

# A Prospective Open-Label Study of the Antifungal Activity of External Forms of Activated Zinc Pyrithione in the Treatment of *Malassezia*-Associated Skin Diseases

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**Abstract:** *Background:* There are insufficient data on the antifungal activity of active zinc pyrithione, which is widely used in practice. Considering the reported role of *Malassezia* spp. in the pathogenesis of several dermatologic diseases, it is of scientific and practical importance to investigate this issue. *Aim:* To evaluate the antifungal activity of external forms of activated zinc pyrithione in the treatment of psoriasis, seborrheic dermatitis, and pityriasis versicolor. *Method:* An open-label prospective study was conducted between March and July 2022. Patients with psoriasis, seborrheic dermatitis, and pityriasis versicolor were treated with external forms of activated zinc pyrithione for 21 days. Skin scales and circular prints from lesion foci, as well as from skin areas without clinical manifestations before and after therapy were studied. A quantitative assessment of skin colonization by micromycetes of *Malassezia* was performed using microscopic and cultural methods of examination. Clinical efficacy and drug safety of the therapy was assessed using the Dermatological Symptom Scale Index, by recording adverse events at weeks 0, 1, 2, and 3. *Results:* 64 patients aged 18 to 65 years with diagnoses of psoriasis, seborrheic dermatitis, and pityriasis versicolor were included. 60 patients completed the study, 4 were excluded due to failure to adhere to the schedule. In patients with seborrheic dermatitis and pityriasis versicolor in the lesion foci after therapy, a significant decrease was observed in the colonization level according to the results of microscopic and cultural studies. In psoriasis patients, a significant decrease in the colonization level was obtained only based on the results of microscopic examination. In all groups, significant differences in comparison to the initial level were observed at the 1st week of treatment. There was no adverse events observed. *Conclusion:* Activated zinc pyrithione in the form of cream and aerosol showed moderate antifungal activity against micromycetes of the genus *Malassezia*.

**Keywords:** Activated zinc pyrithione; *Malassezia*; Psoriasis; Seborrheic dermatitis; Pityriasis versicolor

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## 1. Introduction

*Malassezia* spp., lipophilic (excluding *M. pachydermatis*) yeast fungi, as part of the normal skin microbiome, account for over 90% of the fungal population in different skin regions <sup>[1]</sup>. *Malassezia* spp. is mainly limited to subcutaneous areas and rarely found on limbs and genitalia. Three species (*Pityrosporum orbiculare*, *P. ovale*, and *P. pachydermatis*) were identified over a long period of time and merged into *Malassezia*. In 1995, a total of 7 species have been classified into the *Malassezia* genus (*M. furfur*, *M. obtusa*, *M. globosa*, *M. slooffiae*, *M. sympodialis*, *M. pachydermatis*, *M. restricta*) based on molecular analysis. There are currently 14 species identified <sup>[2]</sup>.

The prevalence of different *Malassezia* species depends on age, localization, and geographic location. In healthy individuals in Canada and Korea, *M. globosa* is found in children under 14 years of age, and *M. sympodialis* is found in the elderly <sup>[3,4]</sup>. *M. globosa* is mainly found on the head, and *M. sympodialis* is found on the trunk <sup>[3]</sup>. According to other data, *M. restricta* is detected on the head, while *M. globosa* is found on the chest <sup>[4]</sup>. *M. sympodialis* is most commonly found in Spain and Sweden <sup>[5,6]</sup>, *M. restricta* is found commonly in Japan <sup>[7]</sup>. *M. restricta* localizes mainly in the external part of the ear canal, in the behind-the-ear folds, and in the bridge of the nose, while *M. globosa* localizes mainly on the back, occipital region, and inguinal folds <sup>[8]</sup>.

*Malassezia* spp. are opportunistic microorganisms that cause dermatologic and systemic diseases. They become pathogens when immune balance is disturbed and interact with the skin by two mechanisms: direct and indirect. In direct exposure, specific metabolites of *Malassezia* are the cause of irritant reactions. Lipases break down triglycerides into fatty acids that cause flaking and release arachidonic acid, which is involved in the development of inflammation. Indirect exposure leads to the activation of immune and allergic reactions leading to the development of inflammation <sup>[1,2]</sup>.

Pedrosa *et al.* distinguish three groups of dermatoses associated with *Malassezia* spp. First group is classical dermatoses (papillary rash and *Malassezia* folliculitis); Second group is dermatoses in which *Malassezia* play a role (seborrheic dermatitis); Third group includes dermatoses that may be associated with *Malassezia* (psoriasis, fusion reticular dermatosis, atopic dermatitis) <sup>[2]</sup>.

*Malassezia* spp. are the cause of pityriasis versicolor. The specific association of *M. globosa* with pityriasis versicolor has been established in various studies in Greece, Spain, and Iran, but in Canada, *M. sympodialis* was most often identified as the cause of the disease <sup>[2]</sup>. Nevertheless, no correlation with the number of the pathogen was observed. It was found that sebum induces the formation of hyphae that promote the penetration of the pathogen into the skin. *Malassezia* spp. may be the cause of hypo- or hyperpigmented spots, affecting melanocytes in pityriasis versicolor <sup>[2]</sup>.

In seborrheic dermatitis, *M. globosa* and *M. restricta* have been detected in the majority of cases <sup>[2]</sup>. Seborrheic dermatitis is currently considered as a chronic inflammatory dermatosis <sup>[9,10]</sup>. Grice and Dawson, and Theelen *et al.* believed that *Malassezia* are the cause of the development of seborrheic dermatitis <sup>[11,12]</sup>, but it should be noted that their elimination does not lead to the cure of the disease. Apparently, it is more appropriate to consider *Malassezia* metabolites as triggers of seborrheic dermatitis. Karakadze *et al.* identified 11 gene mutations associated with seborrheic dermatitis. Most of the encoded proteins play a role in both immune response and epidermal differentiation dysfunction. These dysfunctions lead to the proliferation of *Malassezia*, their spread into the dermis, and the response of innate immunity, which causes inflammation <sup>[13]</sup>.

In recent years, more and more data on the role of *Malassezia* spp. in psoriasis have become available <sup>[14]</sup>. It was found that the microbiome can influence the course and exacerbations of different psoriasis subtypes <sup>[15,16]</sup>. Several authors have shown that *M. japonica* and *M. furfur* are associated with psoriasis vulgaris, and other *Malassezia* are associated with guttate psoriasis and scalp psoriasis <sup>[17-19]</sup>. *M. globosa* is found predominantly

in scalp psoriasis, *M. furfur* and *M. sympodialis* were found less frequently; however, considering that these species exist normally on the above-mentioned areas, it is difficult to explain their role in psoriasis. At the same time, it should be noted that patients with psoriasis have antibodies to *Malassezia* spp. and their antigens<sup>[20]</sup>.

In psoriasis, interleukin-23 (IL-23/Th17) is known to play a key role in the pathogenesis of the disease<sup>[21]</sup>. *Malassezia* can induce the production of cytokines associated with T helper 1 (Th1) cells in peripheral blood<sup>[22]</sup>, as well as influence keratinocyte proliferation and production of pro-inflammatory cytokines involved in the pathogenesis of inflammation<sup>[23]</sup>. According to various data, topical and systemic antifungal preparations can have a significant therapeutic effect in the treatment of psoriasis<sup>[24-27]</sup>. According to Hurabielle *et al.*, in these cases, *Malassezia* spp. play the role of a factor exacerbating the disease, but they are not its cause, which explains the positive results of antifungal therapy. Undoubtedly, additional studies are required to understand the role of *Malassezia* spp. in the pathogenesis of psoriasis<sup>[16]</sup>.

The treatment of dermatoses associated with *Malassezia* is based on antifungal and anti-inflammatory therapy (when involved in the pathogenesis of inflammation). Oral terbinafine is ineffective in prune-like psoriasis, but effective in moderate forms of seborrheic dermatitis. Oral itraconazole has a pronounced clinical effect in treating seborrheic dermatitis and reduces the number of *Malassezia* spp., but these data are few<sup>[2]</sup>. Topical (antifungal agents, corticosteroids, pimecrolimus, tacrolimus, zinc pyrithione, keratolytics) and systemic (imidazoles, terbinafine, isotretinoin) drugs are recommended in the treatment of seborrheic dermatitis<sup>[9]</sup>.

In recent years, there has been renewed interest in zinc preparations, which have the following pharmacologic properties: they influence the differentiation of keratinocytes, have anti-inflammatory, antifungal, and antibacterial effects<sup>[28]</sup>. Thus, zinc preparations may act pathogenetically on dermatoses associated with *Malassezia*.

Activation of zinc pyrithione molecule leads to strengthening of intramolecular bonds, which results in activated zinc pyrithione becoming 50 times more stable in comparison with standard zinc pyrithione. Activated zinc pyrithione can bind to phospholipids and chelate metal cations, activate apoptosis, and inhibit regeneration. The antimicrobial and antifungal actions are based on the ability of this drug to disrupt the integrity of cell membranes<sup>[29]</sup>. In a comparative study of the anti-inflammatory activity of zinc pyrithione in a laboratory model of psoriasis, it was found that the anti-inflammatory effect of activated zinc pyrithione was insignificantly inferior to betamethasone and superior to zinc pyrithione<sup>[30,31]</sup>.

To date, there are very few studies on the antimycotic activity of activated zinc pyrithione in the treatment of skin diseases. New data on the drug in this aspect will allow the expansion of the range of indications for use and better understanding of the pathogenesis of dermatoses.

The aim of the study is to evaluate the antifungal activity of external forms of activated zinc pyrithione in the treatment of psoriasis, seborrheic dermatitis, and pityriasis versicolor. Secondary objectives were to evaluate the clinical efficacy and safety of the drug application.

## 2. Methods

### 2.1. Inclusion and exclusion criteria

This paper conducted a prospective, open-label, interventional study. Inclusion criteria were men and women of any race between the ages of 18 and 65; signed informed consent; outpatients and/or inpatients; an established clinical diagnosis of psoriasis, seborrheic dermatitis, or pityriasis versicolor. Exclusion criteria were widespread forms of psoriasis (skin lesion area more than 10%); the need for systemic antimycotic therapy (for pityriasis versicolor); hypersensitivity to any component of external forms of activated zinc pyrithione; topical or systemic use of antifungal drugs within 3 months prior to the date of inclusion of the patient in the study; pregnancy or breastfeeding; expected violation of the drug regimen by the patient. The following criteria were also excluded:

withdrawal of informed consent; failure of the patient to comply with the visit schedule; development of serious adverse events or medical conditions/diseases for which, in the opinion of the investigator, continuation of the study treatment is not feasible, or is dangerous to the patient, or is not in the interest of maximizing the patient's welfare and safety.

## 2.2. Study methods

The study was carried out at the clinical base of the Department of Skin and Venereal Diseases of the Kirov Military Medical Academy of the Ministry of Defense of the Russian Federation, laboratory support by Research Institute of Medical Mycology named after P.N. Kashkin (Research Institute of Mycological Monitoring and Fungal Biology). Duration of the study was from March to July 2022.

All included patients were treated with topical forms of activated zinc pyrithione (psoriasis and seborrheic dermatitis in the form of a cream, pityriasis versicolor in the form of an aerosol) for 21 days. The drug was applied to the lesion foci 2 times a day. Control examinations were performed on weeks 0 (visit 1), 1 (visit 2), 2 (visit 3), and 3 (visit 4). At visits 1, 2, 3, and 4, basic physical data were recorded, the dermatologic index of the symptom scale was determined, and adverse events were recorded. Laboratory examination (microscopic and culture tests) was performed at visit 1 and 4.

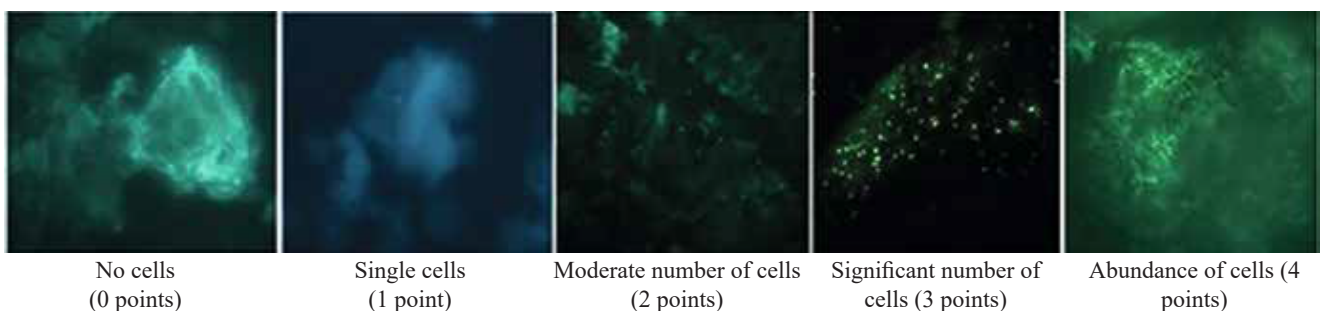
## 2.3. Observation indicators

The colonization of skin by *Malassezia* micromycetes in the lesions before and after application of the study drug was observed, as well as the clinical efficacy and safety of the investigational drug.

Microscopic study was one of the indicators observed in this study. The material was skin scales from lesions and skin areas without clinical manifestations (control), taken with surgical adhesive tape. The number of samples studied was 248 (128 before treatment and 120 after treatment).

Microscopic examination of preparations was performed using light-field and luminescent microscopy. When making native temporary micropreparations according to the "crushed drop" type, the samples were enclosed in a mounting solution of KOH (potassium hydroxide) at a concentration of 10% wt. in 40% vol of DMSO (dimethyl sulfoxide) with the addition of methylene blue. Additionally, a solution of Calcofluor white with Evans blue was added to the pre-preparation before examination as a fluorescent label for chitin and cellulose of the fungal cell wall. Microscopy of preparations from the samples of Object No.1 with subsequent photofixation of the results was carried out using a Leica DM LB2 microscope with a Leica DFC320 camera at magnification ratios of  $\times 200$  and  $\times 400$ .

The results of microscopic examination were evaluated using a 5-point scale developed by us: 0 – absence of cells, 1 – single cells, 2 – moderate number of cells (up to 20 cells in the field of view), 3 – significant number of cells (from 20 to 100 cells in the field of view), 4 – abundance of cells (more than 100 cells in the field of view). Visualization of the score evaluation of microscopy results is presented in **Figure 1**.



**Figure 1.** Visualization of the scale of the degree of colonization of samples under the microscope

Furthermore, culture study is another indicator observed. Material was taken from lesions and skin areas without clinical manifestations (control) in two variants: with the help of adhesive tape with the size of the working area 3.5 cm × 1.5 cm, the number of samples studied was 248 (128 before treatment and 120 after treatment); circular prints taken by the method of contact cups (bacterial prints with elective lipid-containing nutrient medium), the number of samples studied was 248 (128 before treatment and 120 after treatment).

Dense (agarized) lipid-containing elective nutrient medium, modified Leeming-Notman agar (mLNA), was used for isolation and cultivation of lipid-dependent micromycetes of the genus *Malassezia*. Composition of mLNA medium includes distilled water (1 liter), fermentative peptone (10 g), glucose (10 g), yeast extract (2 g), dry bovine bile (8 g), glycerol (10 ml), glycerol monostearate (0.5 g), Tween 60 (5.0 ml), olive oil (20 ml), and agar (15 g). To inhibit bacterial growth, an anti-antibiotic, chloramphenicol at a concentration of 40 mg/L, was added to the mLNA medium. One batch of mLNA medium produced in the laboratory was used to fulfill the entire scope of work.

Samples from adhesive tapes, fixed on the slides, before inoculation on nutrient media were pre-treated from the outside of the slide, as well as from its back side by successive progressive movements with a cotton swab soaked in 95% ethyl alcohol to avoid contamination of the inoculation by fast-growing mycelial (filamentous) micromycetes. Samples on adhesive tapes were removed from the slides with sterile tweezers and inoculated in pairs (from the lesions and control) on nutrient medium in Petri dishes.

The incubation of Petri dishes and contact cups (bakpechatka) was carried out in the incubator at  $32 \pm 2^\circ\text{C}$  for 10 days. After the end of culturing, the counting of the grown colonies of micromycetes in each Petri dishes and contact cups was carried out with photofixation of the results. The results were expressed as the number of colonies per 1 dm<sup>2</sup> (CFU/dm<sup>2</sup>).

Since *Malassezia* micromycetes are representatives of the human skin normobiota and the degree of colonization by these fungi varies considerably in the human population, for laboratory assessment of antifungal treatment efficacy, we additionally took into account the initial degree of colonization of healthy skin without clinical manifestations for each patient individually.

To assess the degree of skin colonization in the foci of *Malassezia* micromycetes infection before and after treatment, patients were ranked into groups based on the results of microscopic and culture studies. For microscopy, patients were ranked into groups (n = 5) according to the results, namely according to the number of scores (0–4). For culture studies, ranking by groups (n = 8) was carried out according to the value of CFU/dm<sup>2</sup>: 0; 1...100; 101...200; 201...400; 401...800; 801...1600; 1601...3200, and more than 3200.

To determine the clinical efficacy of the drug, the modified dermatologic symptom scale index (DSSI) was used, as well as the physician's overall assessment of the patient's clinical condition (physician global assessment, PGA scale) (**Table 1**). Skin itching, erythema, scaling, infiltration, pigmentation, excoriations, cracks, and crusts were taken into account when calculating the DSSI. The severity of symptoms was graded as follows: none (0 points), mild (1 point), moderate severity (2 points), and significant severity (3 points). The total DSSI score is the sum of the values for each indicator and can range from 0 to 24.

## 2.4. Ethical review

The study was approved by the independent ethical committee of I.I. Mechnikov FGBOU VO NWSMU, protocol No. 2 of 16.02.2022.

## 2.5. Statistical analysis

Preliminary calculation of sample sizes was not performed. Statistical processing of the results of the study was carried out using the licensed software package STATISTICA v.10.0 (StatSoft, USA) and StatTech v. 2.8.8

(StatTech LLC, Russia). Culture results (CFU/dm<sup>2</sup>) were evaluated for conformity to normal distribution using the Shapiro-Wilk criterion. Quantitative data were described using median, and lower and upper quartiles (Q1–Q3). The Wilcoxon (*W*) test was used to compare quantitative indices of skin colonization from the lesion and control skin areas at visits 1 and 4. Comparison of two independent groups was performed using the non-parametric Mann-Whitney criterion (*U*-test). Correlation analysis was performed using the non-parametric Spearman correlation coefficient. Differences were considered statistically significant at  $P < 0.05$ .

**Table 1.** Overall physician assessment of the patient’s clinical condition (Physician Global Assessment, PGA)

Point	Degree of severity	Description	Response to therapy
0	Complete clearance	No signs of illness (100% improvement)	Complete response
1	Almost complete clearance	Very significant improvement ( $\geq 90\%$ , $< 100\%$ ), only traces of disease remain	Partial response
2	Marked improvement	Significant improvement ( $\geq 75\%$ , $< 90\%$ ), only isolated signs of disease remain	Partial response
3	Moderate improvement	Condition intermediate between marked minor improvement ( $\geq 50\%$ , $< 75\%$ )	Partial response
4	Slight (weak) improvement	Some improvement ( $\geq 25\%$ , $< 50\%$ ), significant signs of disease remain	Disease stable
5	No change	No change from baseline ( $\pm 25\%$ )	Disease stable
6	Worsening	Disease worsened from comparison to baseline by $\geq 25\%$ or more	Disease progression

### 3. Results

#### 3.1. Participants of the study

At the screening stage, 64 patients aged 18 to 65 years with diagnoses of limited psoriasis (16 males, 8 females, mean age  $32.6 \pm 16.8$ ), seborrheic dermatitis (20 males, mean age  $27.7 \pm 10.1$ ), and pityriasis versicolor (19 males, 3 females, mean age  $27.8 \pm 12.1$ ) meeting the inclusion/exclusion criteria were examined. Sixty patients completed the study, four patients (2 psoriasis and 2 pityriasis versicolor) were excluded due to non-compliance with the visit schedule.

#### 3.2. Main results of the study in psoriasis patients

In the microscopic and culture studies, micromycetes of the genus *Malassezia* were detected in 95% of patients (19/20). No *Malassezia* fungi were detected in the study material from one patient (No. P10 in the database) obtained at the 1st visit; therefore, it was not included in the analysis of the results in this group for the above-mentioned indicators.

In microscopic study, *Malassezia* micromycetes were detected in samples from foci in 16 patients (84.2%) before the start of antimycotic therapy, and in 11 patients (57.9%) after completion. After treatment, a decrease in the degree of colonization was observed: the majority of samples, 8 (42.1%), were in the 2nd rank group (1 point), 2 (10.5%) in the 3rd rank group (2 points), and another 1 (5.3%) in the 4th rank group. Group 5 did not include any of the studied samples either before or after therapy. The number of patients in whom *Malassezia* was not detected microscopically in the foci increased from 3 (15.8%) to 8 (42.1%). Complete elimination of the tested pathogen in samples from the foci after treatment occurred in 5 (26.3%) patients (**Table 2**). The differences between the related groups (before and after treatment) were statistically significant (**Table 3**). On healthy skin (control), *Malassezia* micromycetes were detected at the beginning of the study (visit 1) in 13 patients (68.4%) and at the end (visit 4) in 14 patients (73.7%); no significant differences between the related groups were found in this case (**Table 3**).

**Table 2.** Results of microscopic examination of samples from psoriasis patients at visits 1 and 4 ( $n = 19$ )

Rank groups	Number of points	Analyzed samples, abs. (%)			
		Foci		Control	
		Visit 1	Visit 4	Visit 1	Visit 4
1	0	3 (15.8%)	8 (42.1%)	6 (31.6%)	5 (26.3%)
2	1	6 (31.6%)	8 (42.1%)	12 (63.1%)	14 (73.7%)
3	2	4 (21%)	2 (10.5%)	1 (5.3%)	0 (0%)
4	3	6 (31.6%)	1 (5.3%)	0 (0%)	0 (0%)
5	4	0 (0%)	0 (0%)	0 (0%)	0 (0%)

**Table 3.** Analysis of skin colonization dynamics in psoriasis patients (scores for microscopy, CFU/dm<sup>2</sup> for culture studies) at visits 1 and 4 (Median, Q1–Q3)

Focal point of the lesion						Healthy skin					
Microscopy		Adhesive tape		Contact cups (bak-pechatka)		Microscopy		Adhesive tape		Contact cups (bak-pechatka)	
Visit 1	Visit 4	Visit 1	Visit 4	Visit 1	Visit 4	Visit 1	Visit 4	Visit 1	Visit 4	Visit 1	Visit 4
1.0 (1.0–2.8)	0.5 (0–1.0)	228 (57–676)	38 (8–112)	20 (0–188)	0 (0–10)	1.0 (0–1.0)	1.0 (0–1.0)	61.5 (0–229)	30 (0–76)	0 (0–0)	0 (0–22)
$P = 0.019^*$		$P = 0.053$		$P = 0.06$		$P = 1.0$		$P = 0.198$		$P = 0.575$	

\*significant differences between related groups ( $P < 0.05$ )

In culture study, before antimycotic therapy, micromycetes of the genus *Malassezia* were isolated from adhesive tape in 17 (89.4%) patients and from contact cups samples in 10 (52.6%) patients. After treatment, *Malassezia* micromycetes were isolated from adhesive tape samples in 14 (73.7%) patients and from contact cups samples in 6 (31.6%) patients. There was a decrease trend of the degree of skin colonization in the foci of *Malassezia* spp. lesions in psoriasis patients under the background of treatment according to the culture results of samples on both adhesive tape and contact cups, and no significant differences between the related groups were revealed (**Table 3**).

After exposure to the investigated drug, most of the tested samples on adhesive tape entered the rank groups with the lowest level of colonization: 5 (26.3%) in the 1st (0 CFU) and 7 (36.9%) in 2nd (1...100 CFU/dm<sup>2</sup>). The number of patients in groups 5 (401...800 CFU/dm<sup>2</sup>) and 6 (801...1600 CFU/dm<sup>2</sup>) decreased from 3 (15.8%) to 0%, while the number of patients in group 3 (101...200 CFU/dm<sup>2</sup>) increased by 10.5%. Regarding the results of contact cups cultures, the number of patients with the highest level of skin colonization from group 8 (more than 3200 CFU/dm<sup>2</sup>) decreased from 3 (15.8%) to 1 (5.3%). The number of samples in group 2 (1...100 CFU/dm<sup>2</sup>) increased by 10.5%, from 2 to 4. At the same time, none of the studied samples entered groups 3 (101...200 CFU/dm<sup>2</sup>) and 7 (1601...3200 CFU/dm<sup>2</sup>). Complete elimination of the pathogen from the focus after treatment according to the culture results of samples occurred in 4 (21%) patients.

### 3.3. Additional results of the study in psoriasis patients

Analysis of the dynamics of DSSI in the course of treatment showed the following results: Median<sub>week 0</sub> = 7.5 (6.5; 11.0) points, Median<sub>week 1</sub> = 6.0 (5.0; 9.0) points, Median<sub>week 2</sub> = 5.0 (3.0; 7.0) points, Median<sub>week 3</sub> = 3.0

(2.0; 4.5) points, significant differences between related groups were obtained at the 1st week of treatment ( $P < 0.001$ ). 15% of patients (3/20) achieved complete or almost complete clearance on the PGA scale (physician global assessment) and 45% (9/20) achieved marked improvement (**Figure 2**).



**Figure 2.** Assessment of clinical effectiveness of the study drug (patient 19 years old, psoriasis, number P09 in the database): a — visit 1, DSSI — 10 points; б — visit 4, DSSI — 3 points, overall physician's evaluation — 2 points (marked improvement, partial response)

### 3.4. Main results of the study in patients with seborrheic dermatitis

In microscopic and culture studies, *Malassezia* micromycetes were detected in 100% of patients (20/20). In microscopic study, *Malassezia* micromycetes were detected in samples from foci in 19 patients (95.0%) before antimycotic therapy and in 17 patients (85.0%) after treatment. After treatment, a decrease in the degree of colonization was observed: most of the samples, 12 (60.0%), entered the 2nd rank group (1 point) and another 5 (25.0%) entered the 3rd group (2 points), while none of the studied samples entered the 4th and 5th groups. The number of patients in whom micromycetes of the genus *Malassezia* were not detected microscopically in the foci increased from 1 (5.0%) to 3 (15.0%). Complete elimination of the tested pathogen in samples from the foci after treatment occurred in 2 (10.0%) patients (**Table 4**). The differences between the related groups (before and after treatment) were statistically significant (**Table 4**). *Malassezia* micromycetes were detected on healthy skin areas at the beginning of the study (visit 1) in 14 patients (70.0%) and at the end of the study (visit 4) in 11 patients (55.0%); no significant differences between the related groups were found in this case (**Table 5**).



**Table 4.** Results of microscopic examination of samples from patients with seborrheic dermatitis at visits 1 and 4 ( $n = 20$ )

Rank groups	Number of points	Analyzed samples, abs. (%)			
		Foci		Control	
		Visit 1	Visit 4	Visit 1	Visit 4
1	0	1 (5%)	3 (15%)	6 (30%)	9 (45%)
2	1	9 (45%)	10 (50%)	10 (50%)	10 (50%)
3	2	4 (20%)	5 (25%)	4 (20%)	0 (0%)
4	3	4 (20%)	0 (0%)	0 (0%)	1 (5%)
5	4	2 (10%)	0 (0%)	0 (0%)	0 (0%)

**Table 5.** Analysis of skin colonization dynamics in patients with seborrheic dermatitis (points for microscopy, CFU/dm<sup>2</sup> for culture studies) at visits 1 and 4 (Median, Q1–Q3)

Focal point of the lesion						Healthy skin					
Microscopy		Adhesive tape		Contact cups (bakpechatka)		Microscopy		Adhesive tape		Contact cups (bakpechatka)	
Visit 1	Visit 4	Visit 1	Visit 4	Visit 1	Visit 4	Visit 1	Visit 4	Visit 1	Visit 4	Visit 1	Visit 4
1.5 (1.0–3.0)	1.0 (1.0–1.5)	828 (124–1796)	48 (0–181)	885 (44–2094)	0 (0–10)	1.0 (0–210)	1.0 (0–1.0)	112.5 (38–333)	9.5 (0–66.5)	0 (0–118)	0 (0–10)
$P = 0.027^*$		$P = 0.001$		$P = 0.002^*$		$P = 0.606$		$P = 0.023^*$		$P = 0.228$	

\*significant differences between related groups ( $P < 0.05$ )

Before antimycotic therapy, *Malassezia* micromycetes were culturally isolated from adhesive tape samples in 18 patients (90.0%) and from contact cups samples in 19 patients (95.0%). After treatment, *Malassezia* micromycetes were isolated from adhesive tape samples in 14 patients (70.0%) and from contact cups samples in 9 patients (45.0%). There was a statistically significant decrease in the degree of skin colonization with *Malassezia* spp. in patients under the background of treatment according to the culture results of samples on both adhesive tape and on contact cups (**Table 5**).

Most of the samples on adhesive tape, 11 (55.0%), were included in the 2nd rank group (1...100 CFU/dm<sup>2</sup>), the number of patients in the 6th group (801...1600 CFU/dm<sup>2</sup>) decreased from 5 (25.0%) to 2 (10.0%), and none of the samples entered the groups 5, 7, and 8 after exposure to the drug. The number of samples belonging to the 6th (801...1600 CFU/dm<sup>2</sup>) and 2nd (1...100 CFU/dm<sup>2</sup>) rank groups decreased by 15% according to the results of contact cups cultures. At the same time, none of the studied samples belonged to group 7 (1601...3200 CFU/dm<sup>2</sup>). The number of patients with the highest level of skin colonization from group 8 (more than 3200 CFU/dm<sup>2</sup>) decreased from 3 (15.0%) to 1 (5.0%). Complete elimination of the pathogen from the foci after treatment according to the culture results of samples on adhesive tapes and contact cups occurred in 4 (20.0%) and 10 (50.0%) patients, respectively. The number of samples belonging to high ranks in terms of colonization degree decreased significantly (by more than 50%).

### 3.5. Additional results of the study in patients with seborrheic dermatitis

Analysis of the dynamics of DSSI in the course of treatment showed the following results: Median<sub>week 0</sub> = 5.5 (4.0; 7.0) points, Median<sub>week 1</sub> = 3.0 (3.0; 4.5) points, Median<sub>week 2</sub> = 2.0 (2.0; 3.0) points, Median<sub>week 3</sub> = 1.0 (0.0; 2.5) points, significant differences between related groups were obtained at the 1st week of treatment ( $P < 0.001$ ). 75% of patients (15/20) achieved complete or nearly complete clearance on the PGA scale (physician global assessment), as shown in **Figure 3**.



**Figure 3.** Assessment of clinical efficacy of study drug (patient 20 years old, seborrheic dermatitis, number C05 in the database): a — visit 1, DSSI — 7 points; б — visit 4, DSSI — 1 point, overall physician score — 1 point (almost complete clearance, partial response)

### 3.6. Main results of the study in patients with pityriasis versicolor

Laboratory diagnosis of pityriasis versicolor was confirmed in almost all patients of this group, with the exception of subject O19, who, despite the presence of a clinical picture of the disease, positive results of culture and microscopic studies, did not have a typical tissue form of *Malassezia* micromycetes characteristic for the diagnosis of pityriasis versicolor. Therefore, the results for this patient were not taken into account in the subsequent group analysis.

*Malassezia* micromycetes were detected microscopically in samples from all 19 patients (100%), both before and after antimycotic therapy. After treatment, a decrease in the degree of colonization of the patients' skin was observed: most of the samples, 9 (47.4%), entered the 2nd rank group (1 point), 5 samples (26.3%) in the 3rd group (2 points), and 4 samples (21%) in the 4th group. The most significant decrease in colonization was observed in rank group 5, which included samples with an abundance of *Malassezia* spp. cells, from 15 (78.9%) to 1 (5.3%). Complete elimination of the tested pathogen in the samples from the lesions after treatment did not occur in any of the patients (**Table 6**). The differences between the related groups (before and after treatment) were statistically significant (**Table 7**). *Malassezia* micromycetes were detected on healthy skin areas at the beginning of the study (visit 1) in 14 patients (70.0%), and at the end of the study (visit 4) in 11 patients (55.0%); no significant differences between the related groups were found in this case (**Table 7**).

**Table 6.** Results of microscopic examination of samples from patients with pityriasis versicolor at visits 1 and 4 ( $n = 19$ )

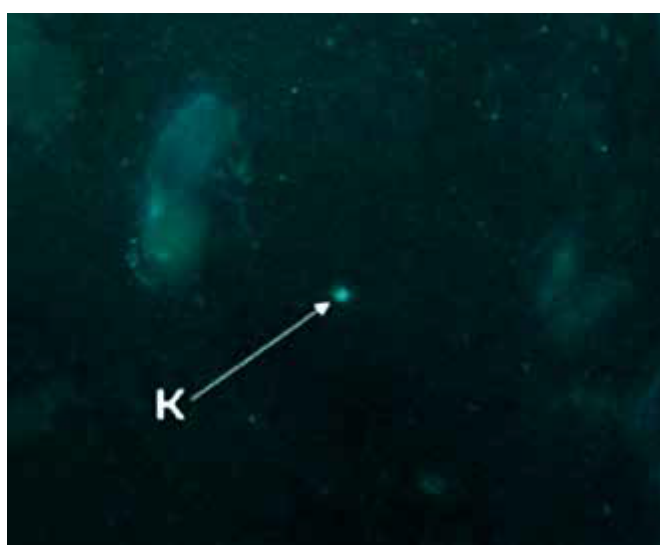
Rank groups	Number of points	Analyzed samples, abs. (%)			
		Foci		Control	
		Visit 1	Visit 4	Visit 1	Visit 4
1	0	0 (0%)	0 (0%)	2(10.5%)	5 (26.4%)
2	1	0 (0%)	9 (47.4%)	13 (68.5%)	12 (63.1%)
3	2	1 (5.3%)	5 (26.3%)	4 (21%)	2 (10.5%)
4	3	3 (15.8%)	4 (21%)	0 (0%)	0 (0%)
5	4	15 (78.9%)	1 (5.3%)	0 (0%)	0 (0%)

**Table 7.** Analysis of skin colonization dynamics in patients with pityriasis versicolor (points for microscopy, CFU/dm<sup>2</sup> for culture studies) at visits 1 and 4 (Median, Q1–Q3)

Focal point of the lesion						Healthy skin					
Microscopy		Adhesive tape		Contact cups (bakpechatka)		Microscopy		Adhesive tape		Contact cups (bakpechatka)	
Visit 1	Visit 4	Visit 1	Visit 4	Visit 1	Visit 4	Visit 1	Visit 4	Visit 1	Visit 4	Visit 1	Visit 4
4.0 (3.5–4.0)	2.0 (1.0–3.0)	648 (296–1188)	38 (19–104)	155 (0–510)	0 (0–44)	1.0 (1.0–1.0)	1.0 (1.0–1.0)	38 (19–114)	19 (0–76)	0 (0–22)	0 (0–11)
$P < 0.001^*$		$P < 0.001^*$		$P = 0.033^*$		$P = 0.593$		$P = 0.17$		$P = 0.415$	

\*significant differences between related groups ( $P < 0.05$ )

It was noted that microscopy of samples from patients after drug therapy (visit 4) revealed *Malassezia* spp. cells predominantly with a destructive cell wall (**Figure 4**). Thus, despite a moderate number of cells of this micromycetes in some samples, most of the cells detected were damaged and therefore incapable of growth and multiplication, i.e., of sustaining the infection process.



**Figure 4.** *Malassezia* sp. cell (K) with a destructive cell wall (Magnification  $\times 400$ )

In culture study, prior to antimycotic therapy, micromycetes of the genus *Malassezia* were isolated from adhesive tape of patient samples in 19 (100%) and from contact cups samples in 10 (52.7%) patients. After treatment, *Malassezia* micromycetes were isolated from adhesive tape samples in 15 (78.9%) patients and from contact cups samples in 5 (26.3%) patients of this group. There was a statistically significant decrease in the degree of skin colonization by *Malassezia* spp. in patients under the background of treatment according to the culture results of samples on both adhesive tape and on contact cups (**Table 7**).

Most of the samples on adhesive films, 10 (52.7%), were included in the 2nd rank group (1...100 CFU/dm<sup>2</sup>), the number of patients in the 8th group (more than 3200 CFU/dm<sup>2</sup>) decreased from 3 (15.8%) to 1 (5.3%), groups 3 and 4 included 2 (10.5%) patients each, and groups 5, 6, and 7 did not include any of the samples after exposure to the tested preparation.

When evaluating the results obtained on contact cups, the number of samples in Group 1 increased from 9 (47.4%) to 14 (73.7%). The number of samples in the 3rd rank group (101...200 CFU/dm<sup>2</sup>) decreased by 10.5%, from 3 (15.8%) to 1 (5.3%). In groups 5 and 7, the number of samples remained unchanged (5.3%). At the same time, none of the tested samples entered groups 4 (201...400 CFU/dm<sup>2</sup>), 6 (801...1600 CFU/dm<sup>2</sup>), and 8 (more than 3200 CFU/dm<sup>2</sup>). Complete elimination of the pathogen from the foci after treatment according to the culture results of samples on adhesive tapes and contact cups occurred in 4 (21.0%) and 5 (26.3%) patients respectively. The number of samples belonging to high ranks in terms of colonization degree decreased significantly (by more than 50%).

### 3.7. Additional study findings in patients with pityriasis versicolor

The analysis of the dynamics of DSSI during treatment showed the following results: Median<sub>week 0</sub> = 4.0 (3.5; 5.5) points, Median<sub>week 1</sub> = 3.0 (2.0; 4.0) points, Median<sub>week 2</sub> = 2.0 (2.0; 3.0) points, Median<sub>week 3</sub> = 1.0 (1.0; 2.0) points, significant differences between related groups were obtained at the 1st week of treatment ( $P < 0.001$ ). 50% of patients (10/20) achieved complete or nearly complete clearance on the PGA scale (physician global assessment) and 25% (5/20) achieved marked improvement (**Figure 5**).



**Figure 5.** Assessment of clinical efficacy of study drug (patient 32 years old, pityriasis versicolor, number O06 in database): a — visit 1, DSSI — 4 points; б — visit 4, DSSI — 1 point, overall physician evaluation — 1 point (almost complete clearance, partial response)

### 3.8. Adverse events

No adverse events, including those associated with the use of activated zinc pyrithione, have been reported.

## 4. Discussion

The obtained results showed a high prevalence of *Malassezia* fungi in patients with psoriasis and seborrheic dermatitis. In the case of psoriasis, according to the results of three variants of investigations (microscopy, inoculation with adhesive tape, inoculation with a contact cup), *Malassezia* spp. were isolated in 95% of patients (19/20), and 100% (20/20) of patients in the case of seborrheic dermatitis. Quantitative indicators of colonization according to the culture data in the foci of psoriasis lesions and on control areas (healthy skin) testify to ambiguous results. No significant differences were obtained when inoculated from adhesive tapes (Median<sub>foci</sub> = 228 CFU/dm<sup>2</sup>, Median<sub>healthy</sub> = 66 CFU/dm<sup>2</sup>,  $P = 0.103$ ), while the differences from contact cups inoculation were statistically significant (Median<sub>foci</sub> = 20 CFU/dm<sup>2</sup>, Median<sub>healthy</sub> = 0 CFU/dm<sup>2</sup>,  $P = 0.031$ ). In patients with seborrheic dermatitis, colonization by fungi of the genus *Malassezia* in the lesion foci was significantly higher in both cases compared to the control (adhesive tape: Median<sub>foci</sub> = 828 CFU/dm<sup>2</sup>, Median<sub>healthy</sub> = 113 CFU/dm<sup>2</sup>,  $P = 0.032$ ; contact cups: Median<sub>foci</sub> = 825 CFU/dm<sup>2</sup>, Median<sub>healthy</sub> = 0 CFU/dm<sup>2</sup>,  $P < 0.001$ ).

The correlation analysis between the level of *Malassezia* spp. colonization in the lesions and DSSI in psoriasis did not reveal any correlation (adhesive tape:  $R = -0.34$ ,  $P = 0.156$ ; contact cups:  $R = -0.06$ ,  $P = 0.816$ ). The opposite results were observed in patients with seborrheic dermatitis, where a direct moderate correlation between the degree of severity of dermatosis (DSSI) and the level of colonization of *Malassezia* spp. in the lesions (adhesive tape:  $R = 0.48$ ,  $P = 0.03$ ; contact cups:  $R = 0.53$ ,  $P = 0.017$ ) was found. Thus, we can conclude about the involvement of yeast fungi in the pathogenesis of seborrheic dermatitis, while this issue requires further study for psoriasis.

The use of activated zinc pyrithione led to a decrease in the colonization of *Malassezia* spp. in the lesions in all studied dermatoses, which indicates a moderate antifungal effect, since it was not possible to achieve complete elimination of *Malassezia* spp. in the foci of lesions in most cases. In psoriasis and seborrheic dermatitis, the decrease in the contamination can be associated with the anti-inflammatory effect of the drug, but in pityriasis versicolor, such mechanism is impossible by definition. An important fact registered in the course of the study that should be considered is the detection of *Malassezia* spp. cells with destructive cell wall, i.e., incapable of growth and multiplication, and, therefore, incapable of maintaining the infectious process.

## 5. Limitations

The sample of psoriasis patients was not large enough, and therefore no statistically significant reduction in skin colonization was obtained. To obtain accurate conclusions on this issue, it is necessary to conduct an additional study with the inclusion of a larger number of patients.

## 6. Conclusion

Activated zinc pyrithione in the form of cream and aerosol showed a moderate antifungal activity against *Malassezia* micromycetes. The use of the drug confirmed its high clinical efficacy and safety in the treatment of psoriasis and seborrheic dermatitis.

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## Disclosure statement

The authors declare no conflict of interest.

## Author contributions

A.V.P., T.S.B., and T.V.B. were involved in the concept and design of the study. A.V.P., A.Y.A., and T.V.B., were involved in collection and processing of material. X.O. A.V.P., K.O.C., T.V.B., and A.Y.A. were involved in text writing. A.V.S. and N.V.V. were involved in editing.

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