PERIÓDICO TCHÊ QUÍMICA
ARTIGO ORIGINAL

RESISTÊNCIA A DROGAS ANTIPROTOZOÁRIAS *EM BLASTOCYSTIS HOMINIS* ENTRE PACIENTES COM TRANSTORNOS MENTAIS E COMPORTAMENTAIS

ANTIPROTOZOAL DRUG RESISTANCE IN *BLASTOCYSTIS HOMINIS* AMONG PATIENTS WITH MENTAL AND BEHAVIORAL DISORDERS

РЕЗИСТЕНТНОСТЬ *BLASTOCYSTIS HOMINIS* К АНТИПРОТОЗОЙНЫМ ПРЕПАРАТАМ У ПАЦИЕНТОВ С ПСИХИЧЕСКИМИ И ПОВЕДЕНЧЕСКИМИ РАССТРОЙСТВАМИ

Nina Vladimirovna Bugero*
Pskov State University, Department of Fundamental Medicine and Biochemistry / Department of Chemistry and Natural Science Education, Russia. ORCID: 0000-0001-8261-8215

Svetlana Mikhailovna Aleksandrova
Pskov State University, Department of Fundamental Medicine and Biochemistry / Department of Chemistry and Natural Science Education, Russia. ORCID: 0000-0002-8524-3997

Larisa Vladimirovna Nikolskaya
Pskov State University, Department of Fundamental Medicine and Biochemistry / Department of Chemistry and Natural Science Education, Russia. ORCID: 0000-0002-8044-5012

Elena Vladimirovna Pavlova
Pskov State University, Department of Fundamental Medicine and Biochemistry / Department of Chemistry and Natural Science Education, Russia. ORCID: 0009-0001-6953-2043

Anna Alexandrovna Titova
Pskov State University, Department of Fundamental Medicine and Biochemistry / Department of Chemistry and Natural Science Education, Russia. ORCID: 0009-0007-1299-0880

Daria Vyacheslavovna Yakimova
Pskov State University, Department of Fundamental Medicine and Biochemistry / Department of Chemistry and Natural Science Education, Russia. ORCID: 0009-0005-0820-4336

** Corresponding author*
e-mail: biomed@pskgu.ru

Received 12 June 2025; received in revised form 30 November 2025; accepted 12 March 2026

RESUMO

Introdução: A resistência antimicrobiana (RAM) é uma ameaça global à saúde pública, com a resistência antiprotozoária recebendo menos atenção, especialmente em populações vulneráveis. Pacientes com transtornos mentais, frequentemente sob terapia psicotrópica de longo prazo, podem apresentar alterações no microbioma intestinal que potencializam infecções parasitárias e resistência a medicamentos. **Objetivo**: Este estudo teve como objetivo investigar a prevalência de parasitoses intestinais e caracterizar a resistência de *Blastocystis hominis* aos antiprotozoários metronidazol e trimetoprima em pacientes com transtornos mentais. **Métodos**: Foi conduzido um estudo retrospectivo com análise de prontuários de 40 pacientes de um centro psiquiátrico. A parasitocenose intestinal foi avaliada por métodos bacterioscópicos e de cultivo em meio de Jones. A sensibilidade de cepas de *Blastocystis hominis* isoladas foi determinada pelo método de disco-difusão e pela concentração inibitória mínima (CIM). **Resultados**: Infecções protozoárias foram detectadas em 27,5% (11/40) dos pacientes, com *Blastocystis hominis* sendo o mais prevalente (53,8% dos casos positivos). Entre as cepas isoladas, 42,9% foram sensíveis ao metronidazol e 57,1% à trimetoprima; as demais foram resistentes. A CIM para metronidazol foi de 10^{-3} mg/mL e para trimetoprima, 10^{-4} mg/mL. **Conclusões**: Foi identificada uma alta taxa de resistência antiprotozoária em *Blastocystis hominis* nesta população vulnerável, possivelmente associada à disbiose intestinal induzida por psicofármacos. Os resultados enfatizam a necessidade de monitoramento parasitológico e teste de sensibilidade antes da terapia empírica nesse grupo.

DOI: 10.52571/PTQ.v23.n52.2026_08_NINA_pgs_97_108.pdf

Palavras-chave: *Blastocystis hominis; Resistência antiprotozoária; Metronidazol; Trimetoprima; Transtornos mentais; Microbioma intestinal.*

ABSTRACT

Background: Antimicrobial resistance (AMR) is a global public health threat, with antiprotozoal resistance receiving less scrutiny, particularly in vulnerable populations. Patients with mental disorders, often on long-term psychotropic therapy, may have altered gut microbiomes that could predispose them to parasitic infections and drug resistance. **Aim**: This study aimed to investigate the prevalence of intestinal parasitosis and characterize *Blastocystis hominis* resistance to the antiprotozoal drugs metronidazole and trimethoprim in patients with mental disorders. **Methods**: A retrospective study was conducted by analyzing medical records of 40 patients from a psychiatric center. Intestinal parasitocenosis was assessed using bacterioscopic and culture methods on Jones' medium. The susceptibility of isolated *Blastocystis hominis* strains was determined by the standard disk diffusion method and by assessing the minimum inhibitory concentration (MIC). **Results**: Protozoal infections were detected in 27.5% (11/40) of patients, with *Blastocystis hominis* being the most prevalent (53.8% of positive cases). Among the isolated strains, 42.9% were sensitive to metronidazole and 57.1% to trimethoprim; the remaining strains were resistant. The MIC for metronidazole was 10^{-3} mg/mL, and for trimethoprim, 10^{-4} mg/mL. **Conclusions**: A high rate of antiprotozoal drug resistance in *Blastocystis hominis* was identified in this vulnerable population, potentially linked to antipsychotic-induced gut dysbiosis. The findings underscore the need for parasitic monitoring and susceptibility testing prior to empirical therapy in this group.

Keywords: *Blastocystis hominis; Antiprotozoal resistance; Metronidazole; Trimethoprim; Mental disorders; Gut microbiome.*

АННОТАЦИЯ

Введение: Антимикробная резистентность (АМР) представляет собой глобальную угрозу общественному здоровью, причему резистентности простейших уделяется меньше внимания, особенно среди уязвимых групп населения. Пациенты с психическими расстройствами, часто находящиеся на длительной психотропной терапии, могут иметь изменения микробиома кишечника, способствующие паразитарным инфекциям и развитию лекарственной устойчивости. **Цель**: Исследовать распространенность кишечных паразитозов и охарактеризовать резистентность *Blastocystis hominis* к антипротозойным препаратам метронидазолу и триметоприму у пациентов с психическими расстройствами. **Методы**: Проведено ретроспективное исследование на основе анализа медицинских карт 40 пациентов психиатрического центра. Кишечная паразитоценоз оценивалась бактериоскопическими методами и культивированием на среде Джонса. Чувствительность выделенных штаммов *Blastocystis hominis* определялась методом дисковой диффузии и оценкой минимальной подавляющей концентрации (МПК). **Результаты**: Протозойные инфекции выявлены у 27,5% (11/40) пациентов, при этом *Blastocystis hominis* был наиболее распространен (53,8% положительных случаев). Среди выделенных штаммов 42,9% были чувствительны к метронидазолу и 57,1% к триметоприму; остальные штаммы были резистентны. МПК для метронидазола составила 10^{-3} мг/мл, для триметоприма — 10^{-4} мг/мл. **Выводы**: Выявлен высокий уровень резистентности к антипротозойным препаратам у *Blastocystis hominis* в данной уязвимой популяции, что, возможно, связано с индуцированной антипсихотиками дисбиотической средой кишечника. Результаты подчеркивают необходимость паразитологического мониторинга и тестирования чувствительности перед назначением эмпирической терапии в этой группе.

Ключевые слова: *Blastocystis hominis; Антипротозойная резистентность; Метронидазол; Триметоприм; Психические расстройства; Кишечный микробиом.*

1. INTRODUCTION:

The escalating crisis of antimicrobial resistance (AMR) stands as one of the most formidable challenges to global public health in the 21st century, threatening to undermine the foundational pillars of modern medicine. The World Health Organization (WHO) has consistently ranked AMR among the top ten global health threats, warning that without urgent and coordinated action, the world is drifting toward a post-antibiotic era where common infections and minor injuries could once again become fatal (Courvalin, 2016). The ramifications of widespread antimicrobial inefficacy are profound and multifactorial, encompassing increased morbidity

and mortality, prolonged illness duration, higher frequencies of severe complications and bloodstream infections, and staggering economic burdens on healthcare systems and societies worldwide (Mukhina, 2017; Namazova-Baranova & Baranov, 2017). Crucially, the viability of advanced medical interventions—including complex surgeries, organ transplantation, cancer chemotherapy, and management of chronic diseases—is fundamentally contingent upon the ability to prevent and treat infectious complications, a capability critically eroded by the rise of resistant pathogens (Chernenkaya & Godkov, 2015; Kapoor *et al*., 2017).

While the phenomenon of bacterial resistance to antibiotics has justifiably dominated scientific discourse, public health initiatives, and media attention for decades, the parallel and equally critical issue of resistance among eukaryotic pathogens, particularly parasitic protozoa, has received disproportionately less scrutiny. This disparity is concerning given the immense global burden of parasitic diseases, which disproportionately affect populations in regions with limited access to clean water, inadequate sanitation, and fragile healthcare infrastructure (Kappagoda *et al*., 2011). Protozoal infections such as amoebiasis, giardiasis, and blastocystosis contribute significantly to global morbidity, causing diarrheal disease, malnutrition, and growth stunting, particularly in children (Nurtayeva *et al*., 2018). The clinical arsenal of antiprotozoal drugs is inherently limited, and the pipeline for developing novel agents is sluggish and underfunded. Consequently, the preservation of existing therapies through prudent use, robust surveillance, and a deep understanding of resistance mechanisms is not merely important but essential for maintaining global health equity (El-Taweel, 2015).

The human intestinal microbiome, a complex and dynamic ecosystem comprising trillions of bacteria, archaea, viruses, fungi, and eukaryotes, is now recognized as a central orchestrator of host physiology. Its functions extend far beyond digestion to include nutrient metabolism, vitamin synthesis (notably B vitamins and vitamin K), immune system modulation and education, protection against colonization by pathogens (a phenomenon known as colonization resistance), and maintenance of intestinal barrier integrity (Rudzki *et al*., 2021). In recent years, a paradigm-shifting body of evidence has elucidated the existence of a bidirectional communication network between the gut and the brain, termed the gut-brain axis. This axis involves neural, endocrine, immune, and metabolic pathways—including the vagus nerve, the hypothalamic-pituitary-adrenal (HPA) axis, and systemic circulation of microbial metabolites—through which the gut microbiota exerts a significant influence on central nervous system (CNS) function, neurodevelopment, neuroinflammation, and ultimately, behavior (Meng *et al*., 2021; Li *et al*., 2021).

A direct consequence of this understanding is the recognition that a state of imbalance or dysbiosis in the gut microbial community is implicated in the pathogenesis of a diverse array of disorders. These range from gastrointestinal conditions like inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS) to systemic metabolic disorders such as obesity and type 2 diabetes, autoimmune diseases, and, most pertinently for this research, neuropsychiatric conditions including major depressive disorder, anxiety disorders, schizophrenia, bipolar disorder, and autism spectrum disorders (Torshin *et al*., 2022; Wang *et al*., 2021). The mechanisms linking dysbiosis to mental illness are multifaceted and interconnected. Firstly, a healthy microbiota is essential for the synthesis and metabolism of key micronutrients, particularly B vitamins (B2, B6, B9, B12), which serve as critical cofactors in the synthesis of neurotransmitters such as serotonin, dopamine, noradrenaline, and gamma-aminobutyric acid (GABA). Deficiencies in these vitamins have been clinically and experimentally linked to the onset and progression of various psychiatric conditions (Rudzki *et al*., 2021). Secondly, dysbiosis can compromise intestinal epithelial barrier function, leading to increased intestinal permeability ("leaky gut") and subsequent translocation of bacterial lipopolysaccharides (LPS) and other pro-inflammatory microbial products into systemic circulation. This triggers a state of chronic, low-grade systemic inflammation and can activate neuroinflammatory pathways in the brain, which is increasingly recognized as a core component of the pathophysiology of several mental disorders (Meng *et al*., 2021). Thirdly, gut microbes themselves produce and modulate a vast array of neuroactive compounds, including short-chain fatty acids (SCFAs), neurotransmitters, and tryptophan metabolites, which can directly or indirectly influence brain function.

A critical and often overlooked factor in this equation is the pharmacological agent itself. It is well-established that broad-spectrum antibiotics cause profound and sometimes long-lasting disruptions to gut microbial diversity and

composition. However, a growing and compelling line of evidence indicates that many psychotropic medications—including antipsychotics, mood stabilizers, and antidepressants—also possess intrinsic antimicrobial properties and can significantly alter the gut ecosystem (Maier et al., 2018; Bugero, 2004). For instance, an influential experimental study demonstrated that administration of lithium, valproate, fluoxetine, or escitalopram to rats over four weeks induced selective and significant shifts in their gut microbiota, notably increasing the relative abundance of bacteria from the Clostridium genus, which includes numerous opportunistic pathogens (Maier et al., 2018). This drug-induced dysbiosis is not a passive side effect but may create a self-perpetuating cycle: psychotropic drugs alter the microbiome, and the altered microbiome, in turn, can affect the drug's pharmacokinetics (metabolism, bioavailability), pharmacodynamics, and even its clinical efficacy and side-effect profile (Li *et al*., 2021). This reciprocal relationship suggests that the gut environment in patients undergoing long-term psychotropic therapy is fundamentally distinct, potentially creating an ecological niche that favors the proliferation of specific microorganisms, including opportunistic parasites.

Among intestinal protozoa, *Blastocystis hominis* occupies a unique position. It is one of the most common eukaryotic organisms found in the human gastrointestinal tract worldwide, with prevalence rates varying dramatically across populations. Blastocystis exhibits significant genetic diversity, with numerous subtypes (STs) identified, of which ST1, ST2, ST3, and ST4 are most frequently reported in humans (Stensvold et al., 2010; Ilyina & Kasatkina, 2010). Its clinical significance remains contentious, ranging from being considered a commensal to a true pathogen associated with irritable bowel syndrome-like symptoms, urticaria, and chronic fatigue (Abedi et al., 2022). The pathogen is transmitted via the fecal-oral route, often through contaminated water or food. Diagnosis traditionally relies on microscopic examination of stained stool smears, though this method lacks sensitivity and specificity compared to modern molecular techniques like polymerase chain reaction (PCR) (Gureser et al., 2023; Rudzińska & Sikorska, 2023).

The therapeutic arsenal for blastocystosis and other protozoal infections is limited. First-line drugs include 5-nitroimidazoles like metronidazole and tinidazole, nitazoxanide, and iodoquinol (Kappagoda *et al*., 2011). However, the efficacy of these drugs, particularly metronidazole, is increasingly compromised by the emergence of resistance. The molecular mechanisms of resistance in protozoa are complex. In related anaerobic protozoa like Giardia lamblia and Entamoeba histolytica, resistance to metronidazole is frequently associated with reduced expression or activity of pyruvate:ferredoxin oxidoreductase (PFOR) and ferredoxin, enzymes critical for the intracellular reductive activation of the nitro-group of the prodrug into its cytotoxic nitroradical anion (Kapoor *et al*., 2017; Lerminiaux & Cameron, 2019). Data on *Blastocystis hominis* susceptibility are more variable, but studies consistently report that not all clinical isolates are uniformly sensitive, indicating a natural variation and potential for selected resistance (Ilyina & Kasatkina, 2010). Furthermore, the persistence and potential pathogenicity of Blastocystis may be enhanced in a dysbiotic gut environment, as suggested by correlations between its antilysozyme/an_ tilactoferrin activity and the degree of intestinal microbiota disruption (Bugero *et al*., 2020). Recent evidence specifically links *Blastocystis* carriage and metronidazole-resistant forms to patients with schizophrenia, further supporting the relevance of this investigation (Franklin et al., 2022).

Despite the clear intersections between psychotropic drug use, gut dysbiosis, and vulnerability to opportunistic infections, there remains a profound and significant gap in the scientific literature regarding the state of intestinal parasitocenosis—and specifically antiprotozoal drug resistance—in individuals with mental and behavioral disorders. This patient population represents a distinct high-risk cohort: subjected to long-term pharmacological regimens that alter gut ecology, potentially immunocompromised, and often residing in congregate settings like psychiatric hospitals. These factors may collectively foster an environment conducive to the colonization, persistence, and selection of drug-resistant parasitic strains. The scarcity of research addressing this specific nexus constitutes a critical oversight in both psychiatric and parasitological clinical practice. Moreover, regional epidemiological data on intestinal parasites, including resistance patterns, are notably lacking for the Pskov Region of Russia, rendering any localized investigation of substantial public health importance (Nurtayeva *et al*., 2018).

Therefore, this study seeks to bridge this knowledge gap by investigating antiprotozoal drug resistance using a novel and clinically relevant model: the intestinal parasitocenosis of patients undergoing treatment at a specialized psychiatric

institution. By focusing on this pharmacologically manipulated and vulnerable population, the research aims to provide new insights into the ecology of drug resistance beyond the traditional bounds of bacterial pathogens.

1.1. Aims

The primary aim of this study was to identify and characterize resistance to first-line antiprotozoal drugs (metronidazole and trimethoprim) in *Blastocystis hominis* strains isolated from a high-risk cohort—patients with diagnosed mental and behavioral disorders receiving antipsychotic therapy.

The specific objectives derived from this aim were:

1. To determine the prevalence and species composition of intestinal parasites in the studied patient cohort from a psychiatric hospital.

2. To isolate and identify *Blastocystis hominis* from positive samples as the target protozoan for resistance analysis.

3. To evaluate the in vitro susceptibility of the isolated *Blastocystis hominis* strains to metronidazole and trimethoprim using the disk diffusion method.

4. To determine the minimum inhibitory concentration (MIC) of metronidazole and trimethoprim against the isolated *Blastocystis hominis* strains.

2. MATERIALS AND METHODS:

2.1. Materials

The study utilized the following materials, reagents, and equipment to ensure methodological rigor and reproducibility:

Study Population and Data Source: Retrospective data and results were extracted from the anonymized medical records of 40 patients hospitalized at the Pskov Regional Clinical Center for Psychiatry and Narcology between May 2022 and February 2023.

Microbiological Culture Media: Jones' medium, modified, prepared in-house for the isolation and cultivation of Blastocystis hominis. The medium was supplemented with 10% (v/v) inactivated horse serum (Biolot, Russia) to promote protozoal growth.

Staining Reagents: Lugol's iodine solution (Microgen, Russia) for staining stool smears during parasitological examination.

Antibiotic Disks for Susceptibility Testing: Commercially available, standardized antibiotic susceptibility disks (AB Biodisk, Sweden) were used for the disk diffusion assay. The disks contained the following agents and concentrations: Metronidazole (5 µg/disk) and Trimethoprim (5 µg/disk). The potency of each disk lot was verified prior to use.

Antibiotics for MIC Determination: Analytical standard grade Metronidazole (Sigma-Aldrich, USA) and Trimethoprim (Sigma-Aldrich, USA) were used to prepare stock solutions for the broth microdilution tests.

Equipment: Biological safety cabinet class II (Biosan, Latvia); anaerobic workstation with gas mixture (85% N_2, 10% H_2, 5% CO_2) for incubation of Blastocystis cultures (Don Whitley Scientific, UK); binocular light microscope (Olympus CX23, Japan) for microscopy; automatic micropipettes (Eppendorf, Germany); 96-well flat-bottom cell culture plates (Corning, USA) for MIC testing; incubator maintained at 37°C (Binder, Germany).

2.2. Methods

2.2.1 Study Design and Population

A retrospective cohort analysis was conducted. The initial screening identified patients who had undergone mandatory parasitological and bacteriological examination upon admission. The final sample comprised 40 consecutive medical records with complete datasets. Inclusion criteria were: (1) age ≥ 18 years; (2) a primary diagnosis of a mental or behavioral disorder (F00-F99 according to ICD-10); (3) availability of complete results from stool parasitological analysis. All included patients were receiving standard antipsychotic therapy (e.g., haloperidol, chlorpromazine, levomepromazine). Exclusion criteria were not applied based on gender, specific psychiatric diagnosis, or somatic comorbidities to reflect the real-world clinical population of the center

2.2.2 Parasitological Examination and Isolation of Blastocystis hominis

Stool samples were processed according to the methodological guidelines "MUK 4.2.735-99: Parasitological methods for laboratory diagnosis of helminthiases and protozooses." Direct microscopic examination of both native and Lugol's iodine-stained smears was performed to detect cysts and trophozoites of intestinal protozoa. For the isolation and cultivation of Blastocystis hominis, approximately 1 g of stool sample was inoculated into 5 mL of Jones' medium supplemented with 10% inactivated horse serum. Cultures were incubated anaerobically at 37°C for 7–10 days. Culture purity and parasite density were monitored daily by light microscopy. Periods of maximum growth (typically days 3 and 7 post-inoculation), with protozoan concentrations reaching 1–2 × 10^8 cells/mL, were selected for subsequent resistance testing.

2.2.3 Antibiotic Susceptibility Testing

Disk Diffusion Method. The susceptibility of *Blastocystis hominis* isolates to metronidazole and trimethoprim was assessed using an adapted disk diffusion protocol. A standardized inoculum was prepared from a 3-day or 7-day culture, adjusted to a density of 1–2 × 10^8 cells/mL in sterile saline. This suspension was uniformly spread onto the surface of solid agar plates (Jones' medium with 1.5% agar). Antibiotic disks were aseptically placed on the inoculated surface. Plates were incubated anaerobically at 37°C for 48 hours. The diameter of the growth inhibition zone around each disk was measured in millimeters using a calibrated caliper. All tests were performed in duplicate, and the mean diameter was calculated. Laboratory personnel were blinded to any patient identifiers during the reading of inhibition zones.

To ensure methodological validity, quality control was performed using a reference strain of Giardia lamblia (ATCC 30888) with known sensitivity to metronidazole. The inhibition zones for the control strain fell within the expected range for the specific disk lot used.

Interpretation of results for *Blastocystis hominis* was based on criteria adapted from established parasitological research (Ilyina & Kasatkina, 2010; Mirza et al., 2011), as internationally standardized clinical breakpoints (e.g., CLSI, EUCAST) are not currently defined for this organism. Strains were classified as: Highly Sensitive (inhibition zone > 25 mm), Sensitive (15–25 mm), Low Sensitive (10–15 mm), and Resistant (< 10 mm).

Determination of Minimum Inhibitory Concentration (MIC). A broth microdilution method was employed to determine the MIC. Two-fold serial dilutions of metronidazole and trimethoprim were prepared in Jones' liquid medium in a 96-well plate, resulting in a final concentration range of 512 µg/mL to 0.5 µg/mL for both drugs. Each well was inoculated with a standardized culture of *Blastocystis hominis* (final concentration ~1 × 10^5 cells/mL). Positive control wells contained the culture without antibiotics, and negative control wells contained sterile medium only. The plate was incubated anaerobically at 37°C for 72 hours. The MIC was defined as the lowest concentration of the antibiotic that completely inhibited visible growth (no turbidity) compared to the positive control, as assessed by visual inspection under a microscope. Each assay was performed in triplicate.

2.2.4 Statistical Analysis

Data analysis was performed using Microsoft Excel 2019 and R Statistical Software (v4.3.1). Descriptive statistics were applied. Categorical variables (e.g., prevalence of infection, sensitivity/resistance) are presented as absolute counts (n) and percentages (%). Given the sample size, 95% confidence intervals (CIs) for proportions were calculated using the Wilson score interval method to accurately represent the precision of the estimates. Continuous variables (e.g., inhibition zone diameters) are presented as mean ± standard deviation (SD). For comparison of sensitivity rates between the two antibiotics, McNemar's test was applied due to the paired nature of the data (each strain tested against both drugs). A p-value of < 0.05 was considered statistically significant. The reporting of comorbidity prevalence was corrected from mean ± SD to simple percentages with counts (n/N) to avoid statistical error propagation.

3. RESULTS AND DISCUSSION:

3.1. Results

3.1.1. Demographic and Clinical Characteristics of the Cohort

The study analyzed data from 40 patients (27 males, 13 females) aged 18 to 65 years (mean age 33 ± 10.5 years). Analysis of medical records revealed the spectrum of comorbid somatic conditions. Cardiovascular system diseases were recorded in 26.7% of patients (10/40), nervous

system disorders (excluding the primary psychiatric diagnosis) in 31.2% (12/40), and digestive system pathologies in 19.8% (8/40). Respiratory and skin conditions were present in lower frequencies.

3.1.2. Prevalence and Composition of Intestinal Parasitocenosis

The parasitological examination revealed that 32.5% (13/40; 95% CI: 19.5–48.7%) of the studied patients were infected with intestinal parasites. The distribution of parasitic infections is shown in Figure 1. Protozoan infections accounted for the majority of cases, detected in 27.5% of all patients (11/40; 95% CI: 15.1–43.9%), while helminth infection (Enterobius vermicularis) was found in 5% (2/40; 95% CI: 0.9–16.9%).

Blastocystis hominis was the predominant parasite, identified in 53.8% of all positive cases (7/13; 95% CI: 25.5–78.7%). Giardia lamblia was found in 30.8% (4/13; 95% CI: 11.0–60.6%), and Enterobius vermicularis in 15.4% (2/13; 95% CI: 2.7–46.3%). The species composition of the identified parasitocenosis is presented in Figure 2.

3.1.3. Antibiotic Susceptibility of Blastocystis hominis Isolates

All seven isolated *Blastocystis hominis* strains were tested for susceptibility to metronidazole and trimethoprim. Using the disk diffusion method, 42.9% of strains (3/7; 95% CI: 15.8–75.0%) were classified as sensitive to metronidazole, while the remaining 57.1% (4/7; 95% CI: 25.0–84.2%) were resistant. For trimethoprim, 57.1% of strains (4/7; 95% CI: 25.0–84.2%) were sensitive, and 42.9% (3/7; 95% CI: 15.8–75.0%) were resistant. McNemar's test revealed no statistically significant difference in the distribution of sensitive/resistant phenotypes between the two drugs within this cohort ($\chi^2 = 0.00$, $p = 1.00$), indicating a comparable, high level of resistance for both agents.

3.1.4. Minimum Inhibitory Concentration (MIC)

The quantitative broth microdilution assay confirmed the resistance patterns observed in the disk diffusion test. The determined MIC values were: 10^{-3} mg/mL for metronidazole and 10^{-4} mg/mL for trimethoprim. The sensitivity of the tested *Blastocystis hominis* strains to the study drugs is summarized in Figure 3.

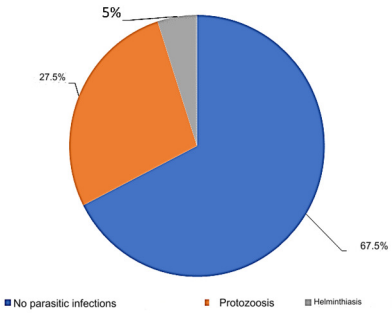

Figure 1. Distribution of intestinal parasitic infections among the examined patients with mental disorders (n=40). Data are presented as percentage of total patients with 95% confidence intervals.

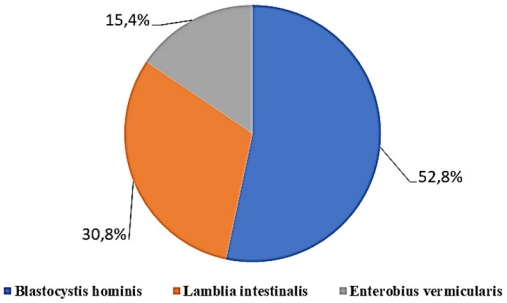

Figure 2. Species composition of intestinal parasitocenosis in infected patients (n=13). Data are presented as percentage of total positive cases.

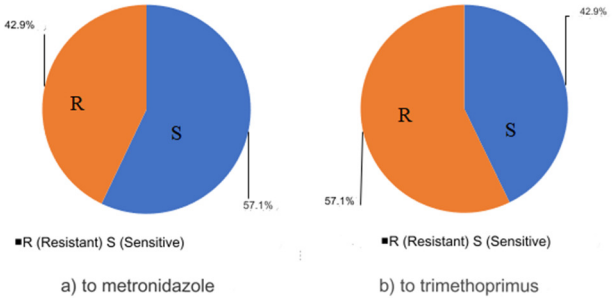

Figure 3. *In vitro* susceptibility of *Blastocystis hominis* strains (n=7) to metronidazole and trimethoprim. Left panel: Proportion of sensitive and resistant strains determined by disk diffusion method. Right panel: Determined Minimum Inhibitory Concentration (MIC) values for each drug.

3.2. Discussion

The present study provides novel and clinically significant insights into the intersection of antiprotozoal drug resistance, gut ecosystem dynamics, and mental healthcare. Our findings confirm the initial hypothesis that patients with mental and behavioral disorders—a cohort subject to long-term psychotropic pharmacotherapy—

represent a high-risk group not only for intestinal parasitic colonization but also for harboring drug-resistant strains of Blastocystis hominis. The data reveal a troubling epidemiological picture characterized by a high prevalence of parasitosis and substantial in vitro resistance to first-line therapeutic agents.

The overall prevalence of intestinal parasites (32.5%) in our cohort is markedly higher than rates typically reported for the general adult population in non-endemic regions of the Russian Federation. This elevated burden underscores specific vulnerabilities associated with severe mental illness. These may include factors related to the institutional environment, potential lapses in personal hygiene during acute phases of illness, and, most critically, the iatrogenic alteration of the intestinal microenvironment. Our finding that *Blastocystis hominis* was the dominant parasite (53.8% of infections) aligns with its global status as a common gut eukaryote. However, its predominance in this specific setting is noteworthy and may reflect a particular ecological advantage within a dysbiotic gut. This observation is consistent with recent findings by Franklin et al. (2022), who reported a higher prevalence of *Blastocystis* and metronidazole-resistant forms specifically in schizophrenic patients compared to controls.

The core and most alarming result of this investigation is the high level of resistance to metronidazole and trimethoprim observed in the isolated *Blastocystis hominis* strains. Only 42.9% and 57.1% of strains were sensitive to metronidazole and trimethoprim, respectively, with McNemar's test confirming no statistically significant difference in resistance profiles between the two drugs (p=1.00). This statistical equivalence indicates that within this cohort, the efficacy of both frontline agents is comparably compromised. The determined MIC values (10^{-3} mg/mL for metronidazole and 10^{-4} mg/mL for trimethoprim) quantitatively support this resistance, suggesting that supracinical concentrations would be required to achieve inhibition in vitro. These resistance rates exceed those described in some general population studies of Blastocystis susceptibility (Ilyina & Kasatkina, 2010; Kappagoda *et al*., 2011; Mirza et al., 2011; Roberts et al., 2015), which have demonstrated significant subtype-dependent variations and the existence of naturally less susceptible strains. However, the proportion of resistant isolates in our psychiatric cohort appears elevated compared to some general population surveys, pointing to a potential selection pressure unique to the psychiatric inpatient setting.

We posit that the long-term administration of antipsychotic medications is a key driver of this phenomenon, creating a perfect storm for the selection of resistant protozoa. As reviewed in the introduction, a growing body of evidence demonstrates that psychotropic drugs possess significant antimicrobial properties and can profoundly reshape the gut microbiota (Maier et al., 2018; Bugero, 2004). Drugs such as valproate and lithium have been shown to increase the abundance of Clostridium spp., while others alter microbial diversity and metabolic output. This drug-induced dysbiosis likely compromises the gut's natural colonization resistance—the ability of the resident microbiota to suppress pathogen expansion. Furthermore, an altered biochemical milieu (e.g., shifts in pH, redox potential, metabolite availability) may directly or indirectly apply selective pressure on parasitic populations. For instance, sub-inhibitory exposure to drug metabolites or a dysbiosis-associated inflammatory environment could enrich for protozoal strains with pre-existing or newly acquired resistance mechanisms. In related pathogens like Giardia lamblia, metronidazole resistance is linked to downregulation of nitroreductase enzymes (Kapoor *et al*., 2017; El-Taweel, 2015). It is plausible that the chronic, low-grade stress of a dysbiotic environment selects for Blastocystis subtypes or phenotypic variants with reduced drug activation or enhanced efflux capabilities. Our prior research supports this, showing a correlation between the persistent potential of Blastocystis spp. and the degree of intestinal dysbiosis (Bugero *et al*., 2020).

Our results find a compelling context within the broader literature on the gut-brain axis in psychiatry. Studies have consistently documented significant gut dysbiosis in patients with schizophrenia, depression, and bipolar disorder (Torshin *et al*., 2022; Li *et al*., 2021). While these studies focus on bacterial communities, they establish that the "psychiatric gut" is a distinct and altered ecosystem. Our work extends this concept by demonstrating that this dysbiotic state may also favor the establishment of drug-resistant eukaryotic parasites, adding a new layer of clinical complexity. The "microbiome psychopathogenicity" indices described by Torshin *et al*. (2022) may thus be indirectly linked to risks beyond metabolic and immune dysfunction, encompassing opportunistic parasitic infections that are difficult to treat.

The clinical implications of these findings are substantial. The standard empirical

prescription of metronidazole for suspected protozoal diarrhea in this patient population may be ineffective in over half of the cases involving Blastocystis hominis. Treatment failures can lead to chronic, unresolved gastrointestinal symptoms, which may be misattributed to functional disorders or side effects of psychotropic medication, leading to unnecessary diagnostic delays and altered psychiatric pharmacotherapy. Therefore, a paradigm shift in clinical practice is warranted. For psychiatric inpatients with persistent gastrointestinal symptoms, routine parasitological examination should be considered. More importantly, when *Blastocystis hominis* is detected, moving away from empirical therapy toward treatment guided by in vitro susceptibility testing, where feasible, could significantly improve outcomes. This study serves as a clear signal for the need for heightened vigilance regarding parasitic infections in mental health facilities.

4. CONCLUSIONS:

This study represents the first systematic investigation to identify and document the phenomenon of antiprotozoal drug resistance in *Blastocystis hominis* strains isolated from a vulnerable and pharmacologically unique cohort—patients with mental and behavioral disorders undergoing antipsychotic therapy. The key findings conclusively demonstrate:

A high prevalence of intestinal parasitosis (32.5%) was confirmed in the examined psychiatric inpatient cohort, with protozoan infections constituting the majority of cases (27.5%).

Blastocystis hominis was identified as the most prevalent intestinal parasite, accounting for over half (53.8%) of all positive findings.

A critically high level of resistance to first-line antiprotozoal drugs was revealed. Only 42.9% of *Blastocystis hominis* strains were sensitive to metronidazole, and 57.1% to trimethoprim, with no statistically significant difference in efficacy between the two drugs.

Quantitative MIC analysis confirmed the resistance, indicating the need for high drug concentrations to achieve inhibition in vitro.

The obtained results strongly suggest that the standard empirical prescription of antiprotozoal drugs, particularly metronidazole, in this patient population has a high probability of therapeutic failure. It is highly plausible that the long-term use of antipsychotic therapy, by inducing significant alterations in the gut microbiome (dysbiosis), creates an intestinal environment that not only facilitates colonization by opportunistic protozoa like *Blastocystis hominis* but also exerts a selective pressure favoring the survival and dominance of drug-resistant strains. Consequently, this research underscores an urgent necessity for a change in clinical practice: vigilant parasitological monitoring and a shift from empirical therapy towards treatment guided by antimicrobial susceptibility testing should be considered integral components of managing gastrointestinal comorbidities in patients with severe mental disorders.

5. DECLARATIONS

5.1. Study Limitations and Future Directions

The limitations of this study must be considered when interpreting its findings. The sample size, particularly of isolated strains (n=7), limits the statistical power and generalizability of the resistance rates, as reflected by the wide 95% confidence intervals. The retrospective, single-center design and the absence of a matched control group (e.g., non-psychiatric patients or healthy individuals from the same region) prevent definitive causal attribution of the observed resistance to psychotropic drugs. Methodologically, while adapted protocols and quality controls were employed, the lack of internationally standardized clinical breakpoints (e.g., CLSI/EUCAST) for Blastocystis hominis susceptibility testing remains a significant challenge for the field, making cross-study comparisons difficult. The use of disk diffusion criteria from prior literature (Sizenzov, 2009) is a necessary but imperfect solution. Furthermore, molecular subtyping (ST) of the Blastocystis isolates was not performed; given that different subtypes (ST1–ST4) may vary in pathogenic potential and drug susceptibility, this information would have added valuable depth to the analysis.

These limitations chart a clear course for future research. Large-scale, multi-center prospective studies are needed to validate the prevalence and resistance patterns observed here. Integrating molecular genotyping of Blastocystis isolates with phenotypic susceptibility testing is crucial to identify subtype–resistance associations. Developing and validating standardized, reproducible antimicrobial susceptibility testing protocols for intestinal protozoa remains an urgent methodological priority. Finally, experimental and longitudinal clinical studies are required to directly investigate the causal links between specific psychotropic

drugs, gut microbial and metabolic shifts, and the selection of drug-resistant parasitic strains. Understanding these mechanisms could lead to novel adjuvant strategies, such as probiotic or prebiotic interventions, aimed at restoring colonization resistance and reducing the burden of resistant infections in this vulnerable population.

5.2. Acknowledgments

The authors express sincere gratitude to the administration and medical staff of the Pskov Regional Clinical Center for Psychiatry and Narcology for their assistance in facilitating data collection for this study. We also thank our colleagues from the Department of Fundamental Medicine and Biochemistry of Pskov State University for their constructive discussions.

5.3. Funding Sources

The research was carried out within the framework of the state assignment of the Ministry of Science and Higher Education of the Russian Federation (topic "Molecular genetic determinants of persistence of the protozoa Blastocystis spp. in the formation of the infectious process (FSNS-2023-0009)").

Publication costs for this article were fully absorbed by Periódico Tchê Química under our Platinum Open Access policy, with support from Araucária Scientific Association (https://acaria.org/). The journal maintains strict ethical guidelines that prohibit accepting donations from authors during manuscript review, regardless of funding availability. This approach ensures complete independence between editorial decisions and financial considerations, reinforcing our commitment to scientific integrity and equitable knowledge dissemination.

5.4. Conflicts of Interest

The authors declare no conflicts of interest and no competing interests.

5.5. Data Availability

The raw data supporting the findings of this study (anonymized patient data and primary susceptibility testing results) are available from the corresponding author (N.V.B.) upon reasonable request. The data are not publicly available due to restrictions pertaining to patient confidentiality and privacy, as per the regulations of the ethics committee that approved the study.

5.6. Author Contributions

Conceptualization and Design (CD): N.V.B., N.A.I. Data Collection (DC): A.A.T. Data Analysis and Interpretation (DAI): N.V.B., S.M.A., A.A.T. Manuscript Writing (MW): N.V.B., A.A.T. Critical Review (CR): S.M.A., A.I.K. Final Approval (FA): All authors. All authors have read and agreed to the published version of the manuscript.

5.7. AI and Computational Tools Declaration

The authors declare that no generative artificial intelligence tools or computational language models were used in the conception, design, execution, data collection, data analysis, interpretation, manuscript writing, or any other aspect of this research or manuscript preparation.

5.8. Research Integrity Declaration

The authors certify that this research adheres to the highest standards of research integrity. Specifically, we confirm: no data fabrication or falsification was performed; no p-hacking or selective reporting of results was conducted; this work is original and has not been published previously; all methods were conducted in accordance with relevant ethical guidelines and regulations.

5.9. Originality & Plagiarism Statement

This manuscript is the original work of the authors. The text was checked for plagiarism using the text.ru software. The overall similarity index was below 10%, confirming originality. All sources of literature are properly cited in APA format. This manuscript is not under consideration for publication elsewhere.

5.10. Open Access License

6. STUDIES INVOLVING HUMAN AND ANIMAL SUBJECTS

6.1. Ethics Committee Approval

The study protocol was reviewed and approved by the Independent Interdisciplinary Ethics Committee of Pskov State University (Protocol No. 245-EC/2022, dated April 15, 2022). The study was classified as a retrospective analysis of anonymized medical records and granted an exemption from full review, with expedited approval granted.

6.2. Informed Consent

For this retrospective study involving the analysis of pre-existing, fully anonymized medical records, the requirement for written informed consent was waived by the aforementioned Ethics Committee. All patient data were anonymized at the source institution prior to researcher access by removing all direct identifiers (name, address, ID number) and using a unique study code. Confidentiality and data privacy were protected in strict compliance with the Russian Federation's Federal Law № 152-FZ "On Personal Data" and the principles of the Declaration of Helsinki.

7. REFERENCES:

1. Abedi, S. H., Fazlzadeh, A., Mollalo, A., Sartip, B., Mahjour, S., Bahadory, S., Taghipour, A., & Rostami, A. (2022). The neglected role of *Blastocystis* sp. and *Giardia lamblia* in development of irritable bowel syndrome: A systematic review and meta-analysis. *Microbial Pathogenesis*, 162, 105215. https://doi.org/10.1016/j.micpath.2021.105215
2. Aykur, M., Karakavuk, E., Karakavuk, M., Akıl, M., Can, H., Döşkaya, M., Gürüz, Y., & Dağcı, H. (2020). Inhibitory effect of Tunceli garlic (*Allium tuncelianum*) on *Blastocystis* subtype 3 grown in vitro. *Expert Opinion on Orphan Drugs*, 8(11), 489–496. https://doi.org/10.1080/21678707.2020.1857725
3. Bremer Christensen, C., Soelberg, J., Stensvold, C. R., & Jäger, A. K. (2015). Activity of medicinal plants from Ghana against the parasitic gut protist *Blastocystis*. *Journal of Ethnopharmacology*, 174, 569–575. https://doi.org/10.1016/j.jep.2015.03.006
4. Bugero, N. V. (2004). *The role of microbial ecology in the pathogenesis of secondary infections in psychiatric patients* [Unpublished doctoral dissertation]. Pskov State University.
5. Bugero, N. V., Ilyina, N. A., & Alexandrova, S. M. (2020). Persistent potential of protozoa *Blastocystis* spp. *Periódico Tchê Química*, 17(35), 1057–1069. https://doi.org/10.52571/ptq.v17.n35.2020.87_bugero_pgs_1057_1069.pdf
6. Chernenkaya, T. V., & Godkov, M. A. (2015). "Problematic" multidrug-resistant bacteria - pathogens of hospital-acquired infections in patients in critical conditions (literature review). *N.V. Sklifosovsky Journal Urgent Medical Help*, 3, 30-35.
7. Courvalin, P. (2016). Why is antibiotic resistance a deadly emerging disease? *Clinical Microbiology and Infection*, 22, 405–407. https://doi.org/10.1016/j.cmi.2016.01.012
8. El-Taweel, H. A. (2015). Understanding drug resistance in human intestinal protozoa. *Parasitology Research*, 114(5), 1647–1659. https://doi.org/10.1007/s00436-015-4423-1
9. Franklin, F., Rajamanikam, A., Raju, C. S., Gill, J. S., Francis, B., Sy-Cherng, L. W., & Kumar, S. (2022). Higher amoebic and metronidazole resistant forms of *Blastocystis* sp. seen in schizophrenic patients. *Parasites & Vectors*, 15(1). https://doi.org/10.1186/s13071-022-05418-0
10. Gureser, A. S., Karasartova, D., Sarzhanov, F., Kosar, N., Taylan-Ozkan, A., & Dogruman-Al, F. (2023). Prevalence of *Blastocystis* and *Dientamoeba fragilis* in diarrheal patients in Corum, Türkiye. *Parasitology Research*, 122(12), 2977–2987. https://doi.org/10.1007/s00436-023-07987-0
11. Ilyina, N. A., & Kasatkina, N. M. (2010). *Blastocystis hominis* sensitivity to antiprotozoal drugs. *Bulletin of OSU*, 12, 38–

41.

12. Kapoor, G., Saigal, S., & Elongavan, A. (2017). Action and resistance mechanisms of antibiotics: A guide for clinicians. *Journal of Anaesthesiology Clinical Pharmacology*, 33(3), 300–305. https://doi.org/10.4103/joacp.JOACP_349_15
13. Kappagoda, S., Singh, U., & Blackburn, B. G. (2011). Antiparasitic therapy. *Mayo Clinic Proceedings*, 86(6), 561–583. https://doi.org/10.4065/mcp.2011.0203
14. Li, S., Song, J., Ke, P., et al. (2021). The gut microbiome is associated with brain structure and function in schizophrenia. *Scientific Reports*, 11(1), 9743. https://doi.org/10.1038/s41598-021-89166-8
15. Maier, L., Pruteanu, M., Kuhn, M., Zeller, G., Telzerow, A., Anderson, E. E., Brochado, A. R., Fernandez, K. C., Dose, H., Mori, H., Patil, K. R., Bork, P., & Typas, A. (2018). Extensive impact of non-antibiotic drugs on human gut bacteria. *Nature*, 555(7698), 623–628. https://doi.org/10.1038/nature25979
16. Meng, S. Q., Xue, Y. X., Xie, W., Shi, J., et al. (2021). Gut microbiota modulates the inflammatory response and cognitive impairment induced by sleep deprivation. *Molecular Psychiatry*. https://doi.org/10.1038/s41380-021-01113-1
17. Mirza, H., Teo, J. D. W., Upcroft, J., & Tan, K. S. W. (2011). A rapid, high-throughput viability assay for *Blastocystis* spp. reveals metronidazole resistance and extensive subtype-dependent variations in drug susceptibilities. *Antimicrobial Agents and Chemotherapy*, 55(2), 637–648. https://doi.org/10.1128/aac.00900-10
18. Mukhina, E. G. (2017). Social problem of antibiotic resistance. *Universum: Medicine and Pharmacology*, 6(40). http://7universum.com/ru/med/archive/item/4898
19. Namazova-Baranova, L. S., & Baranov, A. A. (2017). Antibiotic resistance in the modern world. *Pediatric Pharmacology*, 14(5), 341–354. https://doi.org/10.15690/pf.v14i5.1782
20. Nurtayeva, K. S., Dzhumasheva, R. T., & A.Zh. (2018). Current state of the role of protozoa and parasitic worms in human parasitocenosis. *Bulletin of the Kazakh National Medical University*, 1, 476–478.
21. Roberts, T., Bush, S., Ellis, J., Harkness, J., & Stark, D. (2015). In vitro antimicrobial susceptibility patterns of *Blastocystis*. *Antimicrobial Agents and Chemotherapy*, 59(8), 4417–4423. https://doi.org/10.1128/aac.04832-14
22. Rudzińska, M., & Sikorska, K. (2023). Epidemiology of *Blastocystis* infection: A review of data from Poland in relation to other reports. *Pathogens*, 12(8), 1050. https://doi.org/10.3390/pathogens12081050
23. Rudzki, L., Stone, T. W., Maes, M., Misiak, B., Samochowiec, J., & Szulc, A. (2021). Gut microbiota-derived vitamins - underrated powers of a multipotent ally in psychiatric health and disease. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, 107, 110240. https://doi.org/10.1016/j.pnpbp.2020.110240
24. Stensvold, C. R., Smith, H. V., Nagel, R., Olsen, K. E. P., & Traub, R. J. (2010). Eradication of *Blastocystis* carriage with antimicrobials: Reality or delusion? *Journal of Clinical Gastroenterology*, 44(2), 85–90. https://doi.org/10.1097/mcg.0b013e3181bb86ba
25. Sizenzov, A. N. (2009). *Methods for determining antibiotic productivity and antibiotic resistance: guidelines for laboratory practical training*. Orenburg State University.
26. Torshin, I. Yu., Gromova, O. A., Vanchakova, N. P., & Semenov, V. A. (2022). Comparative analysis of microbiome parameters in patients with mental disorders and healthy volunteers. *Experimental and Clinical Gastroenterology*, 204(8), 93-95.
27. Wang, Z., Chen, W. H., Li, S. X., et al. (2021). Oral microbiota changes in elderly patients, an indicator of Alzheimer's disease. *International Journal of Environmental Research and Public Health*, 18(8), 4211. https://doi.org/10.3390/ijerph18084211