased. Eight volunteers had increased levels of β-lactamases and six were colonized by *C. difficile.*

Fluoroquinolones

Fluoroquinolones are a class of synthetic antimicrobial agents whose effects on the ecology of colonic microflora have been intensively evaluated (Edlund and Nord 1988b; Edlund and Nord 1999b; Sullivan et al 2001a). By administration of different fluoroquinolones and enumeration of bacteria from different genera before and after exposure, it was shown that fluoroquinolones have a selective effect on the normal colonic bacteria. The effects of ciprofloxacin (Brismar et al 1990), norfloxacin (Edlund et al 1987a, 1987b; Edlund and Nord 1988a), ofloxacin (Pecquet et al 1987; Edlund et al 1988), pefloxacin (Vollaard et al 1992), lomefloxacin (Edlund et al 1990), levofloxacin (Edlund et al 1997b), sparfloxacin (Ritz et al 1994), rufloxacin (D'Antonio et al 1996), sitafloxacin (Inagaki and Yamamoto et al 1995), gatifloxacin (Edlund and Nord 1999a), trovafloxacin (Edlund and Nord 1999b), and moxifloxacin (Edlund and Nord 1999b; Edlund et al 2000b) on the intestinal microflora have been analyzed. All of the fluoroquinolones tested decreased the populations of enterobacteria, and pefloxacin decreased the number of aerobic Gram-positive cocci. In general, none of the fluoroquinolones affected the anaerobic bacterial population. Overgrowth of bacteria as the result of fluoroquinolones was not observed, but overgrowth of *Candida* was occasionally observed. Although *C. difficile* infection was not observed, the use of newer fluoroquinolones has been associated with the emergence of *C. difficile* antibiotic-associated diarrhea in some hospitals. *In vitro*, we have shown that some strains of *C. perfringens* readily become resistant to fluoroquinolones, even those that are generally effective against anaerobes (Rafii et al 2005). Resistant strains also exhibit physiological changes that are different from wild-type strains. Similarly, we have shown *in vitro* that one out of five strains of *C. perfringens* produced a higher amount of toxin in response to gatifloxacin exposure (Rafii et al 2008). The effects of some of the most recently developed fluoroquinolones (garenoxacin, gemifloxacin, and clinafloxacin) on colonic ecology have also been evaluated (Table 4).

Garenoxacin

Nord and colleagues (2003) evaluated the effects of oral administration of garenoxacin on the fecal microfloras of 16 individuals. Administration of 600 mg garenoxacin daily for 6 days decreased the number of enterococci, bacilli, corynebacteria, enterobacteria, bifidobacteria, lactobacilli, clostridia, and *Bacteroides* spp. in the intestinal microflora, and it increased the numbers of eubacteria. The microflora returned to normal 2 weeks after discontinuation of this broad-spectrum fluoroquinolone. The concentrations of garenoxacin in fecal samples were 14–31 mg/kg. Garenoxacin-resistant *Eubacterium lentum* (MIC = 64 mg/ml) was isolated from the subjects; the populations had returned to normal 14 days after discontinuation (Nord et al 2003).

Gemifloxacin

The selective effect of gemifloxacin on the intestinal microflora was similar to that of other fluoroquinolones (Barker et al 2001). Administration of 320 mg gemifloxacin orally for 7 days to 10 healthy subjects resulted in the suppression of enterobacteria, enterococci and streptococci. Anaerobic cocci and lactobacilli also decreased. Overgrowth or selection of resistant bacteria was not detected; the bacterial populations had returned to normal 49 days after treatment had stopped (Barker et al 2001).

Clinafloxacin

Oh and colleagues (2000) evaluated the effects of clinafloxacin on intestinal ecology in 12 healthy individuals and found major ecological disturbances. Oral administration of 200 mg clinafloxacin for 7 days resulted in high drug levels in feces (mean value 176 mg/kg on day 7). It eradicated the aerobic bacteria in 11 of the subjects and suppressed the anaerobic microflora. Clinafloxacin-resistant *Bacteroides* sp. (MIC $>$ 4) also emerged; Oh and colleagues (2000) recommended restricting use of this drug.

Macrolides

Macrolides have a macrocyclic 14 to 16-membered lactone ring attached to a deoxy sugar. They include clarithromycin, dirithromycin, erythromycin, and roxithromycin, which have bactericidal or bacteriostatic activities. Their effects on the intestinal microflora are summarized in Table 5.

Table 4 Effects of fluoroquinolones on intestinal microflora

Compound	Treatment (mg per day)	Number of days	Number of subjects	Bacteria suppressed	Bacteria proliferated	Resistant strains developed	Days to normal after treatment discontinued	Reference
Garenoxacin	600	6	16	Enterococci Bacilli Corynebacteria Enterobacteria Bifidobacteria Lactobacilli Clostridia *Bacteroides*	Eubacteria	Garenoxacin-resistant *Eubacterium lentum*	14 days	Nord and colleagues (2003)
Gemifloxacin	320	7	10	Enterobacteria Enterococci Streptococci Anaerobic cocci Lactobacilli			49 days	Barker and colleagues (2001)
Clinafloxacin	200	7	12	Aerobic bacteria	Anaerobic bacteria	*Bacteroides* spp.		Oh and colleagues (2000)

Dirithromycin

Eckernas and colleagues (1991) evaluated the impact of treatment with dirithromycin of 20 healthy individuals on the colonic microbial population. Administration of 500 mg dirithromycin daily for 7 days resulted in the detection of 12 mg/kg of dirithromycin in feces.

The numbers of enterobacteria and anaerobes (Gram-positive cocci, bifidobacteria, eubacteria, and *Bacteroides* spp.) decreased in the intestinal flora and the numbers of streptococci, staphylococci, and other anaerobes (clostridia and lactobacilli) increased. In addition, dirithromycin-resistant enterobacteria colonized the intestine. Eckernas and colleagues (1991) concluded that dirithromycin had a significant ecological impact on the colonic bacteria.

Clarithromycin

Edlund and colleagues (2000a, 2000b) evaluated the ecological impact of clarithromycin in 12 volunteers. Administration of clarithromycin (500 mg twice daily for 7 days) caused significant reduction of *E. coli*, while the numbers of enterococci, enterobacters, citrobacters, klebsiellas, and pseudomonads increased markedly. No significant changes in the numbers of staphylococci, streptococci, bacilli, or *Candida* were noticed. In the anaerobic microflora, bifidobacteria, lactobacilli and clostridia were suppressed, while no changes in peptostreptococci, *Veillonella*, *Bacteroide*s spp., or fusobacteria were found. The microflora was normal in all volunteers after 35 days. Edlund and colleagues (2000a, 2000b) also found the emergence of *Bacteroides* spp. isolates that were resistant to clarithromycin after administration of 500 mg of clarithromycin for 10 days to 10 patients. These bacteria persisted for 2 weeks after the drug was discontinued. In the same study, the authors also found α-hemolytic streptococci, intestinal enterococci, and enterobacteria with resistance to clarithromycin, but no overgrowth of yeast or *C. difficile*.

A combination of 20 mg of omeprazole, 250 mg of clarithromycin, and 400 mg of metronidazole resulted in alteration of the normal microflora, similar to the combination of omeprazole, amoxicillin, and metronidazole (Adamsson et al 1999). The suppression of anaerobic microflora was more pronounced than that with the amoxicillin group. Substantial increases in clarithromycin-resistant *Bacteroides* strains, from 2% to 76%, were observed during treatment. Both combinations altered the fecal microflora, but the clarithromycin combination therapy had more undesirable effects on the colonic ecosystem (Adamsson et al 1999).

Table 5 Effects of dirithromycin, clarithromycin, tigecycline, and erythromycin on intestinal microflora

Compound	Treatment (mg per day)	Number of days and number of treatments per day	Number of subjects	Bacteria suppressed	Bacteria and yeasts proliferated	Resistant strains developed	Days to normal after treatment discontinued	Reference
Dirithromycin	500	7	20	Enterobacteria Anaerobes Gram-positive cocci Bifidobacteria Eubacteria *Bacteroides* spp.	Streptococci Staphylococci Anaerobes Clostridia Lactobacilli	Dirithromycin-resistant enterobacteria		Eckernas and colleagues (1991)
Clarithromycin	500	7/2	12	*Escherichia coli* Bifidobacteria Lactobacilli Clostridia	Enterococci *Enterobacter Citrobacter Klebsiella Pseudomonas*	None	35 days	Edlund and colleagues (2000a)
Omeprazole/ clarithromycin/ metronidazole	20/250/400	7	16	Anaerobic bacteria	Total anaerobic microflora	*Streptococcus* spp. *Enterococcus* spp. Enterobacteria Clarithromycin-resistant *Bacteroides* spp.		Adamsson and colleagues (1999)
Tigecycline	100/ then 50/2	10	13	Enterococci *Escherichia* Lactobacilli Bifidobacteria	Enterobacteria and yeasts	*Klebsiella pneumoniae* Five resistant *Enterobacter cloacae* strains		Nord and colleagues (2006b)
Erythromycin	1000/500	7/2	10	Streptococci Enterococci Enterobacteria Anaerobic bacteria were also affected	*Staphylococcus* spp.	Erythromycin-resistant enterobacteria, clostridia or yeast		Brismar and colleagues (1991)

Erythromycin

Brismar and colleagues (1991) administered 1 g of erythromycin ethyl succinate orally for 7 days to 10 volunteers and evaluated its effect on the colonic bacteria. They found decreases in the numbers of streptococci, enterococci, and enterobacteria during administration, increases in staphylococci, and alteration of the anaerobic bacteria. In an earlier study (Heimdahl and Nord 1982), it had been shown that the administration of 500 mg of erythromycin twice daily for 7 days to 10 volunteers resulted in decreases in enterobacteria and most anaerobes. In addition, potentially pathogenic erythromycin-resistant enterobacteria, clostridia or yeasts colonized all subjects (Heimdahl and Nord 1982).

Ketolides

This is a new class of compounds related to the macrolides and includes telithromycin (Nord et al 2004), which differs from erythromycin in having a large aromatic side chain and substitution of sugar with a keto group. Ketolides can bind to two sites on the bacterial ribosome; therefore, they are effective even against macrolide-resistant bacteria. They also have reduced potential for inducing ketolide-resistant bacteria. Edlund and colleagues (2000a) studied the effect of telithromycin in ten subjects and found an increase in the MIC of telithromycin for resistant *Bacteroides* spp. isolates, which persisted for 2 weeks after the drug was discontinued. However, overgrowth of yeast and *C. difficile* was not observed.

Aminoglycosides

Amikacin, gentamicin, kanamycin, neomycin, netilmicin, paromomycin, rhodostreptomycin, streptomycin, tobramycin, and apramycin are examples of aminoglycosides. Finegold (1986) noted an association between administration of "intestinal antiseptics," such as oral neomycin, and superinfection by anaerobes.

Glycylcyclines

Glycylcyclines, a new class of antibiotics, are tetracycline analogs developed with features to prevent either their efflux from the cells or the development of ribosomal protection proteins, which result in resistance to tetracyclines.

Tigecycline

Tigecycline is a glycylcycline, which has a potent broad-spectrum activity against most Gram-positive and Gram-negative aerobic and anaerobic bacteria. Administration of 50 mg tigecycline to 20 subjects every 12 hours for 10 days (Nord et al 2006b) resulted in a reduction in the numbers of enterococci, *E. coli*, lactobacilli, and bifidobacteria, and increases in other enterobacteria and yeasts. Two tigecycline-resistant strains of *Klebsiella pneumoniae* and five tigecycline-resistant strains of *Enterobacter cloacae* developed (Nord et al 2006b).

Lincosamides

Lincomycin and clindamycin are lincosamides, which inhibit transpeptidase reactions by binding to the 23 S and 50 S subunits of bacterial ribosomes and inhibiting early elongation of peptide chains.

Clindamycin

Clindamycin is used for the treatment not only of anaerobic infections, but also of parasitic infections, including malaria. Administration of clindamycin to 10 subjects for 7 days resulted in the disturbance of colonic ecology (Kager et al 1981). Clindamycin is also associated with *C. difficile* antibiotic-associated diarrhea. Kager and colleagues (1981) evaluated the effect of 600 mg of intravenous clindamycin at 8-hour intervals for 48 hours, which resulted in the decrease of enterococci, streptococci, and anaerobic bacteria. After treatment, streptococci and anaerobic bacteria proliferated; postoperative infection due to *Enterococcus faecalis* occurred in some of the patients.

Oxazolidones

Linezolid belongs to the class of antimicrobial agents called oxazolidones. It is a synthetic antibiotic, rarely used because of its high price, but it is effective for the treatment of multidrug-resistant bacterial infections, including those caused by *Streptococcus* spp. and MRSA. Administration of 600 mg of linezolid for 7 days to healthy volunteers resulted in the suppression of enterococci and increases in the numbers of *Klebsiella* spp. It also decreased the numbers of the following anaerobes: bifidobacteria, lactobacilli, clostridia, and *Bacteroides* spp. Thirty-five days after termination of treatment, the microflora was normal (Lode et al 2001).

Glycopeptides

This class consists of glycosylated cyclic nonribosomal peptides and includes vancomycin, teicoplanin, telavancin, tramplanin, decaplanin, and dalbavancin, which is a lipoglycopeptide. They inhibit peptidoglycan synthesis.

Vancomycin

This drug has been used as the last resort for treatment of infections caused by Gram-positive bacteria. Edlund and

colleagues (1997a) evaluated the effects of vancomycin in 10 individuals after treatment with 250 mg of cefuroxime axetil twice daily for 7 days. The subjects then received 125 mg of vancomycin four times daily for 7 days. The authors concluded that vancomycin causes ecological disturbances of the intestinal microflora. Vancomycin treatment resulted in decreases in the numbers of *Enterococcus faecium*, *E. faecalis*, and *E. durans* and of anaerobes, including bifidobacteria and *Bacteroides* spp. It also resulted in the emergence of motile enterococci, including *E. gallinarum* and *E. casseliflavus* strains with decreased susceptibility to vancomycin (MIC = 4–16 mg/ml). Some of these strains had the *van*C2 (C3) gene. In addition, vancomycin-resistant strains of *Pediococcus*, *Lactobacillus*, *Klebsiella*, *Citrobacter*, and *Enterobacter* species also emerged. Recently, other investigators have shown that oral vancomycin promotes the overgrowth of vancomycin-resistant enterococci (Al-Nassir et al 2008).

Dalbavancin

This is a novel lipoglycopeptide, related to vancomycin, and similarly to that drug it is used for treatment of MRSA and *S. epidermidis*. Nord and colleagues (2006a) evaluated the effect of this drug in 12 individuals. When 1 g dalbavancin was given to six men and six women, there were some changes in numbers of enterococci and *E. coli* but no changes in numbers of lactobacilli, clostridia, and *Bacteroides* spp. No *C. difficile* strains were recovered. No newly colonizing aerobic or anaerobic bacteria with resistance to dalbavancin were found. Dalbavancin apparently has no major ecological effects on the normal human intestinal microflora (Barker et al 2001, Nord et al 2006a).

Metronidazole

Metronidazole is used for the treatment of anaerobic bacterial infections and is also used in combination with other drugs for treatment of ulcerative colitis. It has been shown to eliminate methanogenic Archaea (Ansorg et al 2003). Use of oral metronidazole for the treatment of *C. difficile*-associated disease has resulted in the overgrowth of enterococci, including vancomycin-resistant strains (Al-Nassir et al 2008).

Conclusions

The colonic microflora provides a number of benefits, including contributing to the host's nutrition and protecting the host from infection. In most cases of antimicrobial therapy, the bacterial populations in some genera are reduced in numbers while those in other genera increase. In some cases, the increased numbers of certain bacteria are accompanied by resistant strains of bacteria or overgrowth by fungi. Treatment with antimicrobial combinations does not necessarily prevent resistance development. It may even result in fungal overgrowth and appearance of bacteria with resistance to all of the drugs in the combination. Examples are the amoxicillin and metronidazole combination and the aztreonam combination with either tobramycin or cloxacillin, all of which result not only in the suppression of anaerobes but also in the overgrowth of fungi. Some of the anaerobic bacteria may keep the fungi in check. A review of the impact of antimicrobial agents on colonic ecology also shows variation among individuals in response to treatment with these drugs and variation in the effects of these drugs on different strains of the same bacterium. Toxin-producing strains of *C. difficile* have been found in some hospitals after fluoroquinolone therapy, and some strains of *C. perfringens* produce toxin after gatifloxacin exposure. The importance of judicious use of these important drugs cannot be overemphasized.

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References

Adamsson I, Edlund C, Sjostedt S, et al. 1997. Comparative effects of cefadroxil and phenoxymethylpenicillin on the normal oropharyngeal and intestinal microflora. *Infection*, 25:154–8.

Adamsson I, Nord CE, Lundquist P, et al. 1999. Comparative effects of omeprazole, amoxycillin plus metronidazole versus omeprazole, clarithromycin plus metronidazole on the oral, gastric and intestinal microflora in *Helicobacter pylori*-infected patients. *J Antimicrob Chemother*, 44:629–40.

Al-Nassir WN, Sethi AK, Li Y, et al. 2008. Both oral metronidazole and oral vancomycin promote persistent overgrowth of vancomycin-resistant enterococci during treatment of *Clostridium difficile*-associated disease. *Antimicrob Agents Chemother*, 52:2403–6.

Aldridge KE, Ashcraft D, Cambre K, et al. 2001. Multicenter survey of the changing *in vitro* antimicrobial susceptibilities of clinical isolates of *Bacteroides fragilis* group, *Prevotella*, *Fusobacterium*, *Porphyromonas*, and *Peptostreptococcus* species. *Antimicrob Agents Chemother*, 45:1238–43.

Alestig K, Carlberg H, Nord CE, et al. 1983. Effect of cefoperazone on faecal flora. *J Antimicrob Chemother*, 12:163–7.

Ambrose NS, Johnson M, Burdon DW, et al. 1985. The influence of single dose intravenous antibiotics on faecal flora and emergence of *Clostridium difficile*. *J Antimicrob Chemother*, 15:319–26.

Ansorg R, Rath PM, Runde V, et al. 2003. Influence of intestinal decontamination using metronidazole on the detection of methanogenic Archaea in bone marrow transplant recipients. *Bone Marrow Transplant*, 31:117–9.

Aronsson B, Möllby R, Nord CE. 1981. Occurrence of toxin-producing *Clostridium difficile* in antibiotic-associated diarrhea in Sweden. *Med Microbiol Immunol*, 170:27–35.

Aronsson B, Möllby R, Nord CE. 1985. Antimicrobial agents and *Clostridium difficile* in acute enteric disease: epidemiological data from Sweden, 1980–1982. *J Infect Dis*, 151:476–81.

Arvidsson A, Alvan G, Angelin B, et al. 1982. Ceftriaxone: renal and biliary excretion and effect on the colon microflora. *J Antimicrob Chemother*, 10:207–15.

Arvidsson A, Leijd B, Nord CE, et al. 1988. Interindividual variability in biliary excretion of ceftriaxone: effects on biliary lipid metabolism and on intestinal microflora. *Eur J Clin Invest*, 18:261–6.

Barker PJ, Sheehan R, Teillol-Foo M, et al. 2001. Impact of gemifloxacin on the normal human intestinal microflora. *J Chemother*, 13:47–51.

Berg RD. 1996. The indigenous gastrointestinal microflora. *Trends Microbiol*, 4:430–5.

Bergan T, Nord CE, Thorsteinsson SB. 1991. Effect of meropenem on the intestinal microflora. *Eur J Clin Microbiol Infect Dis*, 10:524–7.

Birnbaum J, Kahan FM, Kropp H, et al. 1985. Carbapenems, a new class of beta-lactam antibiotics. Discovery and development of imipenem/cilastatin. *Am J Med*, 78:3–21.

Brismar B, Edlund C, Malmborg AS, et al. 1990. Ciprofloxacin concentrations and impact of the colon microflora in patients undergoing colorectal surgery. *Antimicrob Agents Chemother*, 34:481–3.

Brismar B, Edlund C, Nord CE. 1991. Comparative effects of clarithromycin and erythromycin on the normal intestinal microflora. *Scand J Infect Dis*, 23:635–42.

Brismar B, Edlund C, Nord CE. 1993a. Effect of ceftibuten on the normal intestinal microflora. *Infection*, 21:373–5.

Brismar B, Edlund C, Nord CE. 1993b. Impact of cefpodoxime proxetil and amoxicillin on the normal oral and intestinal microflora. *Eur J Clin Microbiol Infect Dis*, 12:714–9.

Brumfitt W, Franklin I, Grady D, et al. 1986. Effect of amoxicillin-clavulanate and cephradine on the fecal flora of healthy volunteers not exposed to a hospital environment. *Antimicrob Agents Chemother*, 30:335–7.

Cerniglia CE, Kotarski S. 1999. Evaluation of veterinary drug residues in food for their potential to affect human intestinal microflora. *J Regulatory Toxicol Pharmacol*, 29:238–61.

D'Antonio D, Pizzigallo E, Lacone A, et al. 1996. The impact of rufloxacin given as prophylaxis to patients with cancer on their oral and faecal microflora. *J Antimicrob Chemother*, 38:839–47.

De La Cochetiere MF, Durand T, Lepage P, et al. 2005. Resilience of the dominant human fecal microbiota upon short-course antibiotic challenge. *J Clin Microbiol*, 43:5588–92.

Deneve C, Delomenie C, Barc MC, et al. 2008. Antibiotics involved in *Clostridium difficile*-associated disease increase colonization factor gene expression. *J Med Microbiol*, 57:732–8.

Eckernas SA, Grahnen A, Nord CE. 1991. Impact of dirithromycin on the normal oral and intestinal microflora. *Eur J Clin Microbiol Infect Dis*, 10:688–92.

Edlund C, Alvan G, Barkholt L, et al. 2000a. Pharmacokinetics and comparative effects of telithromycin (HMR 3647) and clarithromycin on the oropharyngeal and intestinal microflora. *J Antimicrob Chemother*, 46:741–9.

Edlund C, Barkholt L, Olsson-Liljequist B, et al. 1997a. Effect of vancomycin on intestinal flora of patients who previously received antimicrobial therapy. *Clin Infect Dis*, 25:729–32.

Edlund C, Bergan T, Josefsson K, et al. 1987a. Effect of norfloxacin on human oropharyngeal and colonic microflora and multiple-dose pharmacokinetics. *Scand J Infect Dis*, 19:113–21.

Edlund C, Beyer G, Hiemer-Bau M, et al. 2000b. Comparative effects of moxifloxacin and clarithromycin on the normal intestinal microflora. *Scand J Infect Dis*, 32:81–5.

Edlund C, Brismar B, Nord CE. 1990. Effect of lomefloxacin on the normal oral and intestinal microflora. *Eur J Clin Microbiol Infect Dis*, 9:35–9.

Edlund C, Kager L, Malmborg AS, et al. 1988. Effect of ofloxacin on oral and gastrointestinal microflora in patients undergoing gastric surgery. *Eur J Clin Microbiol Infect Dis*, 7:135–43.

Edlund C, Lidbeck A, Kager L, et al. 1987b. Comparative effects of enoxacin and norfloxacin on human colonic microflora. *Antimicrob Agents Chemother*, 31:1846–8.

Edlund C, Nord CE. 1988a. Manipulation of the oropharyngeal and intestinal microflora by norfloxacin: microbiological and clinical aspects. *Scand J Infect Dis Suppl*, 56:14–21.

Edlund C, Nord CE. 1988b. A review on the impact of 4-quinolones on the normal oropharyngeal and intestinal human microflora. *Infection*, 16:8–12.

Edlund C, Nord CE. 1999a. Ecological effect of gatifloxacin on the normal human intestinal microflora. *J Chemother*, 11:50–3.

Edlund C, Nord CE. 1999b. Effect of quinolones on intestinal ecology. *Drugs*, 58(Suppl 2):65–70.

Edlund C, Nord CE. 2000. Effect on the human normal microflora of oral antibiotics for treatment of urinary tract infections. *J Antimicrob Chemother*, 46(Suppl A):41–8.

Edlund C, Sjöstedt S, Nord CE. 1997b. Comparative effects of levofloxacin and ofloxacin on the normal oral and intestinal microflora. *Scand J Infect Dis*, 29:383–6.

Edlund C, Stark C, Nord CE. 1994. The relationship between an increase in beta-lactamase activity after oral administration of three new cephalosporins and protection against intestinal ecological disturbances. *J Antimicrob Chemother*, 34:127–38.

Finegold SM. 1986. Anaerobic infections and *Clostridium difficile* colitis emerging during antibacterial therapy. *Scand J Infect Dis Suppl*, 49:160–4.

Gauthier G, Klein BS. 2008. Insights into fungal morphogenesis and immune evasion. *Microbe*, 3:416–23.

Gipponi M, Sciutto C, Accornero L, et al. 1985. Assessing modifications of the intestinal bacterial flora in patients on long-term oral treatment with bacampicillin or amoxicillin: a random study. *Chemioterapia*, 4:214–7.

Heimdahl A, Nord CE. 1982. Effect of erythromycin and clindamycin on the indigenous human anaerobic flora and new colonization of the gastrointestinal tract. *Eur J Clin Microbiol*, 1:38–48.

Inagaki Y, Yamamoto N, Chida T, et al. 1995. The effect of DU-6859a, a new potent fluoroquinolone, on fecal microflora in human volunteers. *Jpn J Antibiot*, 48:368–79.

Kager L, Brismar B, Malmborg AS, et al. 1986. Impact of cefbuperazone on the colonic microflora in patients undergoing colorectal surgery. *Drugs Exp Clin Res*, 12:983–6.

Kager L, Brismar B, Malmborg AS, et al. 1988. Effect of imipenem treatment versus imipenem surgical prophylaxis on the intestinal microflora. *Int J Clin Pharmacol Res*, 8:441–7.

Kager L, Brismar B, Malmborg AS, et al. 1989. Imipenem concentrations in colorectal surgery and impact on the colonic microflora. *Antimicrob Agents Chemother*, 33:204–8.

Kager L, Liljeqvist L, Malmborg AS, et al. 1981. Effect of clindamycin prophylaxis on the colonic microflora in patients undergoing colorectal surgery. *Antimicrob Agents Chemother*, 20:736–40.

Kager L, Malmborg AS, Nord CE, et al. 1982. The effect of short-term cefoxitin prophylaxis on the colonic microflora in patients undergoing colorectal surgery. *Infection*, 10:338–40.

Kager L, Malmborg AS, Nord CE, et al. 1983. The effect of piperacillin prophylaxis on the colonic microflora in patients undergoing colorectal surgery. *Infection*, 11:251–4.

Kennedy MJ, Volz PA. 1985. Effect of various antibiotics on gastrointestinal colonization and dissemination by *Candida albicans*. *Sabouraudia*, 23:265–73.

Kennedy MJ, Volz PA, Edwards CA, et al. 1987. Mechanisms of association of *Candida albicans* with intestinal mucosa. *J Med Microbiol*, 24:333–41.

Lambert-Zechovsky N, Bingen E, Aujard Y, et al. 1985. Impact of cefotaxime on the fecal flora in children. *Infection*, (13 Suppl 1):S140–S144.

Leigh DA. 1979. Pharmacology and toxicological studies with amoxycillin, talampicillin, and ampicillin and a clinical trial of parenteral amoxycillin in serious hospital infections. *Drugs Exptl Clin Res*, 5:29–139.

Leigh DA, Nash JG. 1979. Parenteral amoxycillin treatment of severe infections in hospitalized patients. *J Antimicrob Chemother*, 5:109–12.

Lode H, Müller C, Borner K, et al. 1992. Multiple-dose pharmacokinetics of cefprozil and its impact on intestinal flora of volunteers. *Antimicrob Agents Chemother*, 36:144–9.

Lode H, Von der Höh N, Ziege S, et al. 2001. Ecological effects of linezolid versus amoxicillin/clavulanic acid on the normal intestinal microflora. *Scand J Infect Dis*, 33:899–903.

Louie TJ, Chubb H, Bow EJ, et al. 1985. Preservation of colonization resistance parameters during empiric therapy with aztreonam in the febrile neutropenic patient. *Rev Infect Dis*, 7(Suppl 4):S747–S761.

Nikolaeva IV, Anokhin VA, Bondarenko VM, et al. 2001. [Drug resistance of *Staphylococcus aureus* strains, isolated from children with intestinal dysbacteriosis]. *Zh Mikrobiol Epidemiol Immunobiol*, 1:9–13.

Nilsson-Ehle I, Nord CE, Ursing B. 1985. Ceftriaxone: pharmacokinetics and effect on the intestinal microflora in patients with acute bacterial infections. *Scand J Infect Dis*, 17:77–82.

Nord CE. 1993. The effect of antimicrobial agents on the ecology of the human intestinal microflora. *Vet Microbiol*, 35:193–7.

Nord CE. 2008. Antimicrobial susceptibility patterns of anaerobic bacteria in Europe (abstract). *9th Biennial Congress of the Anaerobe Society of the Americas*, Long Beach, CA. p. 137.

Nord CE, Bergan T, Aase S. 1986a. Impact of azlocillin on the colon microflora. *Scand J Infect Dis*, 18:163–6.

Nord CE, Bergan T, Thorsteinsson SB. 1989. Impact of ticarcillin/clavulanate on the intestinal microflora. *J Antimicrob Chemother*, 24(Suppl B):221–6.

Nord CE, Brismar B, Kasholm-Tengve B, et al. 1992. Effect of piperacillin/tazobactam therapy on intestinal microflora. *Scand J Infect Dis*, 24:209–13.

Nord CE, Brismar B, Kasholm-Tengve B, et al. 1993. Effect of piperacillin/tazobactam treatment on human bowel microflora. *J Antimicrob Chemother*, 31(Suppl A):61–5.

Nord CE, Edlund C. 1990. Impact of antimicrobial agents on human intestinal microflora. *J Chemother*, 2:218–37.

Nord CE, Farrell DJ, Leclercq R. 2004. Impact of ketolides on resistance selection and ecologic effects during treatment for respiratory tract infections. *Microb Drug Resist*, 10:255–63.

Nord CE, Heimdahl A, Kager L, et al. 1984a. The impact of different antimicrobial agents on the normal gastrointestinal microflora of humans. *Rev Infect Dis*, 6(Suppl 1):S270–S275.

Nord CE, Heimdahl A, Lundberg C, et al. 1987. Impact of cefaclor on the normal human oropharyngeal and intestinal microflora. *Scand J Infect Dis*, 19:681–5.

Nord CE, Kager L, Heimdahl A. 1984b. Impact of antimicrobial agents on the gastrointestinal microflora and the risk of infections. *Am J Med*, 76:99–106.

Nord CE, Kager L, Malmborg AS. 1988. Effects of antimicrobial prophylaxis on colonization resistance. *J Hosp Infect*, 11(Suppl A):259–64.

Nord CE, Kager L, Philipson A, et al. 1985. Effect of imipenem/cilastatin on the colonic microflora. *Rev Infect Dis*, 7(Suppl 3):S432–S434.

Nord CE, Lahnborg G. 1994. Efficacy of piperacillin/tazobactam in the treatment of experimental intra-abdominal infections. *Eur J Surg Suppl*, (573):45–9.

Nord CE, Meurling L, Russo RL, et al. 2003. Effect of garenoxacin on eubacteria in the normal intestinal microflora when administered concomitantly with digoxin. *J Chemother*, 15:244–7.

Nord CE, Rasmanis G, Wahlund E. 2006a. Effect of dalbavancin on the normal intestinal microflora. *J Antimicrob Chemother*, 58:627–31.

Nord CE, Sillerstrom E, Wahlund E. 2006b. Effect of tigecycline on normal oropharyngeal and intestinal microflora. *Antimicrob Agents Chemother*, 50:3375–80.

Noverr MC, Huffnagle GB. 2005. The 'microflora hypothesis' of allergic diseases. *Clin Exp Allergy*, 35:1511–20.

Oh H, Nord CE, Barkholt L, et al. 2000. Ecological disturbances in intestinal microflora caused by clinafloxacin, an extended-spectrum quinolone. *Infection*, 28:272–7.

Peck JJ, Fuchs PC, Gustafson ME. 1984. Antimicrobial prophylaxis in elective colon surgery. Experience of 1,035 operations in a community hospital. *Am J Surg*, 147:633–7.

Pecquet S, Andremont A, Tancrede C. 1987. Effect of oral ofloxacin on fecal bacteria in human volunteers. *Antimicrob Agents Chemother*, 31:124–5.

Pletz MW, Rau M, Bulitta J, et al. 2004. Ertapenem pharmacokinetics and impact on intestinal microflora, in comparison to those of ceftriaxone, after multiple dosing in male and female volunteers. *Antimicrob Agents Chemother*, 48:3765–72.

Prantera C, Scribano ML, Berto E, et al. 1997. Antibiotic use in Crohn's disease: why and how? *BioDrugs*, 8:293–306.

Pultz NJ, Donskey CJ. 2005. Effect of antibiotic treatment on growth of and toxin production by *Clostridium difficile* in the cecal contents of mice. *Antimicrob Agents Chemother*, 49:3529–32.

Rafii F, Park M, Bryant AE, et al. 2008. Enhanced production of phospholipase C and perfringolysin O (alpha and theta toxins) in a gatifloxacin-resistant strain of *Clostridium perfringens*. *Antimicrob Agents Chemother*, 52:895–900.

Rafii F, Park M, Novak JS. 2005. Alterations in DNA gyrase and topoisomerase IV in resistant mutants of *Clostridium perfringens* found after *in vitro* treatment with fluoroquinolones. *Antimicrob Agents Chemother*, 49:488–92.

Ritz M, Lode H, Fassbender M, et al. 1994. Multiple-dose pharmacokinetics of sparfloxacin and its influence on fecal flora. *Antimicrob Agents Chemother*, 38:455–9.

Rowland IR. 1995. Toxicology of the colon. Role of intestinal microflora. In: Gibson GR, Macfarlane GT (ed). Human Colonic Bacteria. Boca Raton, FL: CRC Press, pp. 155–74.

Schröder O, Gerhard R, Stein J. 2006. [Antibiotic-associated diarrhea]. *Z Gastroenterol*, 44:193–204.

Stark C, Edlund C, Hedberg M, et al. 1995. Induction of beta-lactamase by cefoxitin in anaerobic intestinal microflora. *Eur J Clin Microbiol Infect Dis*, 14:18–24.

Stark CA, Adamsson I, Edlund C, et al. 1996. Effects of omeprazole and amoxycillin on the human oral and gastrointestinal microflora in patients with *Helicobacter pylori* infection. *J Antimicrob Chemother*, 38:927–39.

Stark CA, Edlund C, Sjöstedt S, et al. 1993. Antimicrobial resistance in human oral and intestinal anaerobic microfloras. *Antimicrob Agents Chemother*, 37:1665–9.

Sullivan A, Edlund C, Nord CE. 2001a. Effect of antimicrobial agents on the ecological balance of human microflora. *Lancet Infect Dis*, 1:101–14.

Sullivan A, Edlund C, Svenungsson B, et al. 2001b. Effect of perorally administered pivmecillinam on the normal oropharyngeal, intestinal and skin microflora. *J Chemother*, 13:299–308.

van der Waaij D. 1985. Selective decontamination of the digestive tract with oral aztreonam and temocillin. *Rev Infect Dis*, 7(Suppl 4): S628–S634.

van der Waaij D, Nord CE. 2000. Development and persistence of multiresistance to antibiotics in bacteria; an analysis and a new approach to this urgent problem. *Int J Antimicrob Agents*, 16:191–7.

Vollaard EJ, Clasener HA, Janssen AJ. 1992. Influence of pefloxacin on microbial colonization resistance in healthy volunteers. *Eur J Clin Microbiol Infect Dis*, 11:257–60.

Wagner RD, Johnson SJ, Cerniglia CE. 2008. An i*n vitro* model of colonization resistance by the enteric microbiota: effects of antimicrobial agents used in food-producing animals. *Antimicrob Agents Chemother*, 52:1230–7.

Winberg J, Herthelius-Elman M, Möllby R, et al. 1993. Pathogenesis of urinary tract infection – experimental studies of vaginal resistance to colonization. *Pediatr Nephrol*, 7:509–14.

Zhang X, McDaniel AD, Wolf LE, et al. 2000. Quinolone antibiotics induce Shiga toxin-encoding bacteriophages, toxin production, and death in mice. *J Infect Dis*, 181:664–70.