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SALMONELLA-INDUCED CHANGES OF THE RAT INTESTINAL MICROBIOTA

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Abstract. The gut microbiome profoundly affects the body functioning: it participates in host protection against pathogenic microorganisms, metabolic events, inhibition of inflammatory responses, formation of innate and adaptive immune response in the intestinal mucosa. One of the causes altering microbiota community is due to antibiotics. Therefore, the processes of antibiotics interaction together with *Salmonella enteritidis* and *Salmonella typhimurium* with representatives of normal intestinal microflora are of particular interest. *Materials and methods.* The quantitative and qualitative analysis of the wall microbiota composition in rats was evaluated by bacteriological method, the statistical data analysis was performed using the software StatSoft Statistica v.12. *Results and discussion*. Inoculation of vancomycin and *S. enteritidis*, *S. typhimurium* in groups II, III, IV resulted in quantitatively decreased *E. coli* level by 10-, 7- and 110-fold, respectively ($p \le 0.05$). The count of *P. aeruginosa* decreased markedly only in the group III ($p \le 0.05$). The count of *Bacteroides* spp. members was profoundly decreased by several thousand times (group II) as well as 70- and 87-fold (groups III and IV), respectively ($p \le 0.05$). The count of *E. faecalis* and *E. faecium* decreased by 861-, 6- and several thousand times (groups II, III, IV), respectively ($p \le 0.05$). The count of *Proteus* spp. markedly decreased in group II by 27-fold and rapidly increased in group IV ($p \le 0.05$). Group III revealed a sharp decline in level of *Enterobacter* spp. and *Klebsiella* spp. by 847- and 150-fold, whereas in group II they were increased by 7- and 46-fold, respectively ($p \le 0.05$). The count of *Staphylococcus* spp. decreased by 10-fold only in group II. The level of *Clostridium* spp. decreased by several thousand times (group II) and by 5,500 times (group IV) ($p \le 0.05$). The count of *Lactobacillus* spp. decreased by several thousand times (group II). The count of *Bifidobacterium* spp. members significantly decreased by 10.9-fold and by several thousand times (groups III, IV). The level of *Peptostreptococcus anaerobius* profoundly decreased in all three study groups ($p \le 0.05$). The level of *Salmonella* spp. increased in group II by 49 times, but markedly increased in groups III and IV ($p \le 0.05$). Inoculation of *Salmonella* after vancomycin pretreatment caused dramatic change in the microbiota composition in groups V and VI, namely: increased count of *E. coli* by 65- and 105-fold, markedly increased level of *P. aeruginosa* in group V and VI — by 3-fold. In addition, these groups also showed decreased level of *Bacteroides* spp. by 9- and 10-fold ($p \le 0.05$). The count of *E. faecalis* and *E. faecium* decreased dramatically only in group V ($p \le 0.05$). The count of *Proteus* spp. decreased by 17 times in group V as well as in group VI ($p \le 0.05$). A sharp increase in level of *Enterobacter* spp. and *Klebsiella* spp. members was observed in groups V and VI ($p \le 0.05$). However, representatives of *Peptostreptococcus anaerobius* in groups V and VI decreased by 20 and 9 times, respectively ($p \le 0.05$). The count of *Salmonella* spp. decreased only in group V by 7 times ($p \le 0,05$). Inoculating experimental animals with *B. fragilis* conditioned with *S. enteritidis*, *S. typhimurium* and pretreated with vancomycin resulted in markedly decreased level of *E. coli* in group VII and VIII by 538 times ($p \le 0.05$). The count of *P. aeruginosa* in groups VII and VIII decreased profoundly, whereas level of Bacteroides spp. members was reciprocally increased ($p \le 0.05$). The level of *Lactobacillus* spp. decreased by 10.3 times only in group VI. The count of *E. faecalis* and *E. faecium* increased by 10 and 19 times in groups VII and VIII, respectively, whereas level of *Proteus* spp. decreased only in group VII by 322 times ($p \le 0.05$). In addition, a sharp decrease in level

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of *Enterobacter* spp. and *Klebsiella* spp. members ($p \le 0.05$) was found in groups VII and VIII. The count of *Peptostreptococcus anaerobius* and *Lactobacillus* spp. members was markedly increased by 7-, 12-, several thousand-fold and 40 times (groups VII and VIII, respectively) ($p \le 0.05$). The count of *S. enteritidis* and *S. typhimurium* in groups VII and VIII decreased rapidly ($p \le 0.05$). *Conclusion*. Inoculation of *B. fragilis* can be used in treatment of inflammatory bowel diseases or disorders with impaired gut barrier function.

Key words: *parietal microbiota, microbiome, vancomycin, Salmonella, bacteroids, rats.*

САЛЬМОНЕЛЛА-ИНДУЦИРОВАННЫЕ ИЗМЕНЕНИЯ КИШЕЧНОГО МИКРОБИОМА КРЫС

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Резюме. Микробиом кишечника существенно влияет на функционирование организма: он участвует в защите организма от патогенных микроорганизмов, в процессах обмена веществ, торможении воспалительных реакций, в формировании врожденного и адаптивного иммунного ответа в слизистой оболочке кишечника. Одной из причин изменения микробиоты является использование антибиотиков. Поэтому процессы взаимодействия антибиотиков, *Salmonella enteritidis* и *Salmonella typhimurium*, с представителями нормальной микрофлоры кишечника представляют особый интерес. *Материалы и методы.* Проведен количественный и качественный анализ состава микробиоты стенки у крыс бактериологическим методом, а также статистический анализ данных с использованием программы StatSoft Statistica v.12. *Результаты и обсуждение.* При введении ванкомицина и *S. enteritidis*, *S. typhimurium* в группы II, III, IV наблюдалось снижение количественного содержания кишечной палочки в 10, 7 и 110 раз соответственно ($p \le 0,05$). Количество *P. aeruginosa* значительно уменьшилось только в третьей группе ($p \le 0,05$). Количество представителей *Bacteroides* spp. значительно уменьшилось в несколько тысяч раз (группа II) и в 70 и 87 раз (группы III и IV) ($p \le 0,05$). Содержание *E. faecalis* и *E. faecium* уменьшилось в 861, 6 и в несколько тысяч раз (группы II, III, IV) ($p \le 0,05$). Количество *Proteus* spp. значительно уменьшился во II группе в 27 раз и быстро увеличился в IV группе ($p \le 0,05$). Группа III показала резкое снижение содержания представителей *Enterobacter* spp. и *Klebsiella* spp. в 847 и 150 раз, а во II группе наблюдается увеличение их числа в 7 и 46 раз соответственно ($p \le 0,05$). Количество *Staphylococcus* spp. снизилось в 10 раз только во II группе. Количественное содержание *Clostridium* spp. снизилось в несколько тысяч раз (группа II) и в 5,5 раз (группа IV) ($p \le 0,05$). Количество *Lactobacillus* spp. уменьшилось в несколько тысяч раз (группа II). Количество представителей *Bifidobacterium* spp. значительно снизилось в 10,9 раз и в несколько тысяч раз (группы III, IV). Количественное содержание *Peptostreptococcus anaerobius* значительно уменьшилось во всех трех группах исследования ($p \le 0,05$). Содержание *Salmonella* spp. увеличилось во II группе в 49 раз, а значительное увеличение наблюдалось в III и IV группах ($p \le 0,05$). Введение сальмонеллы на фоне предварительной обработки ванкомицином вызывает резкое изменение состава микробиоты в группах V и VI, а именно увеличение количества кишечной палочки в 65 и 105 раз, значительное увеличение содержание *P. aeruginosa* в V группе, а в VI — в 3 раза. Также в этих группах наблюдается уменьшение количества *Bacteroides* spp. 9 и 10 раз ($p \le 0,05$). Содержание *E. faecalis* и *E. faecium* значительно снизилось только в пятой группе ($p \le 0,05$). Количество *Proteus* spp. уменьшается в 17 раз в группе V, а также значительно снижается в группе VI ($p \le 0,05$). Резкое увеличение количества представителей *Enterobacter* spp. и *Klebsiella* spp. наблюдалось в группах V и VI ($p \le 0,05$). Однако представителей *Peptostreptococcus anaerobius* в V и VI группах стало меньше в 20 и 9 раз соответственно ($p \le 0,05$). Количество *Salmonella* spp. снизилось только в V группе в 7 раз ($p \le 0,05$). При введении подопытным животным *B. fragilis*, получавшим *S. enteritidis* и *S. typhimurium* на фоне предварительной обработки ванкомицином, отмечалось значительное снижение уровня кишечной палочки в группе VII, а в VIII — в 538 раз ($p \le 0,05$). Количество *P. aeruginosa* в группах VII и VIII значительно уменьшилось, а число представителей *Bacteroides* spp. естественно увеличивается ($p \le 0,05$). Содержание *Lactobacillus* spp. снизилось в 10,3 раза только в VI группе. Содержание *E. faecalis* и *E. faecium* увеличилось в 10 и 19 раз в VII и VIII группах соответственно, а количество *Proteus* spp. уменьшилось только в VII группе в 322 раза ($p \le 0,05$). Также в VII и VIII группах наблюдалось резкое снижение количества представителей *Enterobacter* spp. и *Klebsiella* spp. ($p \le 0,05$). Число представителей *Peptostreptococcus anaerobius* и *Lactobacillus* spp. значительно увеличилось в 7, 12 раз и в несколько тысяч и 40 раз (группы VII и VIII соответственно) ($p \le 0,05$). Количество *S. enteritidis* и *S. typhimurium* в VII и VIII группах интенсивно снижалось ($p \le 0,05$). *Выводы.* Введение *B. fragilis* может быть использовано при лечении воспалительных заболеваний кишечника или заболеваний с нарушением барьерной функции кишечника.

Ключевые слова: *пристеночная микробиота, микробиом, ванкомицин, сальмонелла, бактероиды, крысы.*

The intestinal microbiome significantly affects the functioning of the body: it participates in metabolic processes, inhibition of proinflammatory reactions, in the formation of innate and adaptive immune response in the intestinal mucosa [7, 13, 16, 31]. The most important function of the intestinal microbiome is to protect the body from pathogenic microorganisms — pathogens of bacterial intestinal infections [30, 37]. It is known that dysbiotic changes in the intestine lead to increased susceptibility to pathogenic bacterias, such as *Salmonella* [12, 19], which are the etiological factor of gastroenteritis [35]. One of the most common causes of microbiota changes is the use of antibiotics [5, 24, 40]. Therefore, of particular interest are the processes of interaction of antibiotics, *Salmonella enteritidis* and *Salmonella typhimurium* with representatives of the normal intestinal microbiota [1, 4, 38]. Therefore, in our work, vancomycin was used to induce dysbiotic changes in the intestinal microflora, which acts against gram-positive bacteria and does not affect gram-negative *(Salmonella)*. We also paid attention to determining the quantitative and species composition of the microbiota in *Salmonella*-induced intestinal inflammation, which created the basis for further study of the molecular mechanisms of interaction of *Salmonella enteritidis* and *Salmonella typhimurium* with microbiota and gut-associated lymphoid tissue (GALT). The aim of the study is to analyze changes in the quantitative and species composition of the microbiota of the small intestine in rats with *Salmonella*-induced inflammation of the intestine on the background of the introduction of vancomycin and *B. fragilis*.

Materials and methods

Experiments to determine the quantitative and species content of microorganisms in the parietal intestinal microbiota were performed on 120 rats (males) of the line "Wistar" on the basis of the bacteriological department of the microbiological laboratory of Zaporozhye State Medical University in the bound of scientific research 0118U007141 "Molecular genetic analysis of changes in the transcript of the genes of the immune response and intestinal microbiome in the conditions of experimental pathology and the development of methods for their correction". Acclimatization of animals (quarantine) lasted 7 days before the study. All experiments were conducted in the autumn–winter period in the vivarium of Zaporozhye State Medical University. Rats were kept at temperature of 18–21°C, in natural light during daylight from 7am to 5pm, with free access to food and water. Experimental work with rodents was carried out in accordance with the provisions of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986). All rodents, except group I (control, intact), received vancomycin and/or suspension of microorganisms. In order to quickly internalize the bacteria in the intestinal mucosa, the suspension with *Salmonella* was administered orally using a probe with a volume of 5 ml size 16–18, length 5–7.5 mm, tip size 2.25. Vancomycin was administered to animals at the rate of 50 mg per kg of body weight, suspensions of microorganisms in amount of 15 ml with a concentration of 3×10^8 CFU/g. Thus, to simulate the imbalance of the intestinal microflora, only vancomycin (TEVA, Hungary, No. UA/8995/01/02) was introduced to group II rodents, *S. enteritidis* suspension was introduced to group III, and *S. typhimurium* suspension was introduced to group IV. Animals V and VI of experimental groups received vancomycin on the first day, but group V after 24 h was administered a suspension of *S. enteritidis*, and rats of group VI — a suspension of *S. typhimurium*. Rodents of groups VII and VIII were also given vancomycin on the first day, but group VII received *S. enteritidis* suspension on the second day and *B. fragilis* on the third day, while group VIII rats received a suspension of *S. typhimurium* on the second day, and on the third — *B. fragilis*. As a material for bacteriological studies of the intestinal microbiota used swabs from the ileum of rats. Experimental studies were conducted according to the author's method. To isolate the parietal microflora from the ileum, 0.1 ml of swabs were inoculated on nutrient media. Isolation and identification of *Salmonella* was performed according to the order of the Ministry of Health of the Ukraine No. 425 of 24.05.2013 "On approval of guidelines "Methods of isolation and identification of *Salmonella*". For *Salmonella* used a magnesium enrichment medium in which the swabs were dissolved 1:10. Seedings from the obtained suspensions were performed on bismuth sulfite agar (BSA) and Endo medium immediately and after 24 h of incubation in a thermostat at 37°C, after which Endo medium was incubated for 20 h, BSA — 48 h at 37°C. HiCrome™ nutrient media and HiMedia differential diagnostic media (India) were used to isolate different species of microorganisms. Biochemical identification was performed according to the "Determinant of Bacteria Bergi" (1997), in accordance with the instructional and methodological documents and data of modern literature [11]. Identification of members of the genus *Pseudomonas* was performed in accordance with the methodical recommendations "Biological Characteristics and Microbiological Identification of Non-Fermenting Gram-Negative bacteria" (Kharkov, 2010). Determination of bacteroids and peptostreptococci was performed in accordance with the methodical recommendations "Laboratory diagnosis of purulent-inflammatory diseases caused by asporogenic anaerobic microorganisms" (Kharkov, 2000). Belonging of bacteria to the genus *Enterococcus* was carried out in accordance with the methodical recommendations "Microbiological diagnosis

of streptococcal, enterococcal and peptostreptococcal infections" (Kharkov, 2007). The experimental work used strains of *S. enteritidis* and *S. typhimurium*, which were obtained from the Museum of Microorganism Strains "Ukrainian Center for Disease Control and Monitoring" of the Ministry of Health of the Ukraine (Kyiv) and culture of *Bacteroides fragilis* isolated from the intestines of intact rodents. The affiliation of this strain to the genus Bacteroides was established by cultural characteristics, as well as by PCR (bactopol kit for determining *Bacteroides fragilis, vulgatus, thetaiotaomicron, ovatus*). The isolate was confirmed as *Bacteroides fragilis* on a number of biochemical grounds.

Counting the number of microorganisms was performed according to the formula:

$$M = N \times 10n + 1,$$

where M is the number of microorganisms in 1 g of the test material, N is the number of colonies grown on agar, n is the degree of dilution of the test material.

Table 1. Quantitative content of microorganisms (CFU/g) in the parietal content of the small intestine in rats with the introduction of vancomycin and *S. enteritidis*, *S. typhimurium*

Groups of microorganisms	Groups of experimental animals					
	Control (n = 15)	Vancomycin (n = 15)	*S. enteritidis* (n = 15)	*S. typhimurium* (n = 15)	Vancomycin + *S. enteritidis* (n = 15)	Vancomycin + *S. typhimurium* (n = 15)
	The number of microorganisms [Me (Q_{25}–Q_{75})], CFU/g					
E. coli	$2{,}2 \times 10^5$ ($1{,}0 \times 10^5$–$5{,}2 \times 10^5$)	$2{,}1 \times 10^4$ ($1{,}6 \times 10^4$–$9{,}0 \times 10^4$)*	$3{,}0 \times 10^4$ ($1{,}2 \times 10^4$–$8{,}0 \times 10^4$)*	$2{,}0 \times 10^3$ ($1{,}8 \times 10^3$–$2{,}4 \times 10^3$)*	$1{,}95 \times 10^6$ ($1{,}2 \times 10^6$–$2{,}8 \times 10^6$)[a]	$2{,}1 \times 10^5$ ($1{,}5 \times 10^5$–$2{,}9 \times 10^5$)[b]
P. aeruginosa	$7{,}05 \times 10^4$ ($2{,}4 \times 10^4$–$1{,}5 \times 10^5$)	$8{,}7 \times 10^4$ ($3{,}0 \times 10^4$–$3{,}2 \times 10^5$)	$2{,}2 \times 10^1$ ($1{,}6 \times 10^1$–$3{,}2 \times 10^1$)*	$1{,}25 \times 10^5$ ($5{,}0 \times 10^4$–$2{,}0 \times 10^5$)	$2{,}2 \times 10^6$ ($1{,}4 \times 10^5$–$3{,}5 \times 10^6$)[a]	$3{,}7 \times 10^5$ ($2{,}1 \times 10^5$–$1{,}8 \times 10^6$)[b]
***Bacteroids* spp.**	$1{,}65 \times 10^5$ ($1{,}2 \times 10^4$–$3{,}6 \times 10^5$)	$4{,}0 \times 10^1$ ($1{,}3 \times 10^1$–$1{,}0 \times 10^2$)*	$2{,}35 \times 10^3$ ($2{,}1 \times 10^3$–$3{,}1 \times 10^3$)*	$1{,}9 \times 10^3$ ($1{,}2 \times 10^3$–$2{,}1 \times 10^3$)*	$2{,}6 \times 10^2$ ($1{,}8 \times 10^2$–$4{,}0 \times 10^2$)[a]	$1{,}85 \times 10^2$ ($1{,}2 \times 10^2$–$3{,}3 \times 10^2$)[b]
E. faecalis* and *E. faecium	$1{,}55 \times 10^5$ ($4{,}0 \times 10^4$–$5{,}0 \times 10^5$)	$1{,}8 \times 10^2$ ($4{,}4 \times 10^1$–$4{,}3 \times 10^2$)*	$2{,}45 \times 10^4$ ($1{,}8 \times 10^4$–$4{,}0 \times 10^4$)*	$1{,}1 \times 10^2$ ($2{,}9 \times 10^1$–$1{,}4 \times 10^2$)*	$2{,}3 \times 10^1$ ($2{,}1 \times 10^1$–$3{,}1 \times 10^1$)[a]	$2{,}0 \times 10^2$ ($1{,}1 \times 10^2$–$3{,}6 \times 10^2$)
***Proteus* spp.**	$6{,}84 \times 10^4$ ($3{,}4 \times 10^4$–$2{,}4 \times 10^5$)	$2{,}5 \times 10^3$ ($1{,}4 \times 10^3$–$3{,}7 \times 10^3$)*	$1{,}5 \times 10^5$ ($4{,}1 \times 10^4$–$2{,}2 \times 10^5$)	$1{,}5 \times 10^8$ ($4{,}2 \times 10^7$–$2{,}0 \times 10^8$)*	$8{,}7 \times 10^3$ ($4{,}0 \times 10^3$–$2{,}4 \times 10^4$)[a]	$2{,}7 \times 10^1$ ($1{,}6 \times 10^1$–$1{,}0 \times 10^2$)[b]
***Enterobacter* spp.**	$3{,}05 \times 10^5$ ($1{,}1 \times 10^5$–$4{,}5 \times 10^5$)	$2{,}25 \times 10^6$ ($1{,}6 \times 10^6$–$4{,}0 \times 10^6$)*	$3{,}6 \times 10^2$ ($2{,}1 \times 10^2$–$6{,}0 \times 10^2$)*	$2{,}05 \times 10^2$ ($1{,}8 \times 10^2$–$3{,}0 \times 10^2$)*	$1{,}9 \times 10^6$ ($4{,}0 \times 10^5$–$3{,}6 \times 10^6$)[a]	$2{,}8 \times 10^6$ ($2{,}4 \times 10^6$–$6{,}3 \times 10^6$)[b]
***Klebsiella* spp.**	$4{,}05 \times 104$ ($2{,}0 \times 10^4$–$4{,}3 \times 10^5$)	$1{,}85 \times 10^6$ ($4{,}4 \times 10^5$–$2{,}4 \times 10^6$)*	$2{,}7 \times 10^2$ ($1{,}8 \times 10^2$–$4{,}0 \times 10^2$)*	$2{,}85 \times 10^2$ ($1{,}8 \times 10^2$–$4{,}0 \times 10^2$)*	$2{,}2 \times 10^6$ ($6{,}0 \times 10^5$–$3{,}6 \times 10^6$)[a]	$2{,}45 \times 10^6$ ($1{,}4 \times 10^6$–$3{,}6 \times 10^6$)[b]
Peptostreptococcus anaerobius	$9{,}35 \times 10^5$ ($3{,}2 \times 10^5$–$1{,}7 \times 106$)	$2{,}7 \times 10^2$ ($1{,}8 \times 10^5$–$4{,}0 \times 10^5$)*	$5{,}55 \times 10^2$ ($4{,}4 \times 10^2$–$1{,}2 \times 10^3$)*	$2{,}6 \times 10^2$ ($2{,}0 \times 10^2$–$3{,}7 \times 10^2$)*	$2{,}7 \times 10^1$ ($2{,}1 \times 10^1$–$3{,}8 \times 10^1$)[a]	$2{,}9 \times 10^1$ ($1{,}9 \times 10^1$–$4{,}2 \times 10^1$)[b]
***Staphylococcus* spp.**	$2{,}6 \times 10^5$ (1×10^5–5×10^5)	$2{,}65 \times 10^4$ (1×10^4–8×10^4)*	2×10^5 (1×10^5–3×10^5)	$2{,}1 \times 10^5$ (2×10^5–$7{,}2 \times 10^5$)	0	2×10^5 (6×10^4–$3{,}2 \times 10^5$)
***Clostridium* spp.**	$5{,}5 \times 10^6$ (1×10^6–1×10^8)	1×10^4 (1×10^4–1×10^5)*	1×10^6 (1×10^6–1×10^8)	1×10^6 (1×10^4–1×10^6)*	1×10^6 (1×10^6–1×10^8)	$5{,}05 \times 10^5$ (1×10^4–1×10^6)
***Lactobacillus* spp.**	$1{,}6 \times 10^6$ (1×10^6–8×10^6)	$3{,}5 \times 10^4$ (2×10^4–$2{,}8 \times 10^5$)*	$2{,}05 \times 10^6$ ($5{,}2 \times 10^5$–4×10^6)	$3{,}45 \times 10^6$ (8×10^5–6×10^6)	$6{,}2 \times 10^5$ (2×10^5–2×10^6)	2×10^5 (1×10^5–$2{,}5 \times 10^5$)[b]
***Bifidobacterium* spp.**	$5{,}5 \times 10^6$ (1×10^6–1×10^8)	0	$5{,}05 \times 10^5$ (1×10^4–1×10^6)*	1×10^4 (1×10^4–5×10^5)*	0	1×10^4 (1×10^4–5×10^5)
Candida	$1{,}25 \times 10^5$ (2×10^4–2×10^5)	$2{,}2 \times 10^4$ (2×10^4–4×10^4)	$5{,}5 \times 10^5$ (2×10^4–8×10^6)	0	$1{,}05 \times 10^6$ (2×10^5–7×10^6)	0
***Salmonella* spp.**	$6{,}9 \times 10^1$ ($4{,}3 \times 10^1$–$2{,}3 \times 10^2$)	$3{,}4 \times 10^3$ ($2{,}5 \times 10^3$–$4{,}3 \times 10^3$)*	$2{,}2 \times 10^6$ ($1{,}0 \times 10^6$–$4{,}0 \times 10^6$)*	$1{,}45 \times 10^5$ ($1{,}0 \times 10^5$–$4{,}0 \times 10^5$)*	$3{,}0 \times 10^5$ ($2{,}0 \times 10^5$–$4{,}4 \times 10^5$)[a]	$4{,}0 \times 10^5$ ($2{,}0 \times 10^5$–$2{,}2 \times 10^6$)

Note. * — the significance of differences in the parameters $p \le 0.05$ in relation to the control; [a] — reliability of parameters in relation to the group of *S. enteritidis* ($p \le 0.05$); [b] — reliability of parameters in relation to the group *S. typhimurium* ($p \le 0{,}05$).

Statistical analysis of the results was performed using licensed computer programs Microsoft Excel 2010 and StatSoft Statistica v.12. When analyzing the distributions of quantitative data, the level of the central tendency — the median (Me), and the level of variance — the interquantal range in the form of 25 and 75 percentiles were determined. The calculation of the significance of the differences between the mean values was evaluated using the nonparametric Mann–Whitney test (U-test). The criterion of statistical significance was the level of $p \le 0.05$.

Results and discussion

The data obtained during the studies showed that with the introduction of vancomycin and bacterial agents, the quantitative and qualitative composition of the representatives of the parietal microbiota changed dramatically (Table 1). Thus, in groups II,

Table 2. Quantitative composition of the parietal microbiota of the ileum of rats with the introduction of *Salmonella, B. fragilis* on the background of vancomycin

Groups of microorganisms	Groups of experimental animals			
	Vancomycin + *S. enteritidis* (n = 15)	Vancomycin + *S. typhimurium* (n = 15)	Vancomycin + *S. enteritidis* + *B. fragilis* (n = 15)	Vancomycin + *S. typhimurium* + *B. fragilis* (n = 15)
	The number of microorganisms [Me (Q_{25}–Q_{75})], CFU/g			
E. coli	$1{,}95 \times 10^6$ ($1{,}2 \times 10^6$–$2{,}8 \times 10^6$)	$2{,}1 \times 10^5$ ($1{,}5 \times 10^5$–$2{,}9 \times 10^5$)	$1{,}35 \times 10^2$ ($2{,}9 \times 10^1$–$1{,}8 \times 10^2$)*	$3{,}9 \times 10^2$ ($2{,}0 \times 10^2$–$6{,}0 \times 10^2$)*
P. aeruginosa	$2{,}2 \times 10^6$ ($1{,}4 \times 10^5$–$3{,}5 \times 10^6$)	$3{,}7 \times 10^5$ ($2{,}1 \times 10^5$–$1{,}8 \times 10^6$)	$2{,}05 \times 10^2$ ($1{,}2 \times 10^2$–$3{,}0 \times 10^2$)*	$1{,}9 \times 10^2$ ($1{,}4 \times 10^2$–$4{,}0 \times 10^2$)*
***Bacteroids* spp.**	$2{,}6 \times 10^2$ ($1{,}8 \times 10^2$–$4{,}0 \times 10^2$)	$1{,}85 \times 10^2$ ($1{,}2 \times 10^2$–$3{,}3 \times 10^2$)	$4{,}15 \times 10^5$ ($2{,}1 \times 10^5$–$3{,}2 \times 10^6$)*	$8{,}8 \times 10^6$ ($4{,}0 \times 10^6$–$2{,}0 \times 10^7$)*
E. faecalis* and *E. faecium	$2{,}3 \times 10^1$ ($2{,}1 \times 10^1$–$3{,}1 \times 10^1$)	$2{,}0 \times 10^2$ ($1{,}1 \times 10^2$–$3{,}6 \times 10^2$)	$2{,}4 \times 10^2$ ($1{,}2 \times 10^3$–$5{,}6 \times 10^3$)*	$3{,}75 \times 10^3$ ($2{,}5 \times 10^3$–$7{,}0 \times 10^3$)*
***Proteus* spp.**	$8{,}7 \times 10^3$ ($4{,}0 \times 10^3$–$2{,}4 \times 10^4$)	$2{,}7 \times 10^1$ ($1{,}6 \times 10^1$–$1{,}0 \times 10^2$)	$2{,}7 \times 10^1$ ($1{,}2 \times 10^1$–$3{,}2 \times 10^1$)*	$7{,}9 \times 10^1$ ($6{,}0 \times 10^1$–$1{,}2 \times 10^2$)
***Enterobacter* spp.**	$1{,}9 \times 10^6$ ($4{,}0 \times 10^5$–$3{,}6 \times 10^6$)	$2{,}8 \times 10^6$ ($2{,}4 \times 10^6$–$6{,}3 \times 10^6$)	$4{,}3 \times 10^2$ ($1{,}3 \times 10^2$–$7{,}0 \times 10^2$)*	$2{,}3 \times 10^2$ ($1{,}8 \times 10^2$–$2{,}9 \times 10^2$)*
***Klebsiella* spp.**	$2{,}2 \times 10^6$ ($6{,}0 \times 10^5$–$3{,}6 \times 10^6$)	$2{,}45 \times 10^6$ ($1{,}4 \times 10^6$–$3{,}6 \times 10^6$)	$2{,}3 \times 10^2$ ($1{,}4 \times 10^2$–$4{,}0 \times 10^2$)*	$1{,}6 \times 10^2$ ($1{,}2 \times 10^2$–$2{,}8 \times 10^2$)*
Peptostreptococcus anaerobius	$2{,}7 \times 10^1$ ($2{,}1 \times 10^1$–$3{,}8 \times 10^1$)	$2{,}9 \times 10^1$ ($1{,}9 \times 10^1$–$4{,}2 \times 10^1$)	$1{,}9 \times 10^2$ ($1{,}5 \times 10^1$–$2{,}1 \times 10^2$)	$3{,}45 \times 10^2$ ($3{,}0 \times 10^1$–$5{,}2 \times 10^2$)*
***Staphylococcus* spp.**	0	2×10^5 (6×10^4–$3{,}2 \times 10^5$)	$2{,}25 \times 10^5$ ($1{,}6 \times 10^5$–$3{,}2 \times 10^5$)	$2{,}65 \times 10^5$ ($1{,}5 \times 10^5$–5×10^5)
***Clostridium* spp.**	1×10^6 (1×10^6–1×10^8)	$5{,}05 \times 10^5$ (1×10^4–1×10^6)	1×10^5 (1×10^4–1×10^6)	1×10^4 (1×10^4–1×10^6)
***Lactobacillus* spp.**	$6{,}2 \times 10^5$ (2×10^5–2×10^6)	2×10^5 (1×10^5–$2{,}5 \times 10^5$)	$1{,}7 \times 10^7$ ($2{,}3 \times 10^6$–$2{,}9 \times 10^7$)*	8×10^6 (4×10^6–4×10^7)*
Bifidobacterium spp.	0	1×10^4 (1×10^4–5×10^5)	0	0
Candida	$1{,}0^5 \times 10^6$ (2×10^5–7×10^6)	0	0	0
***Salmonella* spp.**	$3{,}0 \times 10^5$ ($2{,}0 \times 10^5$–$4{,}4 \times 10^5$)	$4{,}0 \times 10^5$ ($2{,}0 \times 10^5$–$2{,}2 \times 10^6$)	$1{,}0 \times 10^1$ ($1{,}2 \times 10^1$–$2{,}9 \times 10^1$)*	$9{,}0 \times 10^1$ ($5{,}0 \times 10^1$–$1{,}4 \times 10^2$)*

Note. * — the significance of differences in the parameters $p \le 0.05$ in relation to the groups Vancomycin + *S. enteritidis* and Vancomycin + *S. typhimurium*.

III, IV there was a decrease in the number of *E. coli* by 10, 7 and 110 times, respectively ($p \leq 0.05$), and the frequency of selection of this species was 10 and 14% (groups II, III). The results of studies conducted by scientists showed that the content of *E. coli* after administration of vancomycin to rats decreased several thousand times, and a decrease was observed in *Salmonella* infection 2 times [26]. The number of *P. aeruginosa* decreased significantly only in the third group ($p \leq 0.05$). At the same time, Carroll et al. (2010) in their work showed a 2-fold increase in the number of pseudomonads when administered to rats *S. enteritidis* [41]. The number of representatives of *Bacteroides* spp. significantly decreased several thousand times (group II) and 70 and 87 times (groups III and IV) ($p \leq 0.05$) (Table 1). Our data are consistent with the results of researchers who studied the composition of the parietal microflora of the intestine of rats and showed a sharp decrease in bacteroids in 2 times with the introduction of vancomycin, as well as several thousand times with the introduction of *Salmonella* [2]. During similar experiments, Parkes et al. (2012) concluded that after the introduction of vancomycin and *Salmonella*, the amount of *E. faecalis*, *E. faecium* decreased by a small amount [23]. As a result of our studies, the frequency of enterococci was 16% and only in group II, and the level of *E. faecalis* and *E. faecium* decreased by 861, 6 and several thousand times (groups II, III, IV) ($p \leq 0.05$). The number of *Proteus* spp. significantly decreased in group II 27 times and increased rapidly in group IV ($p \leq 0.05$). However, proteases were isolated with a frequency of 22 and 78% in groups III, IV (Table 3). According to the literature data when administered to mice vancomycin, as well as *Salmonella*, the content of *Proteus* spp. increased by 4 and 48 times [22], which is similar to the data obtained during our experiment. In group III there was a sharp decrease in the content of *Enterobacter* spp. and *Klebsiella* spp. in 847 and 150 times, and in group II there was an increase in their number by 7 and 46 times, respectively ($p \leq 0.05$). The frequency of selection of these representatives was 87% and only in group II. Turnbaugh P.J. et al. (2006) showed in their studies that when vancomycin was administered to rats, the number of members of the *Enterobacteriaceae* family was reduced in 3 times [34]. At the same time, according to Sekirov I. et al. (2008), with the introduction of vancomycin the number of *Enterobacteria* increased 11 times, and with the introduction of *Salmonella* decreased by 2 times [27], which is consistent with our data. The quantitative composition of *Peptostreptococcus anaerobius* decreased significantly in all three experimental groups ($p \leq 0.05$). A group of scientists from America found a 10-fold reduction in the content of peptostreptococci of this species [25], while Kerckhoffs A.P. et al. (2009) in his experiments showed a 5-fold reduction in *P. anaerobius* with the introduction of vancomycin, and their complete absence with *Salmonella* [14]. At the same time, the number of staphylococci in group II decreased 10 times. No significant changes were found when calculating the content of clostridia and lactobacilli, only in group II we found a slight decrease of 550 times in bacteria of the genus *Clostridium* and 46 times in *Lactobacillus*. However, lactobacilli were isolated with a frequency of 2.72, 85% in all three experimental groups. In their studies, Nagpal R. et al. (2018) demonstrated similar changes in the intestinal microbiota in rats with the development of dysbiosis [21]. The frequency of isolation of opportunistic microflora such as: staphylococci, bifidobacteria, *Candida*, was 10, 77, 81; 9% (group II) and 5.68% (group II and III), respectively. The content of representatives of *Salmonella* spp. increased in group II by 49 times and, also, its significant growth was observed in groups III and IV ($p \leq 0.05$). According to the results of studies conducted by European scientists, after treatment of rats with vancomycin, there was a slight increase in *Salmonella*, and

Table 3. Frequency of excretion of microorganisms in the parietal contents of the intestine of rats with the introduction of vancomycin and *S. enteritidis, S. typhimurium*

Groups of microorganisms	Groups of experimental animals					
	Control (n = 15)	Vancomycin (n = 15)	*S. enteritidis* (n = 15)	*S. typhimurium* (n = 15)	Vancomycin + *S. enteritidis* (n = 15)	Vancomycin + *S. typhimurium* (n = 15)
	Frequency of excretion (%) of microorganisms					
E. coli	100	10	14	0	35	95
***Staphylococcus* spp.**	100	10	77	81	0	95
***Enterococcus* spp.**	100	0	16	0	0	19
***Bifidobacterium* spp.**	100	0	9	0	0	0
***Lactobacillus* spp.**	100	2	72	85	30	6
***Klebsiella* spp.**	10	87	0	0	19	15
***Proteus* spp.**	10	0	22	78	0	0
Candida	30	5	68	0	57	0

Note. * — significant differences compared to reference group (RG).

when rats became infected, this figure increased by 1029 times [10], which does not contradict our results.

As a result of comparison of the parameters obtained with the introduction of *Salmonella enteritidis* and *Salmonella typhimurium* in rats on a single dose of vancomycin, there was a sharper change in the quantitative and species composition of the microbiota than with the introduction of *Salmonella* without antibiotics. Thus, in groups V and VI, there was an increase in the amount of *E. coli* in 65 and 105 times ($p \leq 0.05$), and the frequency of their release in these groups was 35 and 95%. This does not contradict the results of Stecher B. et al. (2010), who demonstrated that after administration of *S. enteritidis* and *S. typhimurium* to mice under antibiotic therapy, the amount of *E. coli* increased 2 times [29]. A significant increase in the content of *P. aeruginosa* was observed in the fifth group, and in the sixth, only 3 times ($p \leq 0.05$). Ferreira R.B. et al. (2011), when examining patients with infectious intestinal diseases, found a slight decrease in the number of pseudomonads [9]. The frequency of lactobacilli in groups V and VI was 30 and 6%, but their number did not change significantly and was isolated from the material from rats of group VI, these indicators corresponded to a decrease of 17 times (Table 3). The results of Lleal M. et al. (2019), in the study of the intestinal microflora of rats, also indicate a decrease in the number of representatives of this genus [15].

According to data provided by Turnbaugh P.J. et al. (2006), when administered to mice antibiotics and *Salmonella*, the number of representatives of Bacteroides spp. in the parietal contents of the intestine, decreased in 4 times [33]. These data do not contradict the results of our studies, where the introduction of vancomycin and *Salmonella*, in groups V and VI, also showed a decrease in the number of *Bacteroides* spp. In 9 and 10 times ($p \leq 0.05$) (see Table 1). The level of *E. faecalis* and *E. faecium* decreased significantly only in the fifth group ($p \leq 0.05$), and in group VI the frequency of their release was 19% (Table 3). The number of *Proteus* spp. significantly decreased in 17 times in group V and, also, a significant decrease was observed in group VI ($p \leq 0.05$). According to the literature in mice with the introduction of antibiotics also showed increased susceptibility to infectious agents, which led to a decrease in indigenous groups of microorganisms, including *Proteus* spp., which confirms our data [36].

A sharp increase in the content of *Enterobacter* spp. and *Klebsiella* spp. was observed in groups V and VI ($p \leq 0.05$). The frequency of secretion of *Klebsiella* in the parietal contents in these groups was 19 and 15%, respectively. Song H.J. et al. (2009) in their studies showed that in patients with *Salmonella*-induced inflammation, on the background of antibiotic therapy, the number of *Enterobacter*ia and *Klebsiella* increased in 6 times, which is fully consistent with our results [28]. According to the literature, when co-administered to rats vancomycin and *Salmonella*, the number of peptostreptococci decreased in 5 times [20]. These data do not contradict our results, which also showed a decrease in the content of *Peptostreptococcus anaerobius* in groups V and VI in 20 and 9 times, respectively ($p \leq 0.05$). The frequency of excretion in the parietal content of fungi of the genus *Candida* was 57% (group V) and staphylococci — 95% in the VI experimental group (Table 3). The amount of *Salmonella* spp. significantly decreased only in group V in 7 times ($p \leq 0.05$). However, the data obtained by Barthel M. et al. (2003), when conducting a similar experiment on mice, talk about an increase in *Salmonella* in the parietal contents of the small intestine in 3 and 5 times [3].

During the experiment, we obtained the results of bacteriological studies, which showed pronounced

Table 4. The frequency of excretion of microorganisms in the parietal contents of the intestine of rats with the introduction of *Salmonella, B. fragilis* on the background of vancomycin

Groups of microorganisms	Groups of experimental animals			
	Vancomycin + *S. enteritidis* (n = 15)	Vancomycin + *S. typhimurium* (n = 15)	Vancomycin + *S. enteritidis* + *B. fragilis* (n = 15)	Vancomycin + *S. typhimurium* + *B. fragilis* (n = 15)
	Frequency of excretion (%) of microorganisms			
E. coli	35	95	0	0
***Staphylococcus* spp.**	0	95	0	68
***Enterococcus* spp.**	0	19	10	13
***Bifidobacterium* spp.**	0	0	0	0
***Lactobacillus* spp.**	30	6	27	40
***Klebsiella* spp.**	19	15	0	0
***Proteus* spp.**	0	0	0	29
Candida	57	0	0	0

Note. * — difference of parameters in relation to Vancomycin + *S. enteritidis* and Vancomycin + *S. typhimurium* groups.

changes in the quantitative and species composition of the parietal microbiota when administered to experimental animals *B. fragilis* (Table 2). Thus, a significant decrease in the content of *E. coli* was observed in group VII, and in VIII — 538 times ($p \leq 0.05$). The number of *P. aeruginosa* in groups VII and VIII decreased significantly, and the number of representatives of *Bacteroides* spp. naturally increased significantly ($p \leq 0.05$).

The quantitative content of *E. faecalis* and *E. faecium* increased in 10 and 19 times in groups VII and VIII ($p \leq 0.05$), and the amount of *Proteus* spp. decreased only in group VII in 322 times ($p \leq 0.05$). The frequency of enterococci in these groups was 10 and 13%, and *Proteus* — 29% and only in group VIII. Also, in groups VII and VIII there was a sharp decrease in the content of *Enterobacter* spp. and *Klebsiella* spp. ($p \leq 0.05$). The number of representatives of *Peptostreptococcus anaerobius* significantly increased in 7 and 12 times (groups VII and VIII) ($p \leq 0.05$). Also, bacteriological examination of the intestinal contents of rats revealed an increase in the number of lactobacilli in several thousand (VII) and 40 times (VIII), and their frequency in these experimental groups was 27 and 40%, respectively. El Aidy S. et al., 2012 in their studies showed an increase in the number of indigenous microflora due to its correction of *B. fragilis* [8]. The frequency of staphylococcal excretion was found in 68% and only in group VIII (Table 4). With regard to *S. enteritidis* and *S. typhimurium*, in groups VII and VIII there was a marked decrease in their number ($p \leq 0.05$) (Table 2).

Our results indicate the possibility of using *B. fragilis* to correct *Salmonella*-induced changes in the intestinal microbiome. We observed a decrease in the level of *Salmonella* spp., *E. coli*, *P. aeruginosa*, *Proteus* spp., *Enterobacter* spp., *Klebsiella* spp., as well as an increase in *Bacteroides* spp., *E. faecalis*, *E. faecium* and *Peptostreptococcus anaerobius*. The ability of *B. fragilis* to influence the quantitative content of microorganisms in the development of *Salmonella*-induced inflammatory bowel disease has been shown in a number of other works [6].

B. fragilis is one of the main producers of short-chain fatty acids (SCFA), which activate cells through free fatty acid receptor 2 (FFAR2), which is expressed in immune system cells, intestinal epitheliocytes and plays an important role in immune regulation, metabolic homeostasis and reduction of colitis-associated inflammation [17, 32]. In addition, polysaccharide A of *B. fragilis* is an important inducer of Treg cell differentiation [18, 40].

Conclusion

1. The data obtained in this study indicate that antibiotic-induced changes in the quantitative and qualitative composition of the parietal microbiota are due to the effect of vancomycin on gram-positive microorganisms. There was a decrease in the number of autochthonous obligate anaerobic bacterias (bacteroids), clostridias, elimination of enterococci, peptostreptococci, staphylococci, bifidobacterias, lactobacilli and an increase in the number of enterobacterias, proteus, *Klebsiella* and *Salmonella*. Reducing the number of *E. coli* and *Bacteroides* spp. with the introduction of *S. enteritidis* and *S. typhimurium* was accompanied by an increase in the parietal contents of the intestine of microorganisms such as *P. aeruginosa*, *E. faecalis*, *E. faecium*, *Enterobacter* spp., *Klebsiella* spp., *Peptostreptococcus anaerobius*, which may have occurred due to the latter for intestinal microbiomatter.

2. The introduction of *S. enteritidis* and *S. typhimurium*, on the background of pretreatment with vancomycin, caused a sharp change in the composition of the microbiota in the parietal contents of the small intestine: an increase in *Salmonella* spp., *E. coli*, *P. aeruginosa*, *Enterobacter* spp., *Klebsiella* spp., and also a sharp decrease in the number of *Bacteroides* spp., *E. faecalis*, *E. faecium*, *Proteus* spp., *Lactobacillus* spp., *Peptostreptococcus anaerobius.* These data suggest that the vancomycin-induced imbalance of the parietal intestinal microbiota facilitates the penetration and colonization of pathogenic microorganisms (*S. enteritidis* and *S. typhimurium*) and promotes the development of intestinal diseases.

3. When administered to experimental animals *B. fragilis*, which received *S. enteritidis* or *S. typhimurium* on the background of pretreatment with vancomycin, there was a change in the quantitative composition of the microbiota in the parietal contents of the small intestine, namely: a decrease in *Salmonella* spp., *E. coli*, *P. aeruginosa*, *Proteus* spp., *Enterobacter* spp., *Klebsiella* spp., as well as an increase in *Bacteroides* spp., *E. faecalis*, *E. faecium*, *Lactobacillus* spp. and *Peptostreptococcus anaerobius.*

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