

# Concurrence of ocular infection with *Demodex folliculorum*

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## Research article

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# Abstract

**Background:** The ectoparasite *Demodex* spp. is the most common human parasite detected in skin lesions such as rosacea, lichen, and keratosis. It is also an etiological factor in blepharitis. As *Demodex* spp. is involved in the transmission of pathogens that can play a key role in the pathogenesis of demodecosis, the aim was to assess the concurrence of *Demodex folliculorum* and bacterial infections.

**Methods:** The study involved 232 patients, including 128 patients infected with *Demodex folliculorum* and 104 non-infected patients. The ophthalmological examination consisted of examining the vision of the patient with and without ocular correction, tonus in both eyes) and a careful examination of the anterior segment of both eyes with special emphasis on the appearance of the eyelid edges and the structure and appearance of eyelashes from both eyelids of both eyes. The samples for microbiological examination were obtained from the conjunctival sac. The bacterial culture was obtained by inoculating the samples on chocolate agar and agar with 5% sheep blood. After 24-48 h of incubation at 37 °C, the growth of various colonies of bacteria was identified based on morphological evaluation of the colonies on the media and preparations stained by the Gram method. Species identification was performed biochemical examination ex. coagulase test or VITEK Compact (bioMerieux, Poland).

**Results:** Physiological flora was found in all patients infected with *D. folliculorum* and 9 (8.7%) participants from the control group. Only in patients infected with *D. folliculorum* we isolated *Staphylococcus aureus* (9 patients, 7%), *Acinetobacter baumannii* (one patient, 0.8%), *Streptococcus pneumoniae* (one patient, 0.8%), *Klebsiella oxytoca* (one patient, 0.8%), and *Bacillus* spp. (one patient, 0.8%) in the conjunctival sac.

**Conclusions:** The occurrence of symptoms: dryness and burning sensation in the eye, tearing, conjunctival redness, foreign body sensation, eyelash loss, blockage of the Meibomian glands, cylindrical dandruff, and chalazia may suggest infection with *Demodex* spp.

Patients infected with *Demodex* spp. should also undergo microbiological examination of conjunctival swabs. The treatment of each patient should be individualized, adapted to the clinical condition, and in cases of bacterial co-infection. Furthermore, daily hygiene of the eyelid margins should be recommended.

## Background

*Demodex* mites are obligatory ectoparasites found in the skin, hair follicles, and outer layers of the epidermis of many mammalian species, showing a high species specificity. Two species are found in humans – *Demodex folliculorum* (Simon, 1842) and *Demodex brevis* (Akbulatova, 1963). Considered commensals for a long time, they are now classified as parasites by most researchers. These cosmopolitan mites live in humans of all races [1].

The transmission routes of *Demodex* spp. have not yet been fully investigated. It is likely that infection with *Demodex* spp. occurs through direct contact, use of common toiletries or towels, or by airborne eggs

and dust [2,3]. Skin colonization occurs during childhood or adolescence; no mites are found in the skin of newborns [4,5].

The *Demodex* species found in humans vary in their habitat and morphological structure. *Demodex folliculorum* occurs in the hair follicle and Zeiss glands, usually forming clusters of several individuals. *Demodex brevis* is most often isolated as separate specimens in the depths of sebaceous glands in the skin of the face, in the Meibomian glands, and in the eyelids [5]. Both *Demodex* species are present in the face skin, mainly around the nose, around the eyes, on the forehead and chin [6]. In addition to facial skin, these mites can be found in other parts of the body, including the hairy part of the head, auditory canals, skin in the neckline area, genitals, hands and feet, and nipples [7-9]. It has been noted that *D. folliculorum* is more numerous, but *D. brevis* occupies larger areas of the skin.

Demodectosis usually causes symptoms in the skin and eyes, but it can also be asymptomatic. The symptoms of ocular demodectosis are non-specific. Patients infected with *Demodex* spp. have reported tearing, burning, foreign body sensations, eyelid margin hyperemia, eyelash loss, itching, eye redness, blurred vision, and conjunctivitis with excessive sensitivity to light [2,3,10,11]. Burning and itching of eyelid margins are more frequently reported on warmer days [12].

It was found that *Demodex* mites participate in the transmission of pathogens, which may play a key role in the pathogenesis of demodectosis [13]. Symptomatic demodectosis often occurs with simultaneous bacterial infection, which is confirmed by a decrease in the number of *Demodex* spp. after administration of tetracycline to people with acne [14]. *Demodex folliculorum* contributes to the development of rosacea by transmitting bacteria from insensitive areas to sensitive areas where inflammation may occur [15]. Together with their food, which is mainly epidermal cells and the secretions of sebaceous glands, *Demodex* mites can take up microorganisms from the surface of the skin. Then, through their digestive tract, microbes are transported to the hair follicles of the host [5,16]. In addition, the disintegration of *Demodex* spp. inside the hair follicle can lead to release of the transmitted bacteria and the development of local immune response [17].

The most common bacteria colonizing conjunctival sacs are *Staphylococcus* spp., *Streptococcus* spp., *Micrococcus* spp., and *Corynebacterium* spp., occurring in about 70%, 26%, 22%, and 7% of patients, respectively. Most common among obligate anaerobic bacteria are *Propionibacterium acnes* (~44%), *Peptostreptococcus* spp. (~6%), *Lactobacillus* spp. (~2%), and *Clostridium* spp. (1%) [18].

The composition of the bacterial flora of the conjunctival sac depends on many factors, including patient age and the presence of chronic disease. For example, *Propionibacterium* spp. is more common in adults, whereas *Streptococcus* spp. is more common in children [19]. Chronic diseases, including diabetes, may increase the number of coagulase-negative staphylococci compared to healthy patients. These bacteria produce substances inhibiting the development of pathogenic bacteria, stimulating local immunological processes and the exfoliation and regeneration of epithelial cells of the eye [20]. The aim of the study was to assess the concurrence of *Demodex folliculorum* and bacterial infections.

# Methods

The study was carried out between October 2015 and May 2018, and was approved by the Bioethics Committee of the Pomeranian Medical University in Szczecin (KB-0012/82/15). It conformed to the principles outlined in The Declaration of Helsinki as revised in 2008.

## Characteristics of groups

The study involved 232 patients, including 128 patients infected with *Demodex folliculorum* (16-90 years of age) with blepharitis and 104 non-infected patients (3-81 years of age) from Poland. *Demodex folliculorum* infection was confirmed by collecting four eyelashes from the right and left upper eyelids. Infection was defined as the presence of eggs, larvae, or mature forms of *D. folliculorum* on the eyelashes. The patients with blepharitis included those with at least two symptoms of blepharitis (dryness and burning sensation in the eye, tearing, eyelid hyperemia, foreign body sensation, and excessive loss of eyelashes). Patients in this study were hospitalized in the Ophthalmology Department (n=5) and the Ophthalmology Department of the Regional Hospital (n=45) in Kołobrzeg, and patients of the Ophthalmology Department of the Independent Public Complex of Health Care Centres in Gryfice (n=49). Also, 29 male residents of the Social Welfare Home (SWH) in Jaromin, aged 41 to 80 (mean age 59.0), were examined.

## Clinical examination

Participants in the study were informed about the course of the study and gave written consent. The exclusion criterion was general treatment with oral antibiotics. Participants took part in an ophthalmic interview regarding eye problems and their personal and familial history of eye diseases. The ophthalmological examination consisted of testing of uncorrected and best corrected distance visual acuity using Snellen charts. The examination was performed at a distance of 4 m in a room providing the same lighting for all examinations. The result of the best corrected visual acuity was recorded and converted to the LogMAR scale (decimal logarithm from the minimum angle of resolution). Intraocular pressure was measured with Mackay-Marg Tono-Pen AVIA applanation tonometer (Reichert, USA). The measurement was performed three times and the average used for the analysis. Anterior segment examination was performed using a Haag-Streit L0185 slit lamp (Nikon, Japan).

## Microbiological examination

The samples for microbiological examination were obtained from the conjunctival sac using a sterile swab and AMIES transport medium. The identification methods used in this paper correspond to those used in routine bacteriological diagnostics. Samples were plated on basic microbiological media: Columbia agar with 5% sheep blood (Chapman, MacConkey, and Sabouraud) and then incubated at 37°C for 24-48 hours. Strains were identified based on morphological evaluation of the colonies on the media and preparations stained by the Gram method.

The identification of *Staphylococcus* spp. was performed by determination of hemolytic capacity of colonies on Columbia agar medium with 5% sheep's blood and by evaluation of growth on Chapman medium, allowing for differentiation of staphylococci into mannitol-positive and mannitol-negative strains. All strains showing the ability to ferment mannitol were analyzed for the presence of clumping factor A, protein A, and coagulase. The presence of all three factors indicated *Staphylococcus aureus*.

MacConkey medium was used to isolate and identify strains of Gram-negative bacteria. Due to the lack of pathogenicity of this group of microorganisms in conjunctivitis, only growth morphology on the medium was evaluated, dividing bacteria into lactose-positive and lactose-negative strains. Species identification was performed by VITEK Compact (bioMerieux, Poland).

All the microorganisms showing growth characteristic of *Corynebacteria* on Columbia agar with 5% sheep's blood were analyzed by Gram staining. Gram-positive cells with a characteristic club-like shape were considered to be *Corynebacterium* spp.

Using the disk diffusion test, the drug susceptibility of isolated strains was determined. From single colonies grown after 18-24 h, a suspension of density 0.5 according to McFarland scale ( $1 \times 10^8$  cfu/ml) was prepared and inoculated into Mueller-Hinton agar medium (bioMerieux, Poland). Subsequently, antibiotic discs with erythromycin (15  $\mu$ l), clindamycin (2  $\mu$ l), gentamicin (10  $\mu$ l), neomycin (10  $\mu$ l), tetracycline (10  $\mu$ l), and trimethoprim/sulfamethoxazole (1.25/23.75  $\mu$ l) were placed onto the culture medium. Methicillin resistance of *S. aureus* was determined using a ceftioxylin disk (30  $\mu$ g). Assessment of the growth inhibition zone around the discs and analysis of the results were performed according to the guidelines of the National Reference Centre for Microbial Susceptibility ([www.antybiotyki.pl](http://www.antybiotyki.pl)).

## Statistical analysis

In order to establish possible relationships between *Demodex* spp. infection and the occurrence of symptoms in patients from the two groups, the Chi<sup>2</sup> independence test was used. Differences were deemed statistically significant at  $p < 0.05$ . Calculations were performed using Statistica 12.

## Results

Among control subjects and patients with blepharitis symptoms, hyperopia was observed in 66 and 32 patients, myopia in 14 and 9 patients, and astigmatism in 3 control patients. Moreover, a number of the examined patients had glaucoma ( $n=12$ ), pseudophakia ( $n=16$ ), cataract ( $n=18$ ), chalazia ( $n=41$ ), anisocoria ( $n=2$ ), amblyopia ( $n=3$ ), previous corneal herpes ( $n=2$ ), and divergent strabismus ( $n=1$ ). The residents from the Social Welfare Centre were not examined in detail due to their limited cooperation during the examination.

Statistically significant relationships were observed between the and the occurrence of *Demodex* spp. and the occurrence of burning eyes ( $p < 0.001$ ), eyelash loss ( $p = 0.001$ ), foreign body sensation ( $p < 0.000$ ), conjunctival redness ( $p < 0.001$ ) and tearing ( $p = 0.001$ ), and *Demodex* spp. infection. Moreover, there was a

statistically proven relationship between the blockage of Meibomian glands ( $p < 0.000$ ), chalazia ( $p < 0.001$ ) and cylindrical dandruff ( $p < 0.000$ ).

Physiological flora was found in all patients infected with *D. folliculorum* with blepharitis and in 9 (8.7%) control subjects (Fig. 1C). The aerobic and facultative anaerobic bacterial flora colonizing conjunctival sacs of the examined patients were found to be *Bacillus subtilis*, *Corynebacterium* spp., *Diphtheroids* spp., *Haemophilus influenzae*, *Micrococcus* spp., *Penicillium* spp., *Staphylococcus* spp., *Streptococcus* spp. and *Penicillium* spp.

Four (3.1%) patients were infected with *D. folliculorum* had *Corynebacteriaceae*, three of which also had chalazia.

Only in patients infected with *D. folliculorum* did we isolate *Staphylococcus aureus* (9 patients, 7%), *Acinetobacter baumannii* (one patient, 0.8%), *Streptococcus pneumoniae* (one patient, 0.8%), *Klebsiella oxytoca* (one patient, 0.8%), and *Bacillus* spp. (one patient, 0.8%) in the conjunctival sac. *Staphylococcus aureus* was observed in an adult male patient with no chronic diseases. In microscopic examination, isolated mature forms of *D. folliculorum* were observed on the eyelashes (Fig. 1A). Ophthalmic examination showed that the visual acuity (VA) of the right eye and of the left eye were 1.0 and intraocular pressure (IOP) of the right eye was 11.7 mmHg and 10.7 mmHg, respectively. Anterior segment examination using a slit-lamp showed cylindrical dandruff on the upper eyelid and blockage of the Meibomian glands. *Staphylococcus aureus* was also found in an adult female patient with arterial hypertension and diabetes mellitus. Microscopic examination of her eyelashes revealed numerous larval and mature forms of *D. folliculorum*. Ophthalmic examination showed that VA of the right eye was 0.9 and in the left eye was 1.0. Intraocular pressure was 12.0 mmHg, and 13.0 mmHg, respectively. The patient suffered from irritation of the eye and conjunctiva. *Staphylococcus aureus* sensitive to erythromycin, clindamycin, gentamicin, neomycin, tetracycline, and trimethoprim/sulfamethoxazole were observed in two patients with mature forms of *D. folliculorum*. An adult female patient with thrombocytopenia without ophthalmic symptoms had hyperopia corrected by glasses. Ophthalmic examination showed that VA to be 0.2 in both eyes and IOP was 14.0 and 15.0 mmHg, respectively. Slit lamp examination showed slight follicular irritation of the conjunctiva. In an adult female patient with hyperopia corrected with glasses, VA was 0.6 in the right eye and 0.8 in the left eye. Intraocular pressure was 17.0 mmHg in both eyes. Slit lamp examination showed a cylindrical dandruff on the upper eyelid. *Staphylococcus aureus* was found in an adult male patient from SWH with myopia corrected with eyeglasses. Microscopic examination of his eyelashes revealed mature forms of *D. folliculorum*. Due to poor cooperation, visual acuity of the eyes was not examined. Intraocular pressure was 14.0 in the right eye and 15.0 mmHg in the left eye. Examination with a slit lamp showed a single cylindrical dandruff on the upper eyelid. *Staphylococcus aureus* was found in an adult from SWH patient with a chalazion. Microscopic examination revealed isolated mature forms of *D. folliculorum*. Similarly, visual acuity was not examined due to lack of cooperation, IOP was 15.0 mmHg in both eyes. Slit lamp examination showed irritation of the conjunctiva close to the upper and lower eyelids, and both the upper and lower eyelids were swollen. Additionally, *S. aureus* was found in an adult SWH resident. Around the patient's

eyelashes isolated mature forms of *D. folliculorum* were found in microscopic examination. Visual acuity was 1.0 in both eyes, while IOP was 9.0 in the right eye and 11.0 mmHg in the left. Examination using a slit-lamp showed no specific symptoms. Methicillin-resistant *S. aureus* (MRSA) was found in an adult male patient with hyperopia corrected by glasses, hypertension, and atrial fibrillation. Numerous mature forms of *D. folliculorum* were observed in the patient. Ophthalmic examination showed that VA in both eyes was 1.0 and IOP to be 21.0 in the right eye and 18.0 mmHg in the left. On the upper eyelid, we observed cylindrical dandruff and blockage of the Meibomian glands with an oily secretion at the orifices of the glands; eyelashes were glued together. MRSA was also found in an adult HIV-infected patient from Social Welfare Home in Jaromin. Microscopic examination showed isolated mature forms of *D. folliculorum*. Intraocular pressure was found to be 11.0 mmHg in both eyes. Slit lamp examination showed pale conjunctiva.

*Acinetobacter baumannii* was isolated from an adult patient with hypertension. The patient had numerous eggs (Fig. 1B), as well as larval and mature forms of *D. folliculorum*. Best corrected distance visual acuity was found to be 0.7 in the right eye and 0.6 in the left eye. Intraocular pressure was 20 mmHg in the right eye and 17 mmHg, respectively. The slit lamp test revealed cylindrical dandruff on the upper eyelid.

*Streptococcus pneumoniae* was found in an adult patient with isolated mature forms of *D. folliculorum*. Due to poor cooperation, visual acuity of the eyes was not examined. Intraocular pressure was 12.0 in the right eye and 9.0 mmHg in the left eye. The slit lamp study showed cylindrical dandruff, irritation, conjunctival hyperemia, and Meibomian gland dysfunction.

*Klebsiella oxytoca* were found in an adult patient with hypertension. During microscopic examination of eyelashes, the patient was found to have numerous eggs along with larval and mature forms of *D. folliculorum*. Ophthalmological examination showed that VA was 0.3 in the right eye and 0.4 in the left eye. Intraocular pressure was 21.0 mmHg in the right eye and 18.0 mmHg in the left eye. In addition, the patient had hyperopia corrected with glasses. The patient complained about tearing and periodical occurrence of chalazia. In the ophthalmological examination, cylindrical dandruff was observed, the eyelashes were stuck together, and the Meibomian glands were found to be obstructed with oily secretion.

*Bacillus* spp. was found in adult man without chronic diseases. The patient presented with single mature forms of *D. folliculorum* during microscopic examination. Due to poor cooperation from the patient, vision was not examined, whereas IOP was 14.0 mmHg in the right eye and 21.0 mmHg in the left eye. Slit lamp examination did not show any changes.

## Discussion

Demodectosis is a problem in both dermatology and ophthalmology due to the chronic nature of the infection. Most studies concern the occurrence of *Demodex* spp. infections in the skin of the face, while research on their incidence in the eyelids is scarce. The effect of *Demodex* spp. infection on the visual system is a contentious issue. Some researchers indicate that these mites participate in the

etiopathogenesis of eye diseases, while others disagree [21,22]. However, there are studies which offer evidence that *Demodex* spp. infection may cause changes in the cornea and conjunctiva of the eye [23,24].

The incidence of *Demodex* spp. infection depends on climate, socioeconomic and sanitary conditions, as well as access to medical care and effectiveness of treatment [25]. In human skin, the extensiveness of *Demodex* spp. infection may range from 20% to 80% patients [26]. Studies on potentially healthy subjects showed that 10%-60% of respondents had *Demodex* spp. in eyelash follicles. A study by Kuźna-Grygiel et al. [27] showed that, in Szczecin (i.e. the capital of the region of West Pomerania, the area covered in our research), the frequency of infection with *Demodex* spp. in eyelashes was 61%. A similar prevalence of infection with *Demodex* spp. (59%) was reported by Czepita et al. [10] in their study also performed in Szczecin. Lower incidence of *Demodex* spp. infection was found by Sędzikowska et al. [28] in patients of hospitals in Warsaw (47%). Wesołowska et al. [22] determined the prevalence of infection of *Demodex* spp. in patients (n=95) and staff (n=75) of the Centre for Orthopaedics and Rehabilitation of the Regional Hospital in Wrocław, addicts from the MONAR Addiction Treatment Centre in Wrocław (n=34), and students of the Medical University in Wrocław (n=89). Those authors found *Demodex* spp. infection in 41% of respondents. The greatest prevalence of *Demodex* infection was reported by the staff of the Centre for Orthopaedics and Rehabilitation of the Regional Hospital (40%). Garbacewicz et al. [29] calculated the incidence of *Demodex* spp. infection at 36% people from central Poland.

All surveyed residents of the Social Welfare Home in Jaromin (n=28) were infected with *Demodex* spp. In a study conducted at a similar institution, the Veteran's House in Szczecin [10], infection with *Demodex* spp. was found in 80% of residents. The very high infection rate at the Social Welfare Home in our study may have been caused by the fact that adults with psychiatric illness can restore their mental health. In this group of patients, infection with *Demodex* spp. is generally higher than in control subjects. For example, in a study by Kokaçya et al. [30], patients with schizophrenia had a higher incidence of *Demodex* spp. (29.03%) than control group (6.7%). The same team of researchers found that in depressed patients, *Demodex* spp. infection was also higher than control group (23.8% vs. 9.5%) [31].

*In our study, physiological flora was found in conjunctival sac swab in all patients with symptoms of blepharitis, all residents of the Welfare Center infected with Demodex spp., and in 9% of those not infected with D. folliculorum (control group). This may indicate that Demodex spp. promotes colonization of the conjunctival sac with physiological flora.*

*Spickett [32] showed that D. folliculorum may be a vector organism for leprosy mycobacteria (Mycobacterium leprae). Demodex mites may also transmit Staphylococcus spp. and Streptococcus spp. on its surface [17]. In a study conducted on patients, staff, and visitors of the Optometry Clinic in Oklahoma [33], S. aureus and S. epidermidis were found in 16.8% and 75.8% of subjects. In the study, two or more mites (11.6% and 5.2%, respectively) were reported more frequently in patients infected with Staphylococcus aureus than in uninfected patients. Staphylococcus aureus was found in 21.9% of patients aged 1-29 years, 13.1% aged 30-59, and 15.1% aged 60-89. In another study, Türk et al. [34]*

found *S. aureus* in two patients with blepharitis and infected with *D. folliculorum*. In our study, *S. aureus* was isolated from 7% of patients with symptoms of blepharitis, including 14.3% of residents of the Social Welfare House infected with *D. folliculorum*. We did not find the bacteria in the control group. In contrast, Lee et al. [35] found no differences in the incidence of bacteria between people infected and not infected with *Demodex* spp. Coagulase-negative *Staphylococcus* spp., *Corynebacterium diphtheriae*, and *S. aureus* were found in patients of both groups. There were no differences in the occurrence of MRSA between those infected and not infected with *Demodex* spp. In our study, MRSA was found only in patients infected with *D. folliculorum*, including one resident of the Social Welfare House in Jaromin.

*Acinetobacter baumannii* is one of the most common etiological factors of hospital-acquired infections. It shows natural mechanisms of resistance to antibiotics and chemotherapy. In the present study, *A. baumannii* was isolated from the conjunctival sac of a patient infected with *D. folliculorum* with symptoms of blepharitis.

Lacey et al. [36] isolated *Bacillus oleronius* from inside *D. folliculorum* collected from patients with papulopustular rosacea, and found specific antigens against *B. oleronius* in the serum.

Li et al. [37] on sera from 59 patients with diagnosed rosacea showed a statistically significant correlation between a positive serum reaction with *B. oleronius* antigens and the presence of *Demodex* spp. on eyelashes and face skin lesions. Results of a study by O'Reilly et al. [38] showed that proteins derived from *B. oleronius* may be a neutrophil-activating factor. Such activation of neutrophils could take place if *B. oleronius* proteins released from mites entered the tissues surrounding the hair follicle. This, in turn, could result in the development of local inflammation in the perifollicular tissue [38]. In our study, *Bacillus* spp. were isolated from the conjunctival sac of a patient with *D. folliculorum* infection. In a study by Szkaradkiewicz et al. [39], 23 strains of *B. oleronius* were isolated from eyelashes collected from 18 patients infected with *Demodex* spp. and with symptoms of blepharitis; the authors observed more severe symptoms of blepharitis in patients in whom *B. oleronius* was found. However, *B. oleronius* was also found in five healthy subjects, which may undermine its role in the development of blepharitis. The authors concluded that these bacteria, living inside the intestines of the *Demodex* mites as symbionts, can be excreted by these mites onto the surface of human skin. Due to the fact that *B. oleronius* plays a significant role in the process of digestion in termites, it seems that these bacteria may play a similar role in *Demodex* spp. [36,40]. Lacey et al. [36] stated that two specific antigenic proteins (62 and 83 kDa) produced by *B. oleronius* can stimulate and be responsible for inflammation of the hair follicle. Moreover, Li et al. [37] noted a correlation between *Demodex* spp. infection and serum immunoreactivity to *B. oleronius* proteins 62-kDa and 83-kDa.

*Streptococcus pneumoniae* can cause inflammation of the middle ear, paranasal sinuses, and conjunctiva and cornea of the eye, as well as pneumonia. *S. pneumoniae* infection can cause severe or chronic complications [41,42]. In the presented study *S. pneumoniae* was reported in a resident of a Social Welfare Home infected with *D. folliculorum*.

## Conclusions

The occurrence of the following symptoms: dryness and burning sensation in the eye, tearing, conjunctival redness, foreign body sensation, eyelash loss, blockage of the Meibomian glands, cylindrical dandruff, and chalazia may suggest infection by *Demodex* spp.

*Demodex* spp. can collect microorganisms found on the surface of the skin and transport them to the host's hair follicles. Transmission of bacteria from non-susceptible sites to sensitive areas can contribute to the development of inflammatory reactions. Therefore, patients infected with *Demodex* spp. should also undergo microbiological examination of conjunctival swabs. The treatment of each patient should be individualized, adapted to the clinical condition, and in cases of bacterial co-infection, an antibiotic and/or a topical steroid drug should be additionally prescribed. Furthermore, daily hygiene of the eyelid margins should be recommended.

## Declarations

### Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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### **Authors' contributions**

DIKB and JP conceived and designed the research. DIKB, JP, KG, KK, and NŁA performed laboratory work. DIKB, JP, KG, MC and KK analyzed the data. DIKB, JP, MC, KK and DC contributed to writing the manuscript. DIKB, MC and DC provided scientific supervision of the study. All authors read and approved the final manuscript.

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## **Ethics declarations**

## **Ethics approval and consent to participate**

The study protocol and data collection adhered to the tenets of the Declaration of Helsinki and was approved by the Bioethics Committee of the Pomeranian Medical University in Szczecin (KB-0012/82/15). Written informed consent was obtained from all participants (or their parent in the case of children under 16).

## **Consent for publication**

Not Applicable.

## **Competing interests**

None of the following authors have any proprietary interests or conflicts of interest related to this submission

## **Abbreviations**

MRSA: methicillin-resistant *Staphylococcus aureus*; SWH: the Social Welfare Home in Jaromin; VA: visual acuity, IOP: intraocular pressure

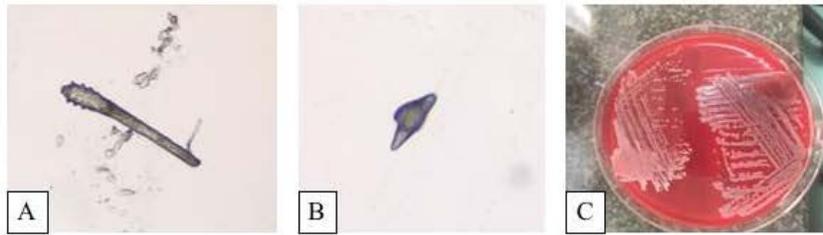
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## Figures



**Fig 1.** Adult of *Demodex folliculorum* (A), egg of *Demodex* sp. (B), macroscopic image of a bacterial colony of physiological flora and gram-negative bacilli grown on a culture medium (C; Columbia agar with 5% sheep blood)

## Figure 1

Adult of *Demodex folliculorum* (A), egg of *Demodex* sp. (B), macroscopic image of a bacterial colony of physiological flora and gram-negative bacilli grown on a culture medium (C; Columbia agar with 5% sheep blood)