We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

4,800
Open access books available

122,000
International authors and editors

135M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

CLARIVATE ANALYTICS BOOK CITATION INDEX INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Chapter 5

Influence of Selected Per Orally Administered ATB on Microflora of GIT in Experimental Animals

Madar Marian, Telepjanová Tatiana and Gancarčíková Soňa

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.71554

Abstract

Composition of gastrointestinal (GIT) microbiota differs in individual parts of GIT. Only 40% of GIT bacteria are cultivable. Fluorescence-in-situ-hybridization (FISH) can detect non-cultivable bacteria. Perorally administered antibiotics (ATB) affect the composition of microbiota in GIT. The absorbed ATB, namely penicillins, tetracyclines, macrolides or fluorochinolons, have different influence in comparison with poorly absorbed oral ATB, such as aminoglycosides, aminocoumarines or polypeptides. This effect is due to retention of high concentration of non-absorbed ATB during passage through GIT and their longer influence on bacteria living in different parts of GIT. Study methods were based on scientific literature review from PubMed, Elsevier databases and Slovak scientific publications. We searched for publications between years 1980 and 2016, with keywords: ATB, influence, microbiota, FISH. The literature review focuses on peroral administration of ATB to humans and animals and its potential effect on composition of GIT microbiota. The relevant studies showed that per orally administered ATB produced many important changes in microbiota of GIT. FISH method was more frequently used for screening the normal composition of microbiota than for studying the effects of ATB although there were some studies dealing also with this issue.

Keywords: peroral ATB, effect, microbiota, GIT, FISH

1. Introduction

Although the use of antibiotics administered antibiotics (ATB) is nowadays often necessary, there is still a number of issues that arise from their abuse. It is known, that excessive use of ATB has a negative impact on physiological composition of intestinal microbiota,

especially when they are administered *per os*. This is due to increase in gastrointestinal (GIT) diseases. To understand the impact of ATB on GIT microbiota it is necessary to know the correct composition of the GIT microbiota and changes induced by various ATB in this convocation. The most common pattern for tracking changes in the microflora is faeces. However, there is little knowledge on microbiological changes in various parts of GIT. Experimental animals, both conventional and gnotobiotic, were used in relevant studies. However, they were fed a different type of food in addition to a number of anatomical and physiological differences. Therefore, for many scientists this issue still remains a great mystery. Also, until the development of sensitive molecular methods, conventional culture methods were used to track these changes. However, since 40–90% of the intestinal bacteria are not cultivable, scientists looked for and tested more sensitive and accurate methods for the detection and quantification of microorganisms [1]. For example, developed were methods based on PCR-DGGE, real-time PCR, and others. However, even these methods have shortcomings that require an amplification process which may introduce an untargeted error. The fluorescent-in-situ-hybridization (FISH) method is independent of the amplification and is sufficiently sensitive to trap even non-cultivable microorganisms. So far scientists have used a number of FISH to determine the physiological composition of microbiota of GIT, either animal or human. In addition, the new development allows one to monitor potential changes under the impact of substances added to the diet in both experimental animals and clinical patients. The aim of this study was to summarise the findings on the impact of ATB on composition of intestinal microbiota by means of FISH method using available sources and compare them with previously published knowledge in this area. The importance of this study consists in finding out whether it is possible to track by this method the changes in GIT microbiota produced by ATB and thus contribute to the body of knowledge in this area.

Recently, the increasing resistance of bacterial agents to ATBs, such as Methicillin-resistant *Staphylococcus aureus* (MRSA) or *Pseudomonas*, stressed the importance of the development of novel ATB derivatives [2]. New classes of antibiotics are urgently needed to treat nosocomial infections. The risk of increasing ATB resistance also increases due to the increased use of broad-spectrum ATBs in unprofessional human and veterinary clinical practice, without detecting the bacterial origin of the disease and its sensitivity to ATBs. ATB residues in food of animal origin from countries not complying with the 2006 EU Directive have an impact on the increased risk of spreading antibiotic resistance. Development of ATB-resistant strains can be prevented by using correct therapeutic dose of ATB and completing the prescribed course of treatment. Properly balanced intestinal microflora prevents the development of resistant microbial strains. Normal microbiota acts as a barrier against the colonisation of potentially pathogenic microorganisms and against the excessive growth of opportunistic microorganisms already present. Administration of ATBs either therapeutically or as a prophylactic measure disturbs the ecological balance between the host and the normal microbiota. The clinically most common symptoms of intestinal microbiota disruption are diarrhoea and fungal infections that usually resolve after the treatment has ended [3]. It is difficult to assess the long-term consequences of microbial symbiosis disorders in the intestine. In addition to

changes in intestinal microbiota, many chronic diseases such as asthma and atopic diseases are associated with the use of ATB in childhood [4].

2. Antibiotics

In 1928, Alexander Fleming discovered that the growth of *Penicillium notatum* suppressed the growth of staphylococci, and then, as this phenomenon was studied, it was found that the cause was an exoproduct of a mould called penicillin that was released into the cultivation medium. In 1938, Howard Florey and Ernst Chain began experimenting with penicillin mould. By 1941, a sufficiently purified form of penicillin was obtained and by early 1942, American pharmaceutical companies were mass producing penicillin for distribution to Allied soldiers during the Second World War [5]. Since the first effects of ATB have been discovered, other substances with ATB properties have appeared and many have found a wide range of applications in medicine for the treatment of infections caused by bacteria, pathogenic fungi, mycoplasmas, rickets, chlamydia and some other agents [6]. Attempts to influence GIT microbiota with ATB date back to the very beginning of their use. The impact of ATB was observed in clinical practice as well as during preoperative patient preparation. With regard to animal production, it raised interest particularly for economic reasons, as it was shown that ATBs accelerate the growth and weight gain in mice, dogs, but also in pigs and calves. Experiments on germ-free chickens revealed that the nutritional effect of ATB is mainly related to suppression of some subclinical infections [7].

Antibiotics are substances of organic origin produced by bacteria and moulds, possibly from higher plants or animal tissues, and can be prepared synthetically or semi-synthetically [8]. Their name was derived from the phenotype of Pasteur, which was described by Pasteur in the 1960s.

According to their biological effect on microorganisms they are divided to two groups, one with bacteriostatic action and another one with bactericidal effect. Bacteriostatic ATBs arrest multiplication of bacteria so the bacteria are not killed and natural dying of quiescent bacterial cells is not affected. Bactericidal effect of ATBs results in death of bacterial cells. The bactericidal effect during the first 4 hours of action of ATBs is of specific importance. If at least 99% of bacteria is killed within this time we can speak about clinically relevant bactericidal action.

ATBs are divided into 5 groups according to the mechanism of action:

1. Inhibition of cell wall synthesis (bactericidal effect), (typical of penicillins, vancomycin, cycloserin)
2. Effect of cell wall function (bactericidal effect), (typical of polymyxins)
3. Inhibition of protein synthesis (bacteriostatic and bactericidal effect) (chloramphenicol, tetracyclines, aminoglycosides, macrolide ATBs)

4. Inhibition of nucleic acid synthesis (bactericidal effect), (griziofulvin, rifampicin)
5. Interference in the intermediary metabolism of bacteria (sulfonamides)

2.1. Oral antibiotics

Not all ATBs can be administered orally, but ATBs capable of influencing GIT microbiota must be available in the form suitable for oral administration. The most commonly used orally administered ATBs include: penicillins, cephalosporins, tetracyclines, polypeptide ATBs, aminoglycosides, macrolides, Lincosamide ATB, ansamycin ATB, diterpenes, aminocoumarin ATBs, steroid antibiotics, sulfonamides and quinolones. Among these, we include the following representatives:

1. **Penicillins**:

   (A) **Phenoxypenicillins**: Phenoxymethylpenicillin—Penicillin V, Penamecillin, Penetacillin, Benetaminpenicillin, Phenticillin, Propicillin, Phenbenicillin, Klometocillin

   (B) **Wide spectrum of penicillins**:

   1. **Aminopenicillins**: Ampicillin, Bakampicillin, Pivampicillin, Talampicillin, Amoxicillin, Epicillin, Cyclaclin
   2. **Carboxypenicillins:** Carbenicillin Esters: Indanyl Carbenicillin, Carfecili
   3. **Amidopenicillins:** Mecilinam esters: Bakmecilinam, Pivmecilinam
   4. **Isoxazolylpenicillins:** Oxacillin, Dicloxacillin, Kloxacillin, Flucloxacillin, Pirazocillin

2. **Cephalosporins**: Cefalexin, Cefadroxil, Cefixim, Metacyclin, Tiacycline
3. **Amphenicols**: Chloramphenicol, Tiamfenicol, Florfenicol
4. **Tetracyclines**: Chlortetracycline, Oxytetracycline, Tetracycline, Doxycycline, Minocycline,
5. **Polypeptide antibiotics**: Polymyxins: Polymyxin B
6. **Aminoglycosides**: Streptomycin, Neomycin, Kanamycin, Apramycin, Gentamicin, Tobramycin and Aminocyclitols: Spectinomycin
7. **Macrolides**: Erythromycin, Spiramycin, Tylozine, Oleandromycin, Troleandromycin, Josamycin, Tilmicosine, Clarithromycin, Roxithromycin and Azalides: Azithromycin
8. **Linkozamide antibiotics**: Linkomycin, Klindymycin
9. **Ansamycin antibiotics**: Rifampicins: Rifampicin, Rifaximin, Rifabutin, Rifapentin
10. **Diterpenes**: Tiamulin, Valnemulin
11. **Aminocoumarin antibiotics**: Novobiocin
12. **Antibiotics with steroid structure**: Fusidic acid

According to some authors, other peroral drugs with antibacterial activity are considered antibiotics:

http://dx.doi.org/10.5772/intechopen.71554

13. Other antimicrobials

1. Nitroimidazole derivatives: Metronidazole, Tinidazole Nimorazole
2. Sulfonamides:

   Short-acting: Sulfathiazole, Sulfacetamide, Sulfisoxazole.

   Medium-effective sulphonamides: Sulfadimidine, Sulfadiazine, Sulfamerazine, Sulfamethoxazole + Trimetoprim = Kotrixomazole, Sulfachloropyridazine.

   Long-term effective: Sulfamethoxypyridazine, Sulfadoxine, Sulfadimetoxin Enteric-acting sulfonamides: Phthalylsulfathiazole, Succinylsulfathiazole, Sulfachinoxaline, Sulfaclozine
3. Quinolones: Nalidixic acid, Flumequin, Enrofloxacin, Difloxacin, Ciprofloxacin, Marbofloxacin, Norfloxacin, Sarafloxacin, Pefloxacin, Ofloxacin, Ibafloxacin, Orbifloxacin

3. Materials and methods

Search method: we searched the PubMed, and Elsevier databases and Slovak scientific literature for the studies dealing with the effect of ATBs on GIT composition. We searched for publications in the period from 1980 to 2016 using keywords related to ATB, Influence, microbiota, FISH. A literature review was produced aimed to identify association between peroral administration of ATB to humans or animals and its effect on composition of normal microbiota in GIT.

4. Influence of ATB on GIT microflora

Administration of ATBs can seriously disturb the balance of the intestinal microbiota in terms of multiplication of bacteria and development of resistant microorganisms. This can lead to infections and to the transfer of resistance factors between bacteria [9]. According to the majority of authors, the effect of ATB on nutrition is mediated by intestinal microbiota. Antibiotics are divided according to their effect on GI microbiota to ATBs capable of absorption across the intestinal wall and to those that cannot be absorbed at all or only in very small amounts. The lower the bioavailability of ATB the more it remains in the colon and thus the risk of suppression of intestinal microflora increases. If ATBs are absorbable (e.g. tetracycline, penicillin, chloramphenicol, etc.), their concentration is lower in the GIT endpoints. In contrast, ATBs incapable of absorption (e.g. streptomycin, polymyxin, neomycin, etc.) may have a strong toxic effect on the microbiota throughout the GIT. The effect of ATB is generally dependent on the dose, the active substance, the duration of administration and other factors. The search results clearly demonstrated that the effect of ATB on the GIT microbiota is as follows:

1. Breach of microbial balance (in GIT, urinary tract, reproduction tract, etc.).
2. Vitamin K hypovitaminosis as result of long-term use ATB (especially p.o.)

3. Resistance of resistant strains, superinfection: *Candida*, *Staphylococcus*, *Pseudomonas*, *Clostridium difficile* and others.
4. Evidence of rapid bacteriolysis, particularly Gram-negative bacteria (endotoxin release)

4.1. Testing of ATB effect on animals

The studies of the effect of ATB on microbiological-clinical microbiota date back more than 50 years ago [10, 11]. The effect on microbiota was investigated with regard to the weight gains of conventional experimental animals. Studies on germ-free animals (without GIT microbes) showed weight gains related to ATB [11]. It is still an up-to-date topic as indicated by recent studies [12]. To demonstrate the presence of bacteria and changes in their numbers, whether under the influence of antibiotics and other substances, conventional cultivation methods are still used. However, these methods have recently been supplemented by more sensitive molecular methods. One of the methods used for quantification of bacterial population is the fluorescent-in-situ-hybridization method (FISH). These methods can be used to accurately identify and quantify the species representation of microorganisms [13]. While radioactive labelling was previously used in the FISH methodology, today we use fluorochrome-labelled probes [14]. The probes serve to specifically bind to that part of the target sequence that exhibits a high degree of sequence complementarity. The probes consist mostly of 15–30 nucleotides and are covalently labelled with a fluorescent dye at the 5 'end—fluorescein, tetramethylrodamine, Texas red, carbocyanine. Up to now, several probes have been standardised, which are currently used to quantify the major intestinal bacteria (**Table 1**). For example, a probe called (S-G-Lab-0158-a-A-20) or abbreviated Lab158 is designed to detect the presence of *Lactobacillus* spp./*Enterococcus* spp. in the monitored samples. It is an oligonucleotide with the sequence 5'X-GGT AAT AGC A (T/C) C TGT TTC-3 'wherein X is fluorochrome [16]. This method is particularly useful in the study of the effect of probiotics, which are often required to identify probiotic bacteria of the commensal microflora [17]. Recently it was reported that the simultaneous use of ATB and supportive probiotic therapy, which can help to restore intestinal microbiota, can also expand the antibiotic resistance of bacterial intestinal bacteria [18, 19].

4.2. Changes in GIT miroflora after ATB treatment in laboratory animals by FISH methods

In addition to scientific papers dealing with the impact of antibiotics on the microflora of GIT by means of conventional culture methods, studies using FISH method were also published focusing mainly on quantification of bacterial representatives in samples of various origin. This later led to the use of this method also for the purpose of monitoring the effect of ATB on the GIT microflora not only in humans [20] but also in experimental animals that were used to determine changes in composition of microbiota. For example, using of FISH for research of effect of amoxicillin potentiated by clavulanic acid on human faecal microflora in germ-free mice [21]. To provide more clear overview, the sources obtained by search were divided on the basis of their ability to absorb across the GIT.

Short name	Full name	Target microorganism	Sequences (5′ - 3′)
Sal 303	L-S-Sal-1717-a-A-18	*Salmonella* spp.	AATCACTTCACCTACGTG
Bif164	S-G-Bif-0164-a-A-18	*Bifidobacterium* spp., *Parascardovia denticolens*	CATCCGGCATTACCACCC
Lab158	S-G-Lab-0158-a-A-20	*Lactobacillus*, *Weissella* spp.; *Lactococcus lactis*; *Vagococcus*, *Enterococcus*, *Melisococcus*, *Tetragenococcus*, *Catellicoccus*, *Pediococcus* a *Paralactobacillus* spp.	GGTATTAGCAYCTGTTTCCA
Bac303	S-Bacto-0303-a-A-17	*Bacteroides sensu stricto*, *Prevotella* spp., *Parabacteroides*; *Barnesiella viscericola* a *Odoribacter splanchnicus*	CCAATGTGGGGGACCTT
Chis150	S-Chis-0150-a-A-23	*Clostridium tyrobutyricum*; *Adhaeribacter aquaticus*, *Flexibacter canadensis*, *Flexibacteriaceae*; *Propionibacteriaceae*	TTATGCGGTATTAATCTYCCTTT
Rbro730	S-Rbro-730-a-A-18	*Ruminococcus bromii-like*; *Clostridium sporosphaeroides* a *Clostridium leptum*	TAAAGCCCAGYAGGCCGC
Rfla729	S-Rfla-729-a-A-18	*Ruminococcus albus* a *Ruminococcus flavefaciens*	AAAGCCCAGTAAGCCGCC
Ato291	S-Ato-0291-a-A-17	*Atopobium*, *Colinsella*, *Olsenella Eggerthella* spp.; *Cryptobacterium curtum*; *Mycoplasma equigenitalium Mycoplasma elephantis*	GGTCGGTCTCTCAACCC
Erec482	S-Erec-0482-a-A-19	*Clostridium saccharolyticum*, *Syntrophococcus sucromutans*, *Bacteroides galacturonicus Bacteroides xylanolyticus Lachnospira pectinschiza*	GCTTCTTAGTCARGTACCG

Source: http://onlinelibrary.wiley.com/doi/10.1111/j.1574-6941.2008.00610.x/pdf
Table processed by the author from the original Table [15].

Table 1. Probes for FISH analysis used to detect bacterial populations in samples from *in vitro* fermentation.

4.2.1. Absorbable ATBs

4.2.1.1. Penicillin ATBs: aminopenicillins

4.2.1.1.1. Amoxicillin

The results of studies dealing with the effect of amoxicillin on the microbiota indicate that *per os* administration caused a significant decrease in the number of total faecal bacteria by almost 30%, as determined by the universal Eub338 probe. Major microbiota populations such as *Fusobacterium*,

Eubacterium and *Atopobium* were affected by amoxicillin. There was observed also a percentage increase in *Bacteroides* and *Bifidobacterium*. The results also showed that not all evaluated populations were affected by the ATB. The greatest change was observed in *E. coli* counts, which increased significantly during ATB administration [3, 22]. By using FISH, the effect of amoxicillin potentiated by clavulanic acid on human faecal microbiota in germ-free mice was observed [21]. In this study, amoxicillin with clavulanic acid was administered orally for 7 days and the results were compared with the control group of mice not treated with ATB. Molecular analysis of digestive microbiota was performed in a 2-week experiment using FISH in combination with flow cytometry (FC) using specific 16S rRNA target probes for *Bacteroides-Porphyromonas-Prevotella*, *Clostridium coccoides-Eubacterium rectale*, *Clostridium histolyticum*, *Faecalibacterium prausnitzii*, *Enterobacteriaceae*, *Lactobacillus*, *Enterococcus*, and *Bifidobacterium*. *Clostridium coccoides-Eubacterium rectale* and *Bacteroides-Porphyromonas-Prevotella*, which represented the dominant flora, were found to be the most abundant bacterial groups. The *Clostridium coccoides* group was stable in control mice (from 40.7 ± 1.6% to 45.6 ± 2.8%) but significantly decreased in the treated mice on the second day of treatment and remained at a low level throughout the ATB treatment (3.9 ± 0.8%). At the end of ATB administration, the levels increased (17.7 ± 4.7%) and by day 14 reached 36 ± 1.8%. The *Bacteroides-Porphyromonas-Prevotella* group in control mice persisted at 35.9 ± 4.3%, whereas in treated mice it increased from 1 to 6 days when it reached 58.5 ± 0.4.5%. From day 9, the level decreased to 38.6 ± 5.7% until it reached the same level as in control mice at the end of the experiment [21]. This animal model allowed the authors to conclude that amoxicillin potentiated by clavulanic acid disrupts the balance of the dominant anaerobic microflora and that the *Clostridium coccoides* group is very susceptible to amoxicillin potentiated by clavulanic acid. No *Enterobacteriaceae* bacteria were detected in control mice, on the other hand their number increased and they were detectable in the treated mice from day 2 of administration of ATB. From day 8, their counts decreased and from day 11 until the end of the experiment they were no more detectable. *Faecalibacterium prausnitzii* and *Clostridium histolyticum* were present in 1.3 ± 2.1% and 0.4 ± 0.4% of control mice [21]. No bacteria were detected in the treated mice during administration of ATB, i.e. these bacterial groups were sensitive to amoxicillin-clavulanic acid. From day 1 to day 14 after administration of ATB, the counts of these groups of bacteria were similar to those in control mice. The probes for *Bifidobacterium*, *Lactobacillus* and *Enterococcus* did not detect any signals in either treated or control mice [21]. During 7 days of *per os* treatment with amoxicillin potentiated with clavulanic acid, the effect of *Saccharomyces boulardii* yeasts on the composition of intestinal microbiota in mice associated with human microbiota was also investigated. The predominant groups of bacteria were quantified by FISH in combination with flow cytometry. Probes for *Eubacteria*, *Bacteroides-Porphyromonas-Prevotella*, *Clostridium coccoides-Eubacterium rectale*, *Faecalibacterium prausnitzii*, *Clostridium histolyticum*, *Lactobacillus-Enterococcus*, *Enterobacteriaceae* and *Bifidobacterium* species have been used. The observed mice were divided into two groups of mice, the first group received yeast and the second did not. In the second group the level of *Enterobacteriaceae* and *Bacteroides* increased but the numbers of *C. coccoides-E rectale* dropped dramatically. After ATB treatment, the original intestinal flora was restored more rapidly for *C. coccoides-E. rectale* and *Bacteroides-Porphyromonas-Prevotella* in *S. boulardii* mice versus control mice ($p < 0.05$) [21]. The effect of other beta lactam ATBs on the microbiota, in particular of imipenem, was also observed using the FISH method (Dubourg et al., [23]). The susceptibility

of *Akkermansia muciniphila* with respect to the effect of imipenem was also studied. In this case, the FISH method utilised a specific protozoan 5 '[Alexa488/546] GCTGCCACCCGTAGGTGT for *Verrucomicrobium*, which confirmed the presence of the bacterium. EUB338 '[Alexa488/546] 5-GCTGCCTCCCGTAGGAGT-3 [23] was also used. Stool samples with *Akkermansia muciniphila* were susceptible to imipenem.

4.2.1.2. Lincosamide ATBs

4.2.1.2.1. Clindamycin

Clindamycin was used in the study dealing with development of vancomycin-resistant enterococci (VRE) because this ATB inhibits anaerobes in the intestine without the reduction of facultative Gram-negative bacilli and VRE [24]. It has been shown that clindamycin causes VRE growth in mice and colonised patients [25]. In this study, a mouse model was used to test the hypothesis that the anaerobic microflora in the large intestine inhibits development of vancomycin-resistant enterococci. Anaerobic growth of VRE was assessed in the caecal content and cervical mucus of mice receiving subcutaneous clindamycin and in negative control administered saline solution. Following orogastric inoculation of VRE-*Enterococcus Faecium* C68, the mice were sacrificed and tested. To confirm that some Gram-positive cocci visualised in this experiment using light microscopy, a specific commercially available kit for the detection of *E. faecium* by fluorescence in situ hybridization (Microscreen) was used with *E. faecium*. In saline treated mice, no *E. faecium* was detected by in situ hybridization. In contrast, the presence of *E. faecium* was confirmed in clindamycin-treated mice [25].

4.2.1.3. Fluorochinolons

4.2.1.3.1. Ciprofloxacin

In a study investigating the role of intestinal bacteria in the pathogenesis of chronic, immuno-mediated inflammation of the intestine, ciprofloxacin has been shown to affect the inflammation of the intestine but not the inflammation of the colon. This has confirmed the selective effect of ciprofloxacin in the gut. Experimental pathogen free (SPF) mice were used. Furthermore, mice lacking the gene encoding interleukin 10 (IL10) producing colitis have been used. However, this does not occur in germ-free mice. Germ-free, IL-10 deficient mice were colonised by SPF bacteria, and narrowed and broad-spectrum ATBs were observed to influence the development and development of intestinal inflammation in IL10 deficient mice. ATBs were administered to mice orally, either preventively prior to colonisation of SPF with bacteria or therapeutically. Quantitative bacterial analysis using the FISH method used parts of the blind and the colon [26]. BAC303 for *Bacteroides*/*Prevotella*, *E. coli* specific EC1531 and other Enterobacteriaceae, Lab158 for the detection of lactobacilli and enterococci were used for FISH detection. By the FISH method, ciprofloxacin was found to reduce total aerobic bacteria in both the colon and caecum. *E. coli* was not detectable and the number of luminal enterococci was reduced. Reduction of lactobacilli was also confirmed [26].

4.2.1.4. Other antimicrobial substances: imidazole derivatives

4.2.1.4.1. Metronidazole

A study [26] on germ-free, IL10 deficient mice that were colonised by SPF bacteria (no specific pathogens) and were monitored for the effect of a particular narrow spectrum ATB metronidazole on the development of inflammation of the intestine showed a selective effect of metronidazole in the large intestine. The effect of this ATB on inflammation of the cervix was not confirmed. BAC303 for *Bacteroides/Prevotella*, *E. coli* specific EC1531 and other *Enterobacteriaceae*, Lab158 for the detection of lactobacilli and enterococci were used for FISH detection. Metronidazole is selectively effective against anaerobic bacteria, including predominantly *Bacteroides*. FISH revealed that administration of metronidazole reduced the number of *Bacteroides* species to a detectable level. Also, the amount of luminal *E. coli* was significantly reduced. FISH analysis showed that metronidazole had no significant effect on intestinal lactobacilli. Enterococci were confirmed, in particular *E. faecalis*. The study [23] confirmed that *Akkermansia muciniphila* were resistant to metronidazole.

4.2.1.5. Tetracycline ATBs

4.2.1.5.1. Tetracycline

ATBs such as tetracycline have the ability to interfere with bacterial populations in the gut. If the formation of a microbial barrier against pathogens and potential pathogens is impaired, it can lead to the proliferation of undesirable microorganisms such as *Candida albicans*. In *in vitro* studies, growth of *C. albicans* was observed in growth media in the presence of tetracycline, with a significant increase in *C. albicans*. The potency of the probiotic culture of *Lactobacillus plantarum* LPK, which was added to the *in vitro* fermentation system, was also tested to determine whether this organism had any effect on the Candida population. Although *C. albicans* was not completely removed in the presence of this bacterium, its numbers were significantly reduced. This study showed that the use of probiotics, in particular *Lactobacillus plantarum*, had a positive effect on the reduction of undesirable *C. albicans*, the number of which was increased by tetracycline administration. It also pointed out that normal intestinal microflora can itself develop a 'natural' resistance to *C. albicans* (Payne et al., [27]). In the future, it would be necessary to use a probe detecting the presence of *C. albicans* to quantify this bacterium when studying the effect of tetracycline on GIT microbiota. For this purpose, oligonucleotide 020 (5 'CCCCCTTTCCTAAACCAATCCGGA 3') can be used [28].

4.2.1.5.2. Doxycycline

One of the few studies that dealt with the effect of doxycycline on microbiota using the FISH method was a study aimed at monitoring its effect on *Akkermansia muciniphila*. For the FISH method, a specific probe 5 '[Alexa488/546] GCTGCCACCCGTAGGTGT for Verrucomicrobium was used to confirm the presence of the bacterium. Also, EUB338 '[Alexa488/546] 5-GCTGCCTCCCGTAGGAGT-3 [22] was used. In the stool specimen with *Akkermansia muciniphila*, the sensitivity of this bacterium to doxycycline was confirmed.

4.2.2. Not resorbing ATB

4.2.2.1. Aminoglycoside ATB

4.2.2.1.1. Streptomycin

In streptomycin-treated conventional mice most of the facultatively aerobic Gram-negative rods, amounting to about 0.1 to 1% of microbiota, were eliminated by streptomycin treatment [29]. Multiple model experiments were used to study the effect of streptomycin on microbiota of mice. To detect the presence and quantify *E. coli* strains in streptomycin-treated mice, the authors used ribosomal probe ES 1531 specific for *E. coli* 23S rRNA and *E. coli* BJ4 reference strain that was detected in stool samples [29]. Also, the adhesion properties of *E. coli* to colonic mucosa were studied in streptomycin-treated mice and reduced numbers of *E. coli* were detected [30]. Sekirov 2008 used for the study of the effect of streptomycin on the intestinal microbiota the EUB338 mouse probe for all bacteria (*Eubacteriaceae*) with the sequence (5 '[TxRd]-GCT GCC TCC CGT AGT AGG-3'), *Cytophaga-Flavobacterium-Bacteroides* CFB286 '[Fluorescein]-TCC TCT CAG AAC TAC CCC-3') and for the Gammaproteobacteria probe GAM42a (5 'fluorescein-GCC TTC CCA CAT CGT TT-3'). Sekirov investigated the ability to produce *Salmonella* infection after ATB treatment [31]. He demonstrated that after the administration of streptomycin, the equilibrium of the microbial community of the intestine changes, giving the possibility of infection with Salmonella. He also found that increasing doses of streptomycin resulted in a gradual increase in the strains of *Firmicutes* and *Cytophaga-Flavobacterium-Bacteroides* (CFB). At the genus level, the numbers of lactobacilli and enterococci/group D streptococci decreased significantly. Gradually, the number of *Firmicutes* and other bacteria was reduced. Sekirov, however, concluded that ATB treatment changes the composition of intestinal microbiota depending on dose and type of ATB, but does not significantly change the total number of gut microbiota [31]. After 8 days of per oral administration, the use of a combination of streptomycin and penicillin caused a significant reduction in all bacterial counts measured by FISH and intestinal content analysis (Swann et al. [32]). Although almost every ATB treatment induces an increase in pathogenic colonisation, the development of enterocolitis was particularly observed after the use of streptomycin or vancomycin (Ferreira et al. [33]). In the current research, three types of mouse models were used to study the interaction between the host and the given bacterium: gnotobiotic, conventional and streptomycin-treated. Studies have shown that mice pre-treated with ATBs (e.g. streptomycin) have a higher chance of competitive growth of intestinal pathogens in the intestine, although the mechanism is poorly elucidated [34]. Streptomycin-treated mice are the best model for studying the growth and survival of extraneous microorganisms in the intestine without causing pathogenesis [35].

4.2.2.2. Macrolide ATBs

4.2.2.2.1. Erythromycin

Using a FISH method, a study was conducted that investigated the resistance of the *Campylobacter* strain to macrolide ATB erythromycin. This strain is the most common cause of inflammation

of the intestines (enteric). Because its resistance to quinolones rises, macrolides are currently the drug of first choice. In humans, the resistance of *Campylobacter* to macrolides is about 5%, but in some animals it is up to 80%. Probes for the detection of macrolide resistance in *H. pylori* were used [36, 37]. The theoretical applicability of these probes for *Campylobacter* was assessed by controlling previous publications [38, 39]. FISH may also be useful for detecting macrolide resistance in other bacteria, e.g. mycobacteria or haemophiliacs. However, for this purpose, probes must be adapted to different sequences accompanying the mutation point [40].

4.2.2.2.2. Clarithromycin

The effect of clarithromycin as the most commonly used ATB for the treatment and eradication of *Helicobacter pylori* was studied using the FISH method [36]. In this study, FISH methods were used to demonstrate the presence of *H. pylori* and to identify the 23S rRNA spot mutation responsible for macrolide resistance directly from a biopsy specimen. All oligonucleotide probes used in this study were previously described and evaluated [41]. Briefly, the HPY-1 (5′-CACACCTGACTGACTATCCCG-3′) probe targeting 16S rRNA was used to identify *H. pylori*, while the ClaR1 (A2143G) (5′-CGGGGTCTTCCCGTCTT-3′), ClaR2 (5′-CGGGGTCTCTCCGTCTT-3′) and ClaR3 (A2143C) (5′-CGGGGTCTTGCCGTCTT-3′) were used to detect the 23S rRNA spot mutation responsible for the resistance of the bacterium to clarithromycin. A ClaWT probe (5′-CGGGGTCTTTCCGTCTT-3′) was also used to identify *H. pylori* strains sensitive to clarithromycin that were not detected either by ClaR1, ClaR2 or ClaR3. Similar studies have also been addressed [42].

4.2.2.3. Glycopeptide ATBs

4.2.2.3.1. Vancomycin

In mice, the effect of vancomycin on GIT microbiota differs significantly from that of streptomycin [31]. Low doses of vancomycin reduce bacterial counts of *Firmicutes* and *Cytophaga-Flavobacterium-Bacteroidetes* (CFB) strains and cause a small increase in the class of *Gammaproteobacteria*. Higher doses of vancomycin already cause an increase in thecounts of *Gammaproteobacteria*, to nearly 50% of the total microflora, while the counts of CFB remain reduced. The genera *Lactobacillus-Enterococcus*, group D streptococci, are affected by the overgrowth of the *Enterobacteriaceae* and cultivated aerobic bacteria. ATB treatment alone does not cause significant changes in the total number of microbes, although vancomycin administration has a much greater effect on GIT microbiotas than streptomycin [31]. In the study by [43], the broad-spectrum vancomycin-imipenem combination was shown to be effective in mice, both in the cecum and in the colon. Despite the significant decrease in *E. coli* and *E. faecalis*, the total aerobic microflora was not reduced after administration of vancomycin with imipenem. However, the amount of total anaerobic bacteria was significantly reduced. Lactobacilli were eliminated after administration of the vancomycin-imipenem combination. Using FISH, it has also been found that by administering this combination, many *Bacteroides* species have been reduced below a detectable level [43]. Also, vancomycin resistance was investigated in *Akkermansia muciniphila* [23].

Acknowledgements

This publication was supported by the Slovak Research and Development Agency under the contract no. APVV-15-0377, project VEGA no. 1/0009/15 and VEGA no.1/0081/17.

Author details

Madar Marian*, Telepjanová Tatiana and Gancarčíková Soňa

*Address all correspondence to: madarmarian@gmail.com

Department of Microbiology and Immunology, Laboratory of Gnotobiology, University of Veterinary Medicine and Pharmacy in Košice, Košice, Slovakia

References

[1] Hugenholtz P, Goebel BM, et al. Impact of culture-independent studies on the emerging phylogenetic view of bacterial diversity. Journal of Bacteriology. 1998;**180**(18):4765-4774

[2] Brandt C, Makarewicz O, et al. The bigger picture: The history of antibiotics and antimicrobial resistance displayed by scientometric data. International Journal of Antimicrobial Agents. Nov 2014;**44**(5):424-430. DOI: 10.1016/j.ijantimicag.2014.08.001

[3] Sullivan A, Edlund C, et al. Effect of antimicrobial agents on the ecological balance of human microflora. The Lancet Infectious Diseases. 2001;**1**(2):101-114 DOI: S1473-3099(01)00066-4 [pii]

[4] Dethlefsen L, Huse S, et al. The pervasive effects of an antibiotic on the human gut microbiota, as revealed by deep 16S rRNA sequencing. PLoS Biology. 2008;**6**(11):e280 DOI: 08-PLBI-RA-2095 [pii]

[5] Ligon BL. Sir Alexander Fleming: Scottish researcher who discovered penicillin. Seminars in Pediatric Infectious Diseases. 2004;**15**(1):58-64 DOI: S1045187004000184 [pii]

[6] Podolsky SH. Antibiotics and the social history of the controlled clinical trial 1950-1970. Journal of the History of Medicine and Allied Sciences. 2010;**65**(3):327-367 DOI: jrq003 [pii]

[7] Luckey TD. Nutrition and biochemistry of germfree chicks. Annals of the New York Academy of Sciences. 1959;**78**:127-165

[8] Clardy J, Fischbach MA, et al. The natural history of antibiotics. Current Biology. 2009;**19**(11):R437-R441 DOI: S0960-9822(09)00918-X [pii]

[9] Nord CE. The effect of antimicrobial agents on the ecology of the human intestinal microflora. Veterinary Microbiology. 1993;**35**(3-4):193-197

[10] Hejzlar M, Paroubek M. The effect of combined preparation Tetracyclin- Fungicidin-neomycin on intestinal microflora of experimental animals (in Czech). Rozhledy v chirurgii XLIV. 1955;**5**:334

[11] Hrubý S, Turek B. Hygiene Issues Related to Microflora of Digestive Tract in Humans (in Czech). Praha: Avicenum/zdravotnicke nakladatelství; 1989. 136 p

[12] Perez-Cobas AE, Gosalbes MJ, et al. Gut microbiota disturbance during antibiotic therapy: A multi-omic approach. Gut. 2012;**62**(11):1591-1601 DOI: gutjnl-2012-303184 [pii]

[13] Moter A, Gobel UB. Fluorescence in situ hybridization (FISH) for direct visualization of microorganisms. Journal of Microbiological Methods. 2000;**41**(2):85-112 DOI: S0167-7012(00)00152-4 [pii]

[14] Giovannoni SJ, DeLong EF, et al. Phylogenetic group-specific oligodeoxynucleotide probes for identification of single microbial cells. Journal of Bacteriology. 1988;**170**(2): 720-726

[15] Martin-Pelaez S et al. In vitro fermentation of carbohydrates by porcine faecal inocula and their influence on *Salmonella typhimurium* growth in batch culture systems. FEMS Microbiology Ecology. 2008;**66**:608-619 DOI: FEM610 [pii]

[16] Harmsen HJ, Gibson GR, et al. Comparison of viable cell counts and fluorescence in situ hybridization using specific rRNA-based probes for the quantification of human fecal bacteria. FEMS Microbiology Letters. 2000;**183**(1):125-129 DOI: S0378-1097(99)00649-7 [pii]

[17] McCartney AL. Application of molecular biological methods for studying probiotics and the gut flora. The British Journal of Nutrition. 2002;**88**(1):29-37. DOI: 10.1079/BJN2002627

[18] Florez AB, Danielsen M, Korhonen J, Zycka J, von Wright A, Bardowski J, Mayo B . Antibiotic survey of *Lactococcus lactis* strains to six antibiotics by Etest, and establishment of new susceptibility-resistance cut-off values. The Journal of Dairy Research. 2007;**74**:262-268 . DOI: S0022029907002543 [pii]

[19] Saarela M et al. Tetracycline susceptibility of the ingested *Lactobacillus acidophilus* LaCH-5 and *Bifidobacterium animalis* subsp. lactis Bb-12 strains during antibiotic/probiotic intervention. Journal of Antimicrobial Agents. 2007;**29**:271-280 DOI: S0924-8579(06)00436-5 [pii]

[20] Fallani M et al. Intestinal microbiota of 6-week-old infants across Europe: Geographic influence beyond delivery mode, breast-feeding, and antibiotics. Journal of Pediatric Gastroenterology and Nutrition. 2010;**51**:77-84. DOI: 10.1097/MPG.0b013e3181d1b11e

[21] Barc MC et al. Effect of amoxicillin-clavulanic acid on human fecal flora in a gnotobiotic mouse model assessed with fluorescence hybridization using group-specific 16S rRNA

probes in combination with flow cytometry. Antimicrobial Agents and Chemotherapy. 2004;**48**:1365-1368

[22] Brunser O, Gotteland M, Cruchet S, Figueroa G, Garrido D, Steenhout P. Effect of a milk formula with prebiotics on the intestinal microbiota of infants after an antibiotic treatment. Pediatric Research. 2006;**59**(3):451-456 DOI: 59/3/451 [pii]

[23] Dubourg G, Lagier JC, Armougom F, Robert C, Audoly G, Papazian L, Raoult D. High-level colonisation of the human gut by *Verrucomicrobia* following broad-spectrum antibiotic treatment. Journal of Antimicrobial Agents. 2013;**41**:149-155 DOI: S0924-8579(12)00423-2 [pii]

[24] Donskey CJ, Hanrahan JA, Hutton RA, Rice LB. Effect of parenteral antibiotic administration on persistence of vancomycin-resistant *Enterococcus faecium* in the mouse gastrointestinal tract. The Journal of Infectious Diseases. 1999;**180**:384-390 DOI: JID981279 [pii]

[25] Pultz NJ, Stiefel U, Subramanyan S, Helfand MS, Donskey CJ. Mechanisms by which anaerobic microbiota inhibit the establishment in mice of intestinal colonization by vancomycin-resistant *Enterococcus*. The Journal of Infectious Diseases. 2005;**191**:949-956 DOI: JID32925 [pii]

[26] Hoentjen F, Harmsen HJ, Braat H, Torrice CD, Mann BA, Sartor RB, Dieleman LA. Antibiotics with a selective aerobic or anaerobic spectrum have different therapeutic activities in various regions of the colon in interleukin 10 gene deficient mice. Gut. 2003;**52**:1721-1727

[27] Payne S, Gibson G, Wynne A, Hudspith B, Brostoff J, Tuohy K. In vitro studies on colonization resistance of the human gut microbiota to *Candida albicans* and the effects of tetracycline and *Lactobacillus plantarum* LPK. Current Issues in Intestinal Microbiology. 2003;**4**:1-8

[28] Lischewski A, Amann RI, Harmsen D, Merkert H, Hacker J, Morschhauser J. Specific detection of *Candida albicans* and *Candida tropicalis* by fluorescent in situ hybridization with an 18S rRNA-targeted oligonucleotide probe. Microbiology. 1996;**142**(10):2731-2740

[29] Rang CU et al. Estimation of growth rates of *Escherichia coli* BJ4 in streptomycin-treated and previously germfree mice by in situ rRNA hybridization. Clinical and Diagnostic Laboratory Immunology. 1999;**6**:434-436

[30] Poulsen LK, Lan F, Kristensen CS, Hobolth P, Molin S, Krogfelt KA. Spatial distribution of *Escherichia coli* in the mouse large intestine inferred from rRNA in situ hybridization. Infection and Immunity. 1994;**62**:5191-5194

[31] Sekirov I, Tam NM, Jogova M, Robertson ML, Li Y, Lupp C, Finlay BB. Antibiotic-induced perturbations of the intestinal microbiota alter host susceptibility to enteric infection. Infection and Immunity. 2008;**76**:4726-4736 DOI: IAI.00319-08 [pii]

[32] Swann JR et al. Variation in antibiotic-induced microbial recolonization impacts on the host metabolic phenotypes of rats. Journal of Proteome Research. 2011;**10**(8):3590-3603. DOI: 10.1021/pr200243t

[33] Ferreira RB, Gill N, Willing BP, Antunes LC, Russell SL, Croxen MA, Finlay BB. The intestinal microbiota plays a role in Salmonella-induced colitis independent of pathogen colonization. PLoS One. 2011;**6**(5):e20338. DOI: 10.1371/journal.pone.0020338

[34] Que JU, Hentges DJ. Effect of streptomycin administration on colonization resistance to *Salmonella typhimurium* in mice. Infection and Immunity. 1985;**48**:169-174

[35] Wadolkowski EA, Burris JA, O'Brien AD. Mouse model for colonization and disease caused by enterohemorrhagic *Escherichia coli* O157:H7. Infection and Immunity. 1990;**58**:2438-2445

[36] Russmann H, Kempf VA, Koletzko S, Heesemann J, Autenrieth IB. Comparison of fluorescent in situ hybridization and conventional culturing for detection of *Helicobacter pylori* in gastric biopsy specimens. Journal of Clinical Microbiology. 2001;**39**:304-308. DOI: 10.1128/JCM.39.1.304-308.2001

[37] Schmid MW, Lehner A, Stephan R, Schleifer KH, Meier H. Development and application of oligonucleotide probes for in situ detection of thermotolerant *Campylobacter* in chicken faecal and liver samples. Journal of Food Microbiology. 2005;**105**:245-255 DOI: S0168-1605(05)00330-2 [pii]

[38] Alonso R, Mateo E, Churruca E, Martinez I, Girbau C, Fernandez-Astorga A. MAMA-PCR assay for the detection of point mutations associated with high-level erythromycin resistance in *Campylobacter jejuni* and *Campylobacter coli* strains. Journal of Microbiological Methods. 2005;**63**:99-103 DOI: S0167-7012(05)00105-3 [pii]

[39] Vacher S, Menard A, Bernard E, Santos A, Megraud F. Detection of mutations associated with macrolide resistance in thermophilic *Campylobacter* spp. by real-time PCR. Microbial Drug Resistance. 2005;**11**:40-47. DOI: 10.1089/mdr.2005.11.40

[40] Haas M, Essig A, Bartelt E, Poppert S. Detection of resistance to macrolides in thermotolerant campylobacter species by fluorescence in situ hybridization. Journal of Clinical Microbiology. 2008;**46**:3842-3844 DOI: JCM.01155-08 [pii]

[41] Trebesius K et al. Rapid and specific detection of *Helicobacter pylori* macrolide resistance in gastric tissue by fluorescent in situ hybridisation. Gut. 2000;**46**:608-614

[42] Yilmaz O, Demiray E. Clinical role and importance of fluorescence in situ hybridization method in diagnosis of *H. pylori* infection and determination of clarithromycin resistance in *H. pylori* eradication therapy. World Journal of Gastroenterology. 2007;**13**:671-675

[43] Hoentjen F, Harmsen HJ, Braat H, Torrice CD, Mann BA, Sartor RB, Dieleman LA. Antibiotics with a selective aerobic or anaerobic spectrum have different therapeutic activities in various regions of the colon in interleukin 10 gene deficient mice. Gut. 2003;**52**:1721-1727