Article's History: Received: 22.03.2023; Revised: 16.05.2023; Accepted: 26.06.2023

Bulletin of Medical and Biological Research

Vol. 16, No. 2
2023

DOI: 10.61751/bmbr.2706-6290.2023.2.15

Journal homepage: https://bmbr.com.ua/en

UDC 617.55-001.31-005.1-06:616.34-008.87]-092.9

Experimental translocation of intestinal bacteria caused by closed abdominal trauma, acute blood loss, internal haemorrhage

Olena Pokryshko*

PhD in Medical Sciences, Associate Professor
I. Horbachevsky Ternopil National Medical University of the Ministry of Health of Ukraine
46001, 1 Maidan Voli, Ternopil, Ukraine
https://orcid.org/0000-0001-9640-0786

Vitalii Khomenko

Posrgraduate Student
I. Horbachevsky Ternopil National Medical University of the Ministry of Health of Ukraine
46001, 1 Maidan Voli, Ternopil, Ukraine
https://orcid.org/0009-0004-4071-1541

Abstract. Bacterial translocation plays an important role in the development of multiple organ failure, which develops as a result of trauma. The severity of bacterial translocation is proportional to the degree of blood loss and damage. The purpose of the study was to establish the spectrum of microorganisms involved in translocation, their population levels, and explore changes in the microecology of mesenteric lymph nodes and liver tissue in white rats subjected to closed abdominal trauma, acute blood loss, internal haemorrhage, and their combination. Experiments were performed on 36 male rats. Biological material (mesenteric lymph nodes, liver tissue) was collected at 7 and 14 hours post-experiment and the samples underwent homogenisation and were then cultured according to laboratory protocols. Isolated bacteria were identified using a Vitek-2 Compact 15 analyser (bioMérieux, France), and their population level was evaluated in $\log_{10}$ CFU/g. Bacterial translocation was considered present when a positive result was obtained simultaneously in the mesenteric lymph nodes and in the tissue of the right lobe of the liver. It was established that the main role in translocation is played by *E. coli, P. stuartii, P. mirabilis, P. aeruginosa.* The combination of abdominal trauma with acute blood loss was accompanied by translocation and *K. pneumoniae.* Trauma combined with acute blood loss causes additional translocation of *E. faecalis, E. cloacae, E. faecium;* while internal haemorrhage led to *S. aureus and S. epidermidis translocation.* The spectrum of translocating microorganisms was more diverse and multi-component when the trauma was combined with internal haemorrhage. At 14 hours post-trauma, there was a tendency for increased population levels of the isolated bacteria compared to the 7-hour data. In all experimental groups, *E. coli* strains were cultured in the highest concentrations

Keywords: gut microbiota; bacterial translocation; mesenteric lymph nodes; blunt abdominal trauma; haemorrhage

INTRODUCTION

In modern medical science, comprehensive understanding of the mechanisms underlying the interaction between the human body and its own microbiota occupies a prominent position among fundamental research tasks. It has become evident that commensal microorganisms not only reside in the body but also play a crucial role in various important functions of the host organism. Allochthonous bacteria in the gastrointestinal tract of humans serve a dual role as they contribute to digestion, participate in the defence mechanism against pathogens, and exert unique molecular mechanisms that influence the functioning of different organs and systems. However, under certain circumstances, these same bacteria can become potential pathogens. In-depth understanding and investigation of bacterial translocation (BT) help elucidate the translocation processes and facilitate the development and clinical application of new pharmaceutical preparations.

Suggested Citation:
Pokryshko O, Khomenko V. Experimental translocation of intestinal bacteria caused by closed abdominal trauma, acute blood loss, internal haemorrhage. Bull Med Biol Res. 2023;16(2):15–22. DOI: 10.61751/bmbr.2706-6290.2023.2.15

*Corresponding author

According to modern literature sources, it is known that in the body in response to trauma and blood loss, changes in the parameters of homeostasis occur due to both pathological processes and compensatory mechanisms, and their totality is characterised by prolonged action [1, 2]. Moreover, the degree of severity of the physiological response to trauma is proportional to the degree of severity of blood loss and damage [3, 4]. A normally functioning intestinal mucosa prevents intestinal bacteria and endotoxins from entering the bloodstream and other organs [5-7]. Dysfunction of the intestinal barrier is considered the main cause of inflammation, when there is a violation of the intestinal blood supply and insufficient oxygenation of tissues. Furthermore, the loss of intestinal mucosal barrier functions and reduced intestinal blood flow lead to BT. Given the close relationship between BT and inflammatory reactions and their impact on intestinal integrity, it has become essential to explore new aspects of gut microbial ecology and various gastrointestinal and metabolic disorders [8]. The development of translocation phenomena has been demonstrated in a study conducted by Ł. Wlazło *et al.* [9], who performed a microbiological analysis of animal abscesses, revealing a significant increase in intestinal bacteria in all samples which was attributed to the migration of gut bacteria through compromised intestinal permeability and their dissemination into the bloodstream. The majority of studies indicate that the most common routes for bacterial passage from the gut to the systemic circulation and eventually to distant organs are the lymphatic and vascular pathways. It has been proven that the lymphatic collector plays a key role in the implementation of the bacterial translocation mechanism [10].

B.I. Ucar *et al.* [11] investigated the underlying molecular mechanisms of BT in sepsis, diagnosis and evaluation of BT, and ways to improve the underlying molecular mechanisms of BT in sepsis, diagnosis and evaluation of BT, and therapeutic treatments for sepsis. They determined that BT occurs more frequently in patients with intestinal obstruction, endogenous infections, endotoxemia, and immune system disorders, which further leads to the development of sepsis and subsequent multiorgan dysfunction. In some cases, the penetration of bacteria and endotoxins through the intestinal barrier can lead to blood flow infections and multiple organ failure. In the paper, Y.H. Wang [12] highlighted that in chronic kidney diseases, compromised intestinal barrier function may contribute to the translocation of gut microorganisms (endotoxins, antigens, and other microbial products) to the intestinal wall, correlating with signs of chronic inflammation in the gastrointestinal tract, endotoxemia, and systemic inflammation. Therefore, the prevention of BT can become a new important area in the treatment of various diseases. For instance, the application of specific postoperative treatment methods, which are pathogenetically justified and aimed at preventing the migration of enterobacteria into the peritoneal space in patients with acute adhesive small bowel obstruction, has shown a reduction in postoperative complications and mortality rates [13]. Normal gut bacteria have been found to migrate to the draining mesenteric lymph nodes inside host phagocytes. Clinical and experimental evidence confirms that bacteria capable of surviving within macrophages (such as *Salmonella species* and *Listeria monocytogenes*) translocate more easily compared to other bacteria, including obligate anaerobes [14]. Y. Xu *et al.* [15] identified the gut microbiota as a potential mediator of the pathogenesis of heart failure and its associated diseases. Escherichia coli, for example, can increase the permeability of the colonic walls and stimulate inflammation in intestinal tissue, leading to enhanced bacterial translocation. The development of this phenomenon in experiments on mice was established by N. Long *et al.* [16]. There is also evidence that anaerobic bacteria translocate along with facultative anaerobes in situations involving intestinal epithelial damage, such as trauma and acute mesenteric ischemia [17].

The purpose of the study was to establish the taxonomic composition and population levels of microorganisms involved in bacterial translocation and explore the changes in the microecology of mesenteric lymph nodes and liver tissue in white rats with induced closed abdominal trauma, blood loss, and their combination.

MATERIALS AND METHODS

The experimental work was conducted in the Laboratory of Microbiological and Parasitological Research at the I.Ya. Horbachevsky Ternopil National Medical University, Ministry of Health of Ukraine, in 2022. The study utilized 36 sexually mature male Wistar rats weighing 200-250 g, kept on a standard diet in the vivarium. Laboratory animals were divided into 4 groups: control (n = 6), the first – a group of experimental rats with simulated closed abdominal injury (n = 10), the second – a group of experimental rats with simulated closed abdominal injury and acute blood loss (n = 10), the third – a group of experimental rats with simulated closed abdominal injury, acute blood loss and internal haemorrhage (n = 10). Under anaesthesia, mesenteric lymph nodes (MLNs) and liver tissue were collected as biological material for microbiological analysis at 7 and 14 hours after the induction of pathological conditions. To avoid cross-contamination during sample collection from MLNs and liver, instruments were not reused for subsequent sampling. Each tissue sample was homogenised using a sterile 0.9% sodium chloride solution, and the homogenate was further diluted 10 and 100 times.

All diluted samples (100 µl each) were plated on blood agar to determine the population levels of isolated microorganisms. To establish the taxonomic composition of the isolated bacteria, the prepared material was also cultured on selective and differential media and incubated for 24 hours at 37°C. The staphylococci were isolated on egg yolk-salt agar, enterococci on enterococcus agar, enterobacteria on Endo, Olkenitsky, and Simmons media, bifidobacteria on bifidum medium, and lactobacilli on lactoagar. For the detection of obligate anaerobes, thioglycolate medium was used. After 24-96 hours of incubation (for facultative anaerobes) and 5 days (for obligate anaerobes) at 37°C, the number of bacteria was determined by counting colony-forming units (CFU) per gram of tissue. The bacterial population level was calculated using the formula: number of colonies in the sample (CFU/g) = average number of colonies on the medium in a Petri dish × dilution ratio, and represented as $\log_{10}$ CFU/g. Isolation of bacteria in the abdominal cavity in clinically significant concentrations (more than 5 $\log_{10}$ CFU/g) indicated the development of an inflammatory process. The isolated strains were initially

identified based on Gram staining and colony morphology, and finally confirmed using the Vitek 2 Compact 15 automated microbiological analyser (BioMerieux, France) for taxonomic identification. To assess bacterial translocation, the microbial flora of the investigated lymph nodes was compared to its presence in the liver tissue. BT was considered present when a positive result was obtained simultaneously in both mesenteric lymph nodes and liver tissue [18].

During the work with laboratory animals, international requirements for the humane treatment of animals were followed in accordance with the rules of the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes [19], and the methodological recommendations of the State Expert Center of the Ministry of Health of Ukraine on "Preclinical studies of medicinal products" [20]. Euthanasia of the rats throughout the experiment was performed by total exsanguination from the heart after prior propofol anaesthesia (60 mg/kg intravenously).

RESULTS

In the control group of rats (n = 6), only half of the experimental animals had cultured *Escherichia coli* from MLN. However, BT was not considered positive because the liver tissue remained sterile. This phenomenon is natural considering that the normal flora of humans trains the body's immune response. From the MLN of the first group of animals, 12 strains of facultative anaerobic rods were isolated after 7 hours of simulated closed abdominal trauma, and 18 strains after 14 hours. Among them, cultures of Enterobacteria belonging to the following species were identified: *Escherihia coli, Proteus mirabilis, Morganella morganii, Providencia stuartii*, and cultures of non-fermenting rods such as *Pseudomonas aeruginosa*. The population level of microorganisms isolated from MLN after 7 hours of trauma averaged (3.02 ± 0.84) $\log_{10}$ CFU/g (Table 1). After 14 hours, there was a tendency for an increase in bacterial concentration in MLN to (3.58 ± 0.79) $\log_{10}$ CFU/g.

Table 1. Taxonomic composition and population levels of bacteria isolated from rats with closed abdominal trauma (n = 10)

No.	Microorganism	Population level, $\log_{10}$ CFU/g				Number of rats with an existing bacterial translocation, %	
		mesenteric lymph node		liver tissue			
		7 h	14 h	7 h	14 h	7 h	14 h
1	*E. coli*	4.47 ± 0.98	4.56 ± 1.02	2.92 ± 0.78	3.93 ± 0.78	60	80
2	*P. mirabilis*	3.10 ± 0.83	3.84 ± 0.81	1.98 ± 0.62	2.85 ± 0.58	40	30
3	*M. morganii*	2.32 ± 0.78	2.93 ± 0.67	0	2.56 ± 0.44	0	20
4	*P. stuartii*	2.54 ± 0.65	2.86 ± 0.85	0	2.60 ± 0.32	0	10
5	*P. aeruginosa*	3.02 ± 0.94	3.70 ± 0.62	2.32 ± 0.85	2.94 ± 0.56	20	40
Total		**3.02 ± 0.84**	**3.58 ± 0.79**	**2.21 ± 0.37**	**2.98 ± 0.54**	**100**	**100**

Source: compiled by the authors

After 7 hours of trauma simulation in the rats of this group, monospecies cultures of microorganisms were most frequently isolated from the lymph nodes, while after 14 hours with further development of the inflammatory process, most bacteria were found in two-component associations, except for 2 animals where *P. aeruginosa* was isolated as a monospecies. BT in MLN was confirmed by the isolation of the same bacterial species in liver tissues. The dominant species in the lymph nodes was *E. coli*: strains of Escherichia coli were isolated in 60% of the rats in this group after 7 hours and in 80% of the animals after 14 hours. The highest bacterial concentration in MLN was also observed for *E. coli* (4.47 ± 0.98) $\log_{10}$ CFU/g and (4.56 ± 1.02) $\log_{10}$ CFU/g (after 7 and 14 hours, respectively).

From the second group of animals, 18 and 26 strains of facultative anaerobic microorganisms were isolated (after 7 and 14 hours, respectively). After acute blood loss in rats with closed abdominal trauma, the bacterial concentration in MLN averaged (3.22 ± 0.85) $\log_{10}$ CFU/g and (3.49 ± 0.76) $\log_{10}$ CFU/g after 7 and 14 hours, respectively. Although the population level of the bacteria remained virtually unchanged, their taxonomic composition became richer. According to the results of the study, the presence of BT in this group of laboratory rats (n = 10) was caused by strains of gram-negative enterobacteria (*E. coli, Klebsiella pneumoniae*, *M. morganii, P. mirabilis*) and non-fermenting rods (*P. aeruginosa)*, and gram-positive cocci (*Enterococcus faecalis* and *E. faecium*) (Table 2). It is worth noting that gram-positive bacteria were not observed in BT after 7 hours; enterococci penetrated the mesenteric nodes only after 14 hours of the experiment. However, only one rat in this experimental group had a monospecies culture represented by *P. aeruginosa*, while in all other cases, isolated microorganisms were found in two-component associations. Unlike motile pseudomonads, enterobacteria, Proteus, and other enterobacteria, Klebsiella and enterococci are non-motile bacteria, and therefore, their translocation occurred at a slower rate. Although strains of *E. faecalis and E. faecium* were isolated from mesenteric nodes after 7 hours of the experiment, their translocation was not confirmed bacteriologically because they appeared in parenchymal organs, particularly the liver, after 14 hours. Perhaps this is why three-component bacterial associations were determined in half of the experimental rats of this group, compared with animals of the first and second groups. The deficiency of blood supply leads to impaired cell membrane function and increased permeability, which is why conditionally pathogenic Klebsiella and enterococci were not isolated from the lymph nodes of rats with only closed abdominal trauma. The highest population level in MLN was observed for *E. coli*, reaching 4.67 ± 0.7 $\log_{10}$ CFU/g and 5.03 ± 0.30 $\log_{10}$ CFU/g (after 7 and 14 hours, respectively). The lowest population level was observed for enterococci (Table 2). Strains of *E. faecalis and E. faecium* were isolated from mesenteric nodes at concentrations of 2.78 $\log_{10}$ CFU/g and 2.59 $\log_{10}$ CFU/g, respectively.

Table 2. Taxonomic composition, population level of microorganisms isolated from rats with closed abdominal trauma combined with acute bleeding (n = 10)

No.	Microorganism	Population level, $\log_{10}$ CFU/g				Number of rats with existing bacterial translocation, %	
		mesenteric lymph node		liver tissue			
		7 h	14 h	7 h	14 h	7 h	14 h
gram-negative bacteria							
1	*E. coli*	4.67 ± 0.73	5.03 ± 0.30	1.75 ± 0.77	1.89 ± 1.29	90	100
2	*K. pneumoniae*	2.40 ± 0.58	3.22 ± 0.77	2.19	2.38 ± 0.93	10	30
3	*M. morganii*	2.93 ± 0.66	3.11 ± 0.42	2.94 ± 0.22	2.41 ± 0.68	10	20
4	*P. mirabilis*	4.16 ± 0.87	4.56 ± 0.49	2.76 ± 0.38	2.93 ± 0.42	40	50
5	*P. aeruginosa*	2.98 ± 0.78	4.02 ± 0.45	2.75 ± 0.87	2.93 ± 0.56	30	40
gram-positive bacteria							
6	*E. faecalis*	2.78	2.98	0	1.65	0	10
7	*E. faecium*	2.59	2.99	0	1.08	0	10
Total		**3.22 ± 0.85**	**3.49 ± 0.76**	**1.77 ± 2.18**	**1.27 ± 0.68**	**90**	**100**

Source: compiled by the authors

From the animals in the third group, 32 strains of facultative anaerobic bacteria were isolated from MLN after 7 hours of experiment simulation, and 44 strains were isolated after 14 hours. In the group of rats with closed abdominal trauma, combined with acute blood loss and internal haemorrhage, compared to the previous experimental groups, not only was the spectrum of microorganisms isolated from MLN the widest, but the population level of bacteria in the lymph nodes was also significantly higher after 14 hours of the experiment. The average concentration of microorganisms isolated from MLN in this experimental group was (3.30 ± 1.07) $\log_{10}$ CFU/g and (4.18 ± 1.54) $\log_{10}$ CFU/g after 7 and 14 hours, respectively. While the first group identified bacteria belonging to 5 species and the second group to 7 species, the bacterial spectrum in the third group included 10 species. In all experimental rats of this group, BT was confirmed by the presence of the same strains in the liver tissue.

In addition to translocation in mln of Gram-negative rods and enterococci, which were found in all experimental groups of rats, translocation of Gram-positive staphylococci was observed. Staphylococci are characterised by the secretion of enzymes such as coagulase, hyaluronidase, fibrinogen, and toxins such as leukotoxin and enterotoxin, which lead to the release of pro-inflammatory cytokines that trigger and intensify inflammatory processes, causing complications and enhancing the action of endotoxins from gram-negative bacteria. After 7 hours of the experiment, the following strains of staphylococci were isolated from MLN: *Staphylococcus aureus* and *S. epidermidis* from 30% and 40% of all animals in the third group, respectively, at a concentration of (2.56 ± 1.85)-(2.89 ± 1.12) $\log_{10}$ CFU/g. Their population level increased by an order of magnitude after 14 hours (Table 3). *E. coli* strains showed the highest population levels, as in the previous two groups. It reached (5.94 ± 1.58) $\log_{10}$ CFU/g and (8.45 ± 1.81) $\log_{10}$ CFU/g after 7 and 14 hours, respectively. This level indicates the development of an inflammatory process. They were only surpassed in concentration in MLN by cultures of *P. mirabilis*. The population level of Proteus was (4.75 ± 1.09)-(4.79 ± 1.45) $\log_{10}$ CFU/g. Cultures of enterococci were isolated at a concentration of 2.59-(2.85 ± 0.57) $\log_{10}$ CFU/g after 7 hours, and their population level after 14 hours was 2.88-(3.41 ± 0.16) $\log_{10}$ CFU/g. If the isolated microorganisms in the first and second experimental groups were found in mono- and two-component associations, the bacteria isolated from the rats in the third group were found in three-component associations. In contrast to the previous groups, the taxonomic composition of gram-positive microorganisms expanded (Table 3), with bacteria belonging to 5 species being isolated. However, the spectrum of gram-negative bacteria remained unchanged. During acute blood loss combined with internal haemorrhage, where a significant amount of blood is lost externally and accumulates in the peritoneal cavity, there is a substantial decrease in circulating blood volume (CBV). It also affects the diversity of microorganisms and their population levels.

Table 3. Taxonomic composition and population level of microorganisms isolated from rats with closed abdominal trauma combined with acute blood loss and internal haemorrhage (n = 10)

No.	Microorganism	Population level, $\log_{10}$ CFU/g				Number of rats with existing bacterial translocation, %	
		mesenteric lymph node		liver tissue			
		7 h	14 h	7 h	14 h	7 h	14 h
gram-negative bacteria							
1	*E. coli*	5.94 ± 1.58	8.45 ± 1.81	4.67 ± 1.08	5.39 ± 1.56	100	100
2	*K. pneumoniae*	2.79 ± 0.22	3.92 ± 0.37	1.99 ± 0.30	2.43 ± 0.24	20	30
3	*P. stuartii*	2.87	4.41	2.56	3.55	10	10
4	*P. mirabilis*	4.75 ± 1.09	4.79 ± 1.45	3.26 ± 0.94	3.50 ± 1.02	40	60
5	*P. aeruginosa*	3.03 ± 1.52	4.02 ± 1.69	2.76 ± 1.08	2.93 ± 0.87	50	60

Table 3. Continued

No.	Microorganism	Population level, log_{10} CFU/g				Number of rats with existing bacterial translocation, %	
		mesenteric lymph node		liver tissue			
		7 h	14 h	7 h	14 h	7 h	14 h
gram-positive bacteria							
6	*E. faecalis*	2.85 ± 0.57	3.41 ± 0.16	0.78 ± 0.21	1.32 ± 0.30	20	20
7	*E. cloacae*	2.59	2.99 ± 0.30	0.90	1.51 ± 0.34	10	20
8	*E. faecium*	2.61	2.88	0	1.27	0	10
9	*S. aureus*	2.56 ± 1.85	3.71 ± 1.68	1.04 ± 1.13	2.62 ± 1.52	40	70
10	*S. epidermidis*	2.89 ± 1.12	3.18 ± 1.40	1.74 ± 1.48	2.99	30	50
Total		**3.30 ± 1.07**	**4.18 ± 1.54**	**1.99 ± 1.32**	**2.86 ± 1.21**	**100**	**100**

Source: compiled by the authors

Studies have shown that in laboratory animals with closed abdominal trauma, gram-negative intestinal microflora plays a major role in the phenomenon of bacterial translocation (Fig. 1). From the rats in the first experimental group, only gram-negative bacteria were isolated from MLN. In the second experimental group, where acute bleeding was simulated only after 14 hours, gram-positive microorganisms appeared in MLN in small quantities, accounting for only 7.7% of all isolated bacteria. Internal haemorrhage stimulates an increase in bacterial translocation and its diversity, which is why gram-positive cocci accounted for 40.9% of all isolated microorganisms in the third experimental group.

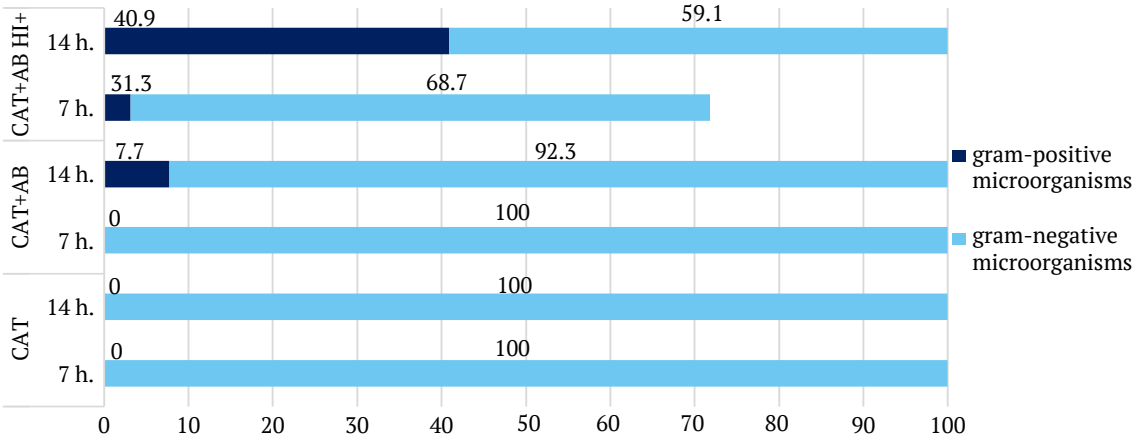

Figure 1. Ratio of gram-positive and gram-negative microorganisms isolated from mesenteric lymph nodes, %
Notes: CAT – closed abdominal trauma; CAT+AB – closed abdominal trauma + acute bleeding; CAT+AB+IH – closed abdominal trauma + acute bleeding + internal haemorrhage
Source: compiled by the authors

In a certain way, the translocation of bacteria from the intestine occurs constantly, even in 5-10% of healthy people [21]. That is why BT was not confirmed in all rats in the experiment as microorganisms from some experimental animals were isolated only from the lymph nodes, which is not a criterion for the presence of BT.

DISCUSSION

In recent years, the influence of gut microbiota on the molecular and pathological mechanisms of inflammation has been the focus of many researchers. Additionally, experiments are being conducted on new treatment methods based on the impact of gut microbiota on trauma, considering the development of BT [22].

T. Komarov *et al.* [18] emphasised that the phenomenon of microbial translocation is a key element in the development of post-traumatic complications. The data obtained in the conducted experiments also showed that closed abdominal trauma combined with blood loss leads to tissue ischemia, which, in turn, contributes to the natural migration of microorganisms inhabiting the intestinal microbiota to the MLN, peritoneal space and sinuses of the liver. This process further contributes to the development of inflammation and complications. E. Nieves *et al.* [23] found a significant statistical difference between postoperative infections in patients with signs of BT (41.6%) compared to patients without BT (12.5%; $p = 0.047$). Bacteria isolated from infection sites were the same as those cultured in MLN in 40% of cases ($n = 2$ out of 5), which allowed them to establish a causal relationship between BT and postoperative infection. M. Schietroma *et al.* [24] demonstrated that risk of bacterial translocation is associated with bleeding ⩾ 1500 ml. Moreover, a higher risk of bacterial translocation and a significantly higher incidence

of postoperative infections exist in patients requiring urgent surgical treatment (splenectomy) after blunt abdominal trauma. The researchers showed that bacterial strains isolated from infection sites were the same as those cultured in MLNs in 48.3% of cases (n = 14 out of 29). In the conducted experiments, it was determined that maximum blood loss is accompanied by the richest spectrum of bacteria involved in BT and an increase in their population level. R.I.D. da Costa *et al.* [25] investigating BT in intestinal obstruction on a rat model, demonstrated that the optimal timing for performing the operation is 24 hours. The phenomenon of bacterial translocation was observed already 7 hours after the start of the experiment. Obviously, this is due to hypoxia, additionally created by simulated blood loss. However, it should be noted that it is precisely at the 24-hour mark that the microecology of MLN and liver tissue in white rats with simulated closed abdominal trauma, haemorrhage, and their combination becomes the most pronounced and multi-component.

According to the literature, gram-negative facultative anaerobic *Enterobacteriaceae*, such as *E. coli*, *K. pneumoniae*, and *P. mirabilis*, are the fastest and easiest to migrate from the gastrointestinal tract to MLN [4, 26]. Among them, according to the data obtained by researchers E. Nieves [23] and Y. Sharapatov [27], intestinal bacteria are most frequently identified in the presence of BT. Exo- and endotoxins enhance the pathogenic action of *E. coli*. It is *this species of Enterobacteriaceae that primarily* triggers the inflammatory process in polytrauma patients [22]. In experiments on research rats, I.V. Strelbytska *et al.* [28] found that simulated limb ischemia-reperfusion, acute blood loss, and their combination lead to the translocation of gut bacteria into the abdominal cavity. These literature data are confirmed by the results of the study. Determination of the spectrum of microorganisms that migrate from the gut during experimental blunt abdominal trauma in combination with haemorrhage showed that strains of *E. coli* migrate the fastest and in greater quantities compared to other bacteria. The translocation of gram-negative bacteria is associated with inflammatory mechanisms induced by lipopolysaccharides (LPS) of their cell walls. These bacterial antigens in tissue damage areas induce an inflammatory process [29]. LPS in combination with lipid-A are mediators of white blood cell activation and inducers of macrophages. The active action of gram-negative flora endotoxins increases the secretion of pro-inflammatory antigens and decreases the production of anti-inflammatory ones. The pro-inflammatory cytokines released during this process can also disrupt tight junctions, promoting the translocation of microorganisms. For example, the presence of proteolytic endotoxin in *P. aeruginosa* often leads to the development of sepsis and septic shock [30]. The results of the study established a correlation between the increasing number of research rats in which strains of *P. aeruginosa* confirming bacterial translocation were detected and the growth of their population level with the development of inflammation. This fact is one of the indications that a closed abdominal injury leads to the development of inflammation. Therefore, the appearance of non-fermenting gram-negative rods in MLN may be a marker of complications in the inflammatory process. C. Doudakmanis *et al*. [4] demonstrated that representatives of the intestinal microbiota, which are obligate anaerobes, virtually do not have the ability to translocate. Only facultative anaerobic bacteria were isolated from rats in all groups, confirming the findings of other researchers, including S.J. Wood *et al*. [31]. According to the results of the study on the dynamics of BT in experimental abdominal trauma in combination with haemorrhage, it was found that it is enterobacteria that will appear outside the boundaries of the gastrointestinal tract.

CONCLUSIONS

As a result of simulated blunt trauma to the abdomen in combination with acute blood loss and internal haemorrhage, an inflammatory process developed in the peritoneum and bacterial translocation occurred to mesenteric nodes and liver tissue.

The main role in BT was played by representatives of the gram-negative intestinal microflora. Strains of gram-negative bacteria migrated first. These included Enterobacteriaceae such as *E. coli, K. pneumoniae, P. stuartii, P. mirabilis,* and non-fermenting rods *P. aeruginosa.* It should be noted that the *K. pneumoniae* strains were capable of migration in the presence of acute haemorrhage in rats with blunt abdominal trauma. In experimental animals with blunt abdominal trauma combined with acute blood loss, the translocation of gram-positive cocci strains, such as *E. faecalis, E. cloacae*, and *E. faecium*, was also observed. Under the condition of additional simulated internal haemorrhage, bacterial translocation of staphylococcal strains occurred: *S. aureus, S. epidermidis.* Thus, the animals with blunt abdominal trauma had the least diverse taxonomic composition, with strains from 5 species being isolated. The group of animals with blunt abdominal trauma combined with acute blood loss and internal bleeding had the richest taxonomic composition, with twice as many species being identified.

The translocation of bacteria from the intestine to the mesenteric lymph nodes and liver tissue led to an increase in the population level of the isolated and identified bacteria. Microorganisms isolated from MLN were found in the highest concentration in experimental animals with blunt abdominal trauma combined with acute blood loss and internal haemorrhage. The highest population level was observed for *E. coli* strains in all three groups of research rats. After 14 hours, the average population level of bacteria isolated from MLN in animals with blunt abdominal trauma was 3.02 ± 0.84 $\log_{10}$ CFU/g, while in the group of rats with blunt abdominal trauma combined with maximal blood loss, the average concentration of microorganisms in MLN was an order of magnitude higher and amounted to 4.18 ± 1.54 $\log_{10}$ CFU/g. In the future, it is worth continuing to investigate bacterial translocation in different pathological conditions, determining bacterial deoxyribonucleic acids.

ACKNOWLEDGEMENTS

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

[1] Pantalone D, Chiara O, Henry S, Cimbanassi S, Gupta S, Scalea T. Facing trauma and surgical emergency in space: Hemorrhagic shock. Front Bioeng Biotechnol. 2022 Jul 1;10:780553. DOI: 10.3389/fbioe.2022.780553
[2] Twardowska A, Makaro A, Binienda A, Fichna J, Salaga M. Preventing bacterial translocation in patients with leaky gut syndrome: nutrition and pharmacological treatment options. Int J Mol Sci. 2022;23(6):3204. DOI: 10.3390/ijms23063204
[3] de Jong PR, González-Navajas JM, Jansen NJG. The digestive tract as the origin of systemic inflammation. Crit Care. 2016;20:279. DOI: 10.1186/s13054-016-1458-3
[4] Doudakmanis C, Bouliaris K, Kolla C, Efthimiou M, Koukoulis GD. Bacterial translocation in patients undergoing major gastrointestinal surgery and its role in postoperative sepsis. World J Gastrointest Pathophysiol. 2021 Nov 22;12(6):106–14. DOI: 10.4291/wjgp.v12.i6.106
[5] Scarsella E, Meineri G, Sandri M, Ganz HH, Stefanon B. Characterization of the blood microbiome and comparison with the fecal microbiome in healthy dogs and dogs with gastrointestinal disease. Vet Sci. 2023 Apr 5;10(4):277. DOI: 10.3390/vetsci1004027
[6] Lu D, Huang Y, Kong Y, Tao T, Zhu X. Gut microecology: Why our microbes could be key to our health. Biomed Pharmacother. 2020 Nov;131:110784. DOI: 10.1016/j.biopha.2020.110784
[7] Lee B, Moon KM, Kim CY. Tight junction in the intestinal epithelium: Its association with diseases and regulation by phytochemicals. J Immunol Res. 2018 Dec 16;2018:2645465. DOI: 10.1155/2018/2645465
[8] Nagpal R, Yadav H. Bacterial translocation from the gut to the distant organs: An overview. Ann Nutr Metab. 2017;71 Suppl 1:11–16. DOI: 10.1159/000479918
[9] Wlazło Ł, Łopuszyński W, Nowakowicz-Dębek B, Ossowski M, Bis-Wencel H. Translocation of intestinal bacteria as a cause of subcutaneous abscesses of the neck and head in American mink (Neovison vison) – a case report. BMC Vet Res. 2020 Nov 10;16(1):434. DOI: 10.1186/s12917-020-02654-3
[10] Ponziani FR, Zocco MA, Cerrito L, Gasbarrini A, Pompili M. Bacterial translocation in patients with liver cirrhosis: Physiology, clinical consequences, and practical implications. Expert Rev Gastroenterol Hepatol. 2018 Jul;12(7):641–56. DOI: 10.1080/17474124.2018.1481747
[11] Ucar BI., Ucar G. Intestinal barrier dysfunction, bacterial translocation and inflammation: Deathly triad in sepsis. in: Infectious diseases and sepsis [Working Title]; August 2021. DOI: 10.5772/intechopen.99554
[12] Wang YH. Current progress of research on intestinal bacterial translocation. Microb Pathog. 2021 Mar;152:104652. DOI: 10.1016/j.micpath.2020.104652
[13] Burak AY, Bedenuyk AD. Study of the features of bacterial translocation in the complex postoperative treatment of patients with acute decompensated adhesive small intestinal Obstruction. Hosp Surg J Kovalchuk. 2021;(2):71–75. DOI: 10.11603/2414-4533.2021.2.12303
[14] Mamic P, Chaikijurajai T, Tang WH. Gut microbiome – a potential mediator of pathogenesis in heart failure and its comorbidities: State-of-the-Art Review. J Mol Cell Cardiol. 2021 Mar;152:105–17. DOI: 10.1016/j.yjmcc.2020.12.001
[15] Xu Y, Kong X, Zhu Y, Xu J, Mao H, Li J, et al. Contribution of gut microbiota toward renal function in sepsis. Front Microbiol. 2022; 13: 985283. Published online 2022 Sep 6. DOI: 10.3389/fmicb.2022.985283
[16] Long N, Deng J, Qiu M, Zhang Y, Wang Y, Guo W, et al. Inflammatory and pathological changes in *Escherichia coli* infected mice. Heliyon. 2022 Dec;8(12):e12533. DOI: 10.1016/j.heliyon.2022.e12533
[17] Chakaroun RM, Massier L, Kovacs P. Gut microbiome, intestinal permeability, and tissue bacteria in metabolic disease: Perpetrators or bystanders? nutrients. 2020 Apr 14;12(4):1082. DOI: 10.3390/nu1204108
[18] Komarov T, Amanova D, Turgunov E. Methods of verification of the phenomenon of microbial translocation in acute mesenterial ischemia (review). [in Russian]. Georgian Med News. 2020 Mar;(300):59–63. Available from: https://pubmed.ncbi.nlm.nih.gov/32383703/
[19] European Convention for the protection of vertebrate animals used for experimental and other scientific purposes. No. 123. Strasbourg; 18.III.1986. [Internet]. [cited 2023 May 31]. Available from: https://rm.coe.int/168007a67b
[20] National Agency of Medicines of Ukraine. Legal Framework for Conducting Ethical Assessment of Preclinical Studies of Medicinal Products Involving Laboratory Animals [Internet]. 2019 Jul 04 [cited 2023 May 31]. Available from: https://amnu.gov.ua/pravova-baza-dlya-provedennya-etychnoyi-ekspertyzy-doklinichnyh-doslidzhen-likarskyh-zasobiv-z-vykorystannyam-laboratornyh-tvaryn/
[21] Huang R, Lu Y, Jin M, Liu Y, Zhang M, Xian S, et al. A bibliometric analysis of the role of microbiota in trauma. Front Microbiol. 2023 Feb 2;14:1091060. DOI: 10.3389/fmicb.2023.1091060
[22] Duffy LC. Interactions mediating bacterial translocation in the immature intestine. J Nutr. 2000 Feb;130(2S Suppl):432S-436S. DOI: 10.1093/jn/130.2.432S
[23] Nieves E, Tobón LF, Ríos DI, Isaza A, Ramírez M, Beltrán JA, Garzón-Ospina D, Patarroyo MA, Gómez A. Bacterial translocation in abdominal trauma and postoperative infections. Auma. 2011 Nov;71(5):1258–61. DOI: 10.1097/TA.0b013e318215140e
[24] Schietroma M, Pessia B, Colozzi S, Carlei F, Amicucci G. Does bacterial translocation influence the postoperative infections in splenectomized patients after abdominal trauma? Surg J (N Y). 2018 Apr;16(2):94–100. DOI: 10.1016/j.surge.2016.09.009
[25] da Costa, RID, Rasslan R, Koike MK, Utiyama EM, Montero EF. Bacterial translocation and mortality on rat model of intestinal ischemia and obstruction. Acta Cir Bras. 2017 Aug;32(8):641–47. DOI: 10.1590/s0102-865020170080000006

[26] Wang F, Jiang H, Shi K, Ren Y, Zhang P, Cheng S. Gut bacterial translocation is associated with microinflammation in end-stage renal disease patients. Nephrology (Carlton). 2012 Nov;17(8):733–38. DOI: 10.1111/j.1440-1797.2012.01647
[27] Sharapatov Y, Turgunov Y, Lavrinenko A. The role of intestinal translocation of *E.coli* in the development of acute obstructive pyelonephritis in an experiment. J Clin Med Kaz. 2023;20(2):9–14. DOI: 10.23950/jcmk/13109
[28] Strelbytska IV, Pokryshko AV, Pokhodun KA. Species composition and level of peritoneal exudate contamination by microorganisms in conditions of acute blood loss complicated by limb ischemia-reperfusion. Hospital Surgery. J Named by L.Ya. Kovalchuk, 2021;2:53-58. DOI: 10.11603/2414-4533.2021.2.12299
[29] Soult MC, Lonergan NE, Shah B, Kim WK, Britt LD, Sullivan CJ. Outer membrane vesicles from pathogenic bacteria initiate an inflammatory response in human endothelial cells. J Surg Res. 2013 Sep;184(1):458–66. DOI: 10.1016/j.jss.2013.05.035
[30] Potruch A, Schwartz A, Ilan Y. The role of bacterial translocation in sepsis: A new target for therapy. Therap Adv Gastroenterol. 2022;15:17562848221094214. DOI: 10.1177/17562848221094214
[31] Wood SJ, Kuzel TM, Shafikhani SH. Pseudomonas aeruginosa: Infections, animal modeling, and therapeutics. Cells. 2023;12(1):199. DOI: 10.3390/cells12010199

Експериментальна транслокація кишкових бактерій спричинена закритою травмою живота, гострою крововтратою, внутрішньою кровотечою

Олена Володимирівна Покришко
Кандидат медичних наук, доцент
Тернопільський національний медичний університет імені І. Я. Горбачевського МОЗ України
46001, майдан Волі, 1, м. Тернопіль, Україна
https://orcid.org/0000-0001-9640-0786
Віталій Станіславович Хоменко
Аспірант
Тернопільський національний медичний університет імені І. Я. Горбачевського МОЗ України
46001, майдан Волі, 1, м. Тернопіль, Україна
https://orcid.org/0009-0004-4071-1541

Анотація. Бактеріальна транслокація відіграє важливу роль у розвитку поліорганної недостатності, яка розвивається в результаті травми. Ступінь вираженості транслокації бактерій пропорційний ступеню крововтрати й ушкодження. Метою дослідження було встановлення спектру мікроорганізмів, які брали участь у явищі транслокації, їх популяційного рівня, та вивчення напрямку змін у мікроекології мезентеріальних вузлів та тканині печінки білих щурів зі змодельованими закритою травмою живота, крововтратами (гостра крововтрата, внутрішня кровотеча) та їх поєднанням. Досліди виконано на 36 щурах-самцях. Біологічний матеріал (мезентеріальні лімфовузли, тканину печінки) брали стерильними інструментами через 7 та 14 год. від початку експерименту, проводили гомогенізацію, після чого висівали на відповідні середовища згідно з лабораторними настановами. Виділені бактерії ідентифікували за допомогою аналізатора Vitek-2 Compact 15 (bioMérieux, France), їх популяційний рівень оцінювали у $\log_{10}$ КУО/г. Бактеріальну транслокацію вважали наявною, коли позитивний результат отримували одночасно в мезентеріальних лімфовузлах і в тканині правої долі печінки. Встановлено, що в транслокації головну роль відіграють *E. coli, P. stuartii, P. mirabilis, P. aeruginosa*. Поєднання травми живота з гострою крововтратою супроводжувалася транслокацією ще й *K. pneumoniae*. Травма, поєднана з гострою крововтратою, викликає додатково транслокацію *E. faecalis, E. cloacae, E. faecium;* а з внутрішньою кровотечою – транслокацію *S. aureus, S. epidermidis*. Спектр мікроорганізмів, здатних до транслокації, був різноманітнішим і багатокомпонентним, якщо травму поєднували із внутрішньою кровотечою. Через 14 год. після нанесення травми спостерігали тенденцію до зростання популяційного рівня виділених бактерій, у порівнянні з даними, отриманими через 7 год. В усіх дослідних групах штами *E. coli* висівали у найбільших концентраціях

Ключові слова: мікробіота кишечника; бактеріальна транслокація; мезентеріальні лімфовузли; тупа травма живота; кровотеча