Man-made load of industrial environment and its impact on human microbiota

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Abstract. People are exposed to a significant amount of man-made hazards upon contact with technological systems, and foundry engineering industrial sites belong undoubtedly to such systems. Foundry shops are characterized by high noise and temperature thresholds that affect the human body. Unsatisfactory condition of the air is considered to be an acute problem in foundry engineering. In this connection, the state of air and its composition change, which can certainly affect health of workers, and the latter may be seen in the respiratory, cardiovascular, nervous systems, and digestive organs functional changes. However, up to date, the effect of health hazards of foundry engineering on the microbiocenosis of human intestine remains poorly studied. The paper apprizes the microbiota of human large intestine being exposed to integrated effects of foundry engineering factors. A structural change of the intestinal coenotype accompanied by a change of absolute dominants and opportunistic microorganisms appearance is established. Intestinal microbiocenosis disorder is accompanied by a high detectability of parasitic protozoa Blastocystis spp. in the sampling material. The obtained blastocyst isolates have pathogenic properties of varying degree of manifestation, which indicates their unequal etiological significance in the development of the pathological process. The isolated strains of protozoa have a high anti-interferon activity characterizing their persistent properties. This characteristic brings along the displacement of normal symbionts by blastocysts and colonization of the intestinal mucous membranes with these protozoa.

1 Introduction

Foundries are characterized by industrial environment adverse factors. They include physical effects (vibration, noise, high temperature, ultrasound, ionizing radiation) and chemical effects (free silicon dioxide, metal oxides, etc.), that create favourable conditions for different diseases [1, 2].

For example, noise factors cause cardiovascular and nervous systems disorders [3]. Vibration is accompanied by changes in acid and pepsin products in the stomach. With longer length of work under vibration conditions, workers show a greater number of erosive gastritis, chronic cholecystitis, parietal digestion disorder, microcirculation of gastric walls, gastrointestinal motility [4]. Ultrasound affects visual and auditory analyzers, causes fatigue and apprehension. [5]. Dust of pheno- and aminoplastics, compounds of lead, zinc, arsenic, silicon dioxide, compounds of rare and non-ferrous metals suppress hematogenesis, disorder metabolism, cause functional changes of nervous system and gastrointestinal tract [6]. The complexity of technological processes, the availability of surplus heat and dust content in the air in the working zone affect the quality of the air environment in the foundry, which usually does not meet sanitary and hygienic requirements [7].

Synthetic materials use in foundry engineering brings forth a pressing problem of detoxication of aerial

phenol, formaldehyde, carbon oxides, benzene, etc. The air environment of foundries has, besides dust, large amounts of carbon oxides, carbon dioxide and sulphur gases, nitrogen and its oxides, hydrogen, aerosols saturated with iron and manganese oxides, hydrocarbon vapours, which, even with solid local exhaust ventilation, enter the working zone, making a threat to workers health [8]. Obviously, in foundry environment, an adverse cumulative effect of the unit factor takes place, and noxious effect of each particular component (dust, gases, temperature, vibration, noise) increases dramatically.

However, up to date, the effect of health hazards foundry engineering on human intestinal microbiocenosis is still little-studied. While the

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important role of normal intestinal microflora in the formation of immune homeostasis of macroorganomas, participation in digestion processes, synthesis of vitamins, its antigonistic effect against pathogenic microorganisms, etc. is known [9]. Intestinal microbiota is a macroorganism state indicator and its changes occur under conditions of adverse environmental factors. And these changes relate to the reduction of the normal symbions representatives number and the growth of opportunistic microorganisms. The latter, at resistance decrease, for example as affected by adverse technological conditions, may show their pathogenicity. Among microorganisms causing human pathology, various types of opportunistic, as well as little-studied agents, have become more common. In this regard, the widespread protozoal invasion - blastocystosis, conditioned by protozoan Blastocystis spp parasitizing mainly in the large intestine, gains ground. [10, 11, 12].

This pathogenic organism as enteropathogen had not attracted specialists' attention for a long time. However, over recent decades, the interest of scientists and practical parasitologists in this protozoan has greatly increased, which is explained by its extremely wide prevalence in the world. It is found in 30-50% of the population of developing countries and 1.5— 10% of the population of developed countries [13, 14]. Besides, blastocysts are often found among patients with gastroenterological diseases, allergoses, etc. [15, 16]. For example, according to Horiki and the co-authors (1999), blastocysts were found in 7.4% of cases when examining practically healthy population of Tokyo (Japan) [17], and in similar research conducted in Perm by N. M. Koza and the co-authors in 1997-2001, blastocyst infestation was found in 13.1% of cases [18]. In Omsk, according to the results of single checkups of the population in 1999-2001 Blastocystis hominis detection rate among healthy population was from 0.9 to 2.3%.

Research by C. H. Zierdt et al. first determined the nature of the protozoan Blastocystis spp., their taxonomic position was established, and indications for the possible etiological role of these microorganisms in the emergence of intestinal invasion-blastocystosis were obtained. Being in the intestine, blastocysts are involved in the microbiocenosis of this biotope formation [19]. Disrupting the balance of microorganisms, these pathogens contribute to the creation of favorable conditions for pathological processes development.

The environmental factors effect modifies not only macroorganism defences, but also changes microorganism properties. However, if nowadays the external impact on the host organism is well studied, then the dynamics of persistent characteristics of microorganisms under anthropogenic environmental factors effect is still unknown, and the parameters that characterize the ability of the parasite to stay in this ecological niche are not determined.

Research goal: Assess the man-made load of the industrial environment on human intestine microbiota in adverse foundry conditions.

Research task:

1. Study qualitative and quantitative changes of intestinal endoflora of workers depending on the length of the service in the foundry.
2. Make an environmental assessment of the intestinal microbiocenosis of foundry workers (floral significance, contagiosity and bio-diversity indexes).
3. Identify the frequency of protozoa Blastocystis spp. parasitism in the intestines of the examined.
4. Investigate foundry conditions effect on pathogenic properties changes of isolated blastocyst strains.
5. Define the anti-interferon activity of Blastocystis spp.

2 Research materials and methods

In the period from 2015 to 2017, a series of studies on microflora of human thick intestine among Kirov plant foundry workers was done based on the polyclinic PJSC "Kirov plant", laboratory "Helix" and the research laboratory "Diagnostika", Saint-Petersburg.

Method of quantitative isolation of types and variants of the microorganisms that are found in the microbiocenosis composition according to the Order of the Ministry of Health of the Russian Federation dated 09.06.2003 № 231 "On approval of the industry-specific standard "Patient management protocol. Intestinal dysbacteriosis" (ISS 91500.11.0004-2003) was used to assess the microbiocenosis.

Cultures grown on nutritional media underwent group, generic, and species identification. Incubation of solid media inoculations to isolate lacto-and bifidumbacteria was carried out in anaerobic jars AE-01 (JSC "NIKI MLT", St. Petersburg.) and OXOID (England) with the use of gas-generating packages «Anaerogaz». Anaerobic microorganisms were identified by using ANAEROTEST 23 (Lachema, Czech Republic). Identification of opportunistic enterobacteria was performed by using conventional identification schemes. To find protozoa, including blastocysts, both methods of traditional parasitological diagnostics (microscopy of fecal extract) and molecular-biological methods (PCR) were used. Microscopy of fecal extracts stained with Lugol's iodine was carried out in accordance with all requirements for mounting a specimen (MOOK 4.2.735-99). To get Blastocystis spp. the Suresh medium was used. Persistence factors study of microorganisms was done at the Institute of cytosymbiosis and intracellular symbiosis UB RAS, Orenburg (ICWN UB RAS). Studies of anti-lactoferrin (alpha) activity were carried out by using research methods proposed by O. V. Bukharin et al. [20]. Ecological analysis of intestinal microbiocenosis was performed by using the contagiosity index (Whittaker, 1980), the index of floral significance of species (Natkevichaite-Ivanauskene, 1985), and the species diversity of microbial communities was calculated by using the Whittaker formula (1980) [21, 22].

The manifestation degree of blastocysts pathogenicity was determined by intra-abdominal injection of 0.5 ml of a suspension of protozoan culture grown on K. Suresh medium to white mice (weighing 23.1+2.2 g).

All the obtained data were subjected to mathematical processing: arithmetic, arithmetic averages errors, standard deviations, confidential intervals, corresponding correlation relations were calculated on the basis of linear correlation analysis, and the validity coefficient was determined according to the method of Student. The results were considered reliable at $p<0.05$. For data processing, the application Software pack for Microsoft Excel 2017 was used.

3 Outcomes and discussions

Intestinal endoflora of 137 of foundry workers (table 1) was examined. A significant reduction of bifidobacteria incidence to 85.4% and lactobacilli to 77.4% was found, whereas, these indicators were 100% among people who do not work in foundry engineering. Another representative of obligate flora - coliform bacterium was found only among 90,5% of foundry workers. A significant increase of E. coli with hemolytic activity is noteworthy, which indicates their high pathogenicity. The degree of incidence of bacteroids in workers and in the control group did not differ significantly, but the density of colonization had a significant difference (lg 8.50.4±CFU / g and lg 10.3±0.1 CFU/g respectively; $p<0.05$). Among workers, Clostridia were found much more often – in 19% of cases, while the indicators of their content did not differ in comparison with the control group.

Table 1. Qualitative and quantitative composition of intestinal biocenosis of the examined.

Microbes number (1g CFU/g	Groups of workers				
	1	2	3	4	Control
Bifidobacterium	8.5±0.3	7.2±0.2	6.3±0.6	5.9±0.4	9.3±0.1
Lactobacillus	7.5±0.1	7.3±0.4	5.7±0.6	5.4±0.5	8.8±0.6
Escherichia	7.7±0.3	7.8±0.6	7.0±0.2	6,0±0.7	7.4±0.3
Bacteroides	9.8±0.4	9.8±0.3	9.3±0.3	9,0±0.6	10.3±0.1
Enterococcus	6.6±0.3	7.1±0.7	7.9±0.1	8,1±0.1	4.7±0.2
Proteus	2.7±0.7	5.1±0.4	5.8±0.2	6,5±0.6	3.5±0.1
Klebsiella	5.3±0.2	5.9±0.4	7.0±0.6	7,3±0.1	3.4±0.7
Staphylococcus	4.9±0.3	5.2±0.3	6.1±0.6	6,8±0.5	4.1±0.4
Candida	6.3±0.4	6.1±0.7	6.1±0.3	6,8±0.3	3.3±0.1
Clostridium	5.5±0.3	6.3±0.7	5.6±0.6	6,7±0.4	6.0±0.8
P	<0.05	<0.05	<0.05	<0.05	<0.05

Opportunistic bacteria took part in the formation of the intestinal microbiocenosis of foundry workers. A significant increase in microorganisms isolation rate quantity and their detection rate were found. This regards to Staphylococcus, Enterococcus, Klebsiella, and fungi of the genus Candida.

In order to find possible dependence of microflora detected changes on the duration of contact with destabilizing working environment factors, all examined were divided into 4 groups in accordance with their work experience in the foundry. The first group consisted of 26 people working at the enterprise for less than 1 year (19%), the second group was of people with work experience from 1 year to 5 years - 32 people (23%), the third group- from 5 to 10 years - 37 people (27%) and the fourth group was with work experience of 10-20 years or more - 42 people (31%).

The data analysis showed that the incidence degree of representatives of the obligate microflora significantly decreases with increasing work experience in the foundry. The incidence degree of transient microflora, depending on the work experience length in the foundry, on the contrary, increased. Thus, enterococci were identified among 46.1% of the 1st group, 46.9% - of the 2nd group, 62.1% - of the 3rd group, and 69.1% - of the 4th group. The incidence degree of Proteus increased among workers of the 4th group in comparison with the 1st group 3 times and amounted to 45.2% and 15.4%, respectively, and Klebsiella-almost 7 times (3.8% and 26.2%, respectively). The dynamics of incidence of staphylococci, Clostridium and fungi of the genus Candida was similar.

Depending on the length of employment at the foundry, the colonization density index of representatives of obligate and transient intestinal endopfora also changed. These changes were already noted in Group 2 of workers, then these changed went up and reached the maximum in Group 4 of workers. However, if the colonization density of normophlora decreased, then, on the contrary, transient flora density increased.

The next stage of the research was to identify the structure of symbiotic relationships of microorganisms involved in the formation of intestinal microbiocenosis of foundry workers (Table 2). It should be noted that opportunistic microorganisms: enterococcus (C = 57.6%) and fungi of Candida genus (C = 55.5%) were part of the dominant species against the declined permanence index of normal microflora representatives. In the control group, these types of bacteria were not included in the dominant species of the control group.

Table 2. Structure of intestinal microbiocenosis of the examined workers.

№	Structure of intestinal microbiocenosis	Permanence indicator
	Dominant groups	%
1	Bifidobacterium	85.4
2	Lactobacillus	77.4
3	Escherichia	90.5
4	Bacteroides	63.5
5	Enterococcus	57.6
6	Candida	55.5
	Additional groups	
1	Staphylococcus	48.9
2	Proteus	30.0
	Transient groups	
1	Klebsiella	20.4
2	Clostridium	19.0

Particular emphasis should be placed on the appearance of the cenotype of Candida fungi in the dominant composition, while they are part of additional species in the control group. Candida fungi act as a permanent co-member of microbiocenosis in the microflora of workers. A change in the hierarchy was also noted in the structure of transient species. So, the

permanence rate for the clostridium genus has increased from 6.2% to 19.0%. Moreover, the transition of staphylococci from the group of transient species into the additional group was found among the workers of the foundry compared with the control group of the examined. Thus, the intestinal coenotype of foundry workers is formed by representatives of normal microflora: bifidobacteria, lactobacilli, Escherichia and bacteroids, as well as enterococci and fungi of the genus Candida.

Workers in contact with hazards of the working environment showed a significant increase in the degree of contagiousness that characterizes the spreading of species in space for obligate microflora: bifidobacteria up to 2.3 ± 0.3 units (in the control group 0.7 ± 0.85 units; p <0.05), lactobacilli 2.5 ± 0.2 units (in the control group 0.9 ± 0.3 units; p <0.05), Escherichia coli - 1.9 ± 0.2 units (in the control group 0.9 ± 0.4 units; p <0.05) and bacteroids - 1.5 ± 0.6 units (in the control group 0.3 ± 0.8 units; p <0.05). On the contrary, among representatives of opportunistic flora, a significant decrease of this indicator was noted. For enterococci, it amounted to 0.6 ± 0.2 units, fungi of the genus Candida - 0.7 ± 0.3 units, and staphylococci - 0.4 ± 0.2 units (in the control group 0.9 ± 0.2; 1.3 + 0.7; 0.8 ± 0.7 units; p <0.05, respectively).

This indicator also went down among clostridia to 0.3Ѓ} 0.2 units (in the control group 0.5 ± 0.1 units; p <0.05), Klebsiella to 0.2 ± 0.7 units. (in the control group 0.5 ± 0.7 units; p <0.05) and proteases to 0.3 ± 0.2 units (in the control group 0.6 ± 04 units; p <0.05). These indicators indicate contagiousness abnormality of major symbionts of intestinal microflora - bifidobacteria, lactobacilli, bacteroids and Escherichia, which leads to the release of ecological niches that are successfully occupied by additional flora. The hierarchical structure evaluation of intestinal microbiocenosis among foundry workers revealed a decrease in the floristic value for obligate flora and an increase for opportunistic microorganisms (Fig. 2).

The study of the micro-landscape of foundry workers intestines showed a certain pattern - dysbiotic changes of bacterial endoflora were accompanied by the detection of protozoa B. hominis in fecal masses. Among 137 workers, 105 of them had (76.6 ± 0.3%) had blastocysts in the fecal masses, while in the group of comparison this indicator did not exceed 5%. Three forms of protozoa B.hominis was found: vacuolar, granular, and amoeboid. Vacuolar forms prevailed both in the examined material (71.4 ± 0.6%) and in cultures (56.2 ± 0.6%). Granular forms were found more often in cultures (26.7 ± 0.4%) than in feces (10.5 ± 0.3%). Amoeboid forms were detected only in culture (5.7 ± 0.3%). The study of parasitological landscape of the intestines of the workers examined revealed along with B.hominis (105 people), other protozoa availability. So, among 18 people (13.14 ± 0.4%) with blastocysts, lamblia was found in feces, among 4 people (3 ± 0.3%) amoeba were found.

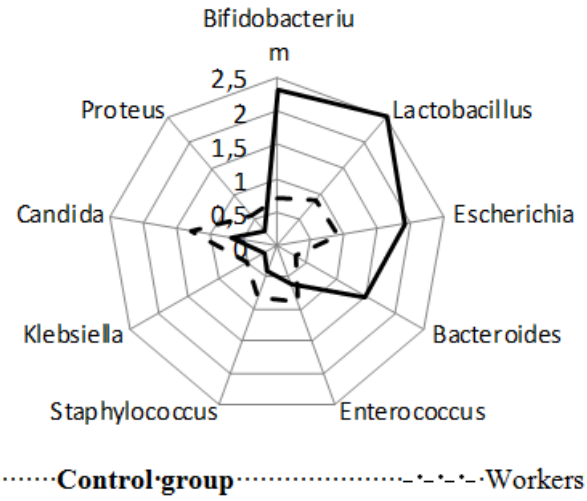

Fig. 2. Indicators of floral significance of intestinal microbiocenosis representatives.

B. hominis pathogenicity identification revealed that not all blastocyst isolates 3 had pathogenic properties. Only 86.7 ± 0.7% of the isolated protozoan strains caused death of mice, besides that, the degree of manifestation of the different strains pathogenicity was not the same. The value of its indicators correlated with work experience in the foundry (table. 3).

Table 3. The value of L. D50 (lg) B.hominis isolated among workers with different work experience.

Groups	Work experience (years)	Number of isolates (abs)	Number of isolates (%)	Value of LD50 (lg)	Correlation coefficient (r)
1	less 1	8	8.8	3.4±0.6	0.97*
2	1-5	22	24.2	3.8±0.7	0.83*
3	5-10	26	28.6	4.4±0.6	0.92*
4	10-20	34	37.4	5.7±0.5	0.85*
*Differences from the control are reliable (P<0.05)					

The largest number of cultures with evident pathogenicity was isolated among people with long work experience at the foundry: from 5 or more years. Blastocysts isolated among workers with 1-5 year- and less than 1 year experience had significantly lower rates of pathogenicity. The research to reveal anti-interferon activity (AIA) in 105 strains of the protozoa B. hominis isolated from the feces of workers in contact with foundry hazards was done to study persistent properties that are aimed at the degradation of host resistance mechanisms.

The studies showed that anti-interferon activity is quite widely represented among protozoa B.hominis. Out of 105 blastocyst strains, 74 (70.5 ± 0.6%) had AIA. The level of AIA among B. hominis varied. To analyze persistent characteristics of blastocysts, 3 groups of protozoa were identified: the first included strains with a low level of AIA - 0-1 units, the second group had an

average level - 1.1-2.0 units, and the third group had a high level - 2.1-3.0 units.

Further research showed that AIA level of blastocysts strains isolated correlated with indicators of blastocysts pathogenicity. (Table 4)

Table 4. AIA level indicators of blastocys with different pathogenicity.

№	Pathogenicity (Lg LD50)	Total number of strains	Number of AIA strains			Correlation coefficient (r)
			AIA 0-1,0	AIA 1,1-2,0	AIA 2,1-3,0	
1	3.6±0.2 – 4.1±0.7	12	6	1	----	0.99**
2	4.1±0.7 – 4.9±0.3	24	9	6	----	0.73*
3	4.9±0.3 – 5.8±0.2	32	12	7	4	0.95*
4	5.8±0.2 – 6.4±0.5	37	14	6	9	0.87**
*Differences from the control are reliable (P<0.05) **Differences from the control are reliable (P<0.01)						

Besides that, AIA of blastocysts isolated in workers was in direct correlation with the duration of industrial hazards effect on the health of the examined. For those with work experience of less than 1 year, the level of AIA was low and amounted to (0-1.1 units), while for the examined workers of group 4 with work experience of 10-20 years and more, this indicator increased to 2.1-3, 0 units.

4 Conclusion

It is known that foundries are characterized not only by complex environmental conditions of the working area, but also by the evidence of toxic aerial effluents, wastewater and solid waste.

In research papers there are a lot of facts on working environment factors effect on workers health, however, little attention is paid to the matters of integrated effects of the entire range of foundry factor. Moreover, there is almost no data on the effect of the entire complex of adverse foundry working conditions on the intestinal microbiota of workers. In turn, the important role of microbiocenosis of the gastrointestinal tract in the human body is well known. The intestinal microflora plays a great role in increasing the immune reactivity of the body, stimulating the lymphoid apparatus, synthesizing immunoglobulins, increasing the activity of lysozyme, and promotes reducing the permeability of vascular tissue barriers for pathogenic microorganisms toxic products. As a result of immune processes activation, normoflora contributes to atypical body cells killing.

This paper shows the role of the man-made load of foundry engineering on the intestinal microbiota of workers, which is manifested in decline of the dominance of the major symbionts and increasing the incidence degree of transient microflora. The revealed changes were directly related to the work experince of the examined.

The experimental data revealed the decline of the dominance and floral significance of the major symbionts secondary to the quantitative increase in representatives of opportunistic flora. This indicates a large degree of violations of biocenotic relations in the studied biotope.

Dysbiotic changes in the intestinal microflora of workers were accompanied by the detection of protozoa Blastocystis spp in the feces. The degree of pathogenicity of blastocyst isolates was determined. Its extent correlated with the duration of contact with environmentally adverse conditions of the in-plant environment. Blastocystis spp. anti-interferon activity was studied for the first time in. The research has shown that the protozoa isolated in foundry workers have a greater ability to persist, which largely determines their pathogenicity. The data obtained in this study expand the understanding of persistence factors role of Blastocystis spp. in the formation of microbial biocenoses of human intestine and allow us to understand the mechanisms of their pathogenicity.

Thus, the whole range of measures that have been taken over recent decades, both in our country and abroad, to keep up and improve normal working conditions in foundry engineering, haven't not yet show high results to reduce risks of various diseases.

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