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JUCILIANE HAIDAMAK

SUSCEPTIBILITY ASPECTS TO PEDICULOSIS

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JUCILIANE HAIDAMAK

SUSCEPTIBILITY ASPECTS TO PEDICULOSIS

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Advisor: Dr Débora do Rocio Klisiowicz Co-Advisor: Dr Vânia Aparecida Vicente

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por

Juciliane Haidamak

Tese aprovada como requisito parcial para obtenção do grau de Doutor no Curso de Pós-Graduação em Microbiologia, Parasitologia e Patologia, pela Comissão formada pelos professores:

Profa. Dra. Débora do Rocio Klisiowicz (Presidente)

Profa. Dra. Maria Adela Valero

Prof. Dr. Marcel Ivan Ramirez Araya

Profa. Dra. Renata Rodrigues Gomes

Prof. Dr. Andrey José de Andrade

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RESUMO

A pediculose, doença causada pelo artrópode Pediculus humanus capitis é considerada um problema de saúde pública, pois afeta milhões de pessoas em todo o mundo, no entanto, crianças em idade escolar são as mais acometidas. É de conhecimento popular que alguns indivíduos são mais suscetíveis à parasitose que outros, porém, fatores inerentes ao hospedeiro, que podem influenciar na suscetibilidade à essa doença não estão totalmente esclarecidos. Assim, o objetivo do presente estudo foi isolar e identificar a microbiota do couro cabeludo e analisar se determinadas caracteríscas da morfologia do cabelo (tipo, cor, comprimento, escamas e diâmetro) podem interferir na predisposição à doença. Para a análise da microbiota do couro cabeludo, 10 crianças com pediculose (grupo A) e 10 crianças sem pediculose (grupo B) foram analisadas. As amostras da microbiota do couro cabeludo foram coletadas através de swabs que foram friccionados na cabeça e acondicionados em meios de transporte Stuart. O isolamento da microbiota fúngica foi feito em Ágar Sabouraud (SBA) com tetraciclina e o isolamento bacteriano foi feito em ágar sangue. A identificação molecular foi realizada através de sequenciamento da região 16S e ITS do DNA ribossomal bacteriano e fúngico, respectivamente. Um total de 186 isolados foram obtidos sendo 35 bactérias e 40 fungos (grupo A) e 47 bactérias e 64 fungos (grupo B). Os resultados sugerem que a microbiota isolada pode estar envolvida na suscetibilidade à pediculose. Em relação à microbiota bacteriana, no grupo A, Staphylococcus captis foi significativamente diferente (P<0.01) em relação Sthaphylococcus epidermidis presente no grupo B. Dentre os fungos isolados, foi observado que Debaryomyces sp. foi o fungo mais frequente (15,6%) no grupo B e (2,5%) no grupo A (P<0.01). No grupo B, ocorreu uma maior diversidade em relação ao grupo A. Essa diversidade foi demonstrada pela presença de bactérias como Staphylococcus hominis (2,1%), Klebsiella oxytoca (4,2%), Pseudomonas stutzeri (4,2%), Acinetobacter nosocomialis (2,1%), e Bacillus muralis (4,2%), enquanto que no grupo A, apenas Pseudomonas sp. (5,7%) e Phychrobacter sp. (2,8%) foram encontrados. Desta forma, acreditase que a diversidade da microbiota do couro cabeludo pode interferir na preferência do piolho. Em relação às análises das características dos fios de

cabelo, das 154 crianças analisadas, através da regressão logística multivariada, O cabelo encaracolado é aquele que apresenta o maior risco de pediculose, quando comparado com o gênero, idade e cabelos longos. O cabelo escuro também está associado ao risco de pediculose. Acredita-se que que a cor escura facilitaria a camuflagem de piolhos em cabelos escuros. Assim, meninas de cabelo comprido, escuro, encaracolado, escamas estreitas e diâmetro do fio de cabelo menor apresentaram maior risco para a pediculose. Referente a prevalência de piolhos, das 658 crianças (338 meninas e 320 meninos) analisadas, as meninas estavam mais infestadas (54,1%) que meninos (36,8%) (P> 0,05). No entanto, quando comparado os grupos etários, meninos com idades de 2-3 anos estavam mais infestados que as meninas.

Palavras-chave: Piolho; cabelo; bactérias; fungos e prevalência.

ABSTRACT

Pediculosis, a disease caused by the arthropod *Pediculus humanus capitis* is considered a public health problem as it affects millions of people around the world. However, school children are the most affected. It is popular knowledge that some individuals are more susceptible to parasitosis than others, but factors inherent to the host that may influence the susceptibility to this disease are not well understood. In this context, the objective of the present study was to isolate and identify the scalp microbiota and to analyze if certain characteristics of hair morphology (scales, type, color, length and diameter) may interfere with predisposition to the disease. For analysis of the scalp microbiota, 10 children with pediculosis (group A) with 10 children without pediculosis (group B) were analyzed. Samples of the scalp microbiota were collected through swabs that were rubbed on the children's heads and packed into Stuart transport medium. Isolation of the fungal microbiota was done in Sabouraud Agar (SBA) with tetracycline and bacterial isolation was done on blood agar. Sequencing was done for the 16S and 18S region of the bacterial and fungal ribosomal DNA, respectively. A total of 186 isolates were obtained from group A, from these 35 bacteria and 40 fungi and for group B, 47 bacteria and 64 fungi were isolated. The results suggest that the isolated microbiota may be involved in pediculosis susceptibility. In relation to the bacterial microbiota, in group A, Staphylococcus capitis was significantly different (p <0.01) in relation to Staphylococcus epidermidis present in group B. Among the isolated fungi, it was observed that *Debaryomyces* sp. was the most frequent fungus (15.6%) in group B and (2.5%) in group A (p <0.01). In group B occurred in a higher diversity in relation to group A, demonstrated by the presence of bacteria such as Staphylococcus hominis (2.1%), Klebsiella oxytoca (4.2%), Pseudomonas stutzeri (4.2%), Acinetobacter nosocomialis (2.1%), and Bacillus muralis (4,2%), while in group A only *Pseudomonas* sp. (5.7%) and *Phychrobacter* sp. (2.8%) were found. Thus, it is believed that the diversity of the scalp microbiota may interfere with the preference of the lice. Regarding the analyzes of hair characteristics by multivariate logistic regression, of the 154 children analyzed, the curly hair is the one that presents the highest pediculosis risk, even exceeding the gender and age. In this study, it was observed that dark hair is

also associated with the risk of pediculosis. It is believed that a higher percentage of dark-colored lice would facilitate the camouflage of dark-colored lice. So, girls with long, dark, curly hair, narrow scales and smaller hair diameter presented greater risk for pediculosis. Regard to the prevalence of lice, of the 658 children (338 girls and 320 boys) analyzed, the girls were more infested (54.1%) than boys (36.8%) (P> 0.05). However, when compared to age groups, boys aged 2-3 years were more infested than girls.

Keywords: Head lice; hair; bacterium; fungi and prevalence

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CHAPTER I: OUTLINE OF THE THESIS

1 GENERAL INTRODUCTION

Head pediculosis is a disease caused by the arthropod *Pediculus humanus capitis* and is considered a public health problem as it affects millions of people worldwide. However, this disease is very common in childhood to which the most affected age group is between 3 to 11 years of age, this is due mainly to social behavior (Gulgun et al., 2013).

The main form of transmission of this disease is through direct head-to-head contact or by sharing objects such as caps, hats, pillows, combs or hairpins, which may serve as fomites in the transmission of the parasite (Gulgun et al., 2013). The lack of parental or guardian participation in the visual head inspection of children in the search for lice, also interferes in the infestation and maintenance of these insects (Catalá et al., 2005a) since the diagnosis is made from the visualization of adult lice, nymphs and / or nits on the hair and / or scalp of the parasitized individual (Burkhart and Burkhart, 2005; Ko and Elston, 2004).

According to Cátala et al. (2004) the worldwide prevalence of head pediculosis varies from 5.8% to 56.7% in countries such as Turkey, Korea, Egypt and Israel. In Brazil, infestation rates in children range from 7.7% to 43.3% (Borges et al., 2007; Borges and Mendes, 2002; Heukelbach et al., 2003; Nunes et al., 2014).

Although pediculosis is as old as mankind (Weiss, 2009) lice infestation is a common problem in schools around the world and, despite high prevalence, lack of information prevents a better understanding of pediculosis, hence the need to study better the characteristics that favor the disease.

It is popular knowledge that some individuals are more susceptible to pediculosis than others. Reports of individuals who have never been affected by the disease are common, even living in the same environment and under the same conditions as people who have lice infestations on a regular basis. It is known that insects are attracted or repelled due to the release of volatile compounds that are produced by the skin's microbiome of their hosts (Verhulst

et al., 2011). However, there are no studies in the literature on the scalp microbiota of healthy individuals with that of individuals affected by pediculosis, nor is there an analysis of the diameter and scales of the hair. It is believed that fungi and bacteria present on the scalp as well as structural characteristics of the wire may be involved in the susceptibility to the disease. Through the understanding of certain factors that modulate this disease, it will be possible to promote more efficient vigilance and control actions aimed at the most susceptible individuals.

1.1 OBJECTIVES

1.1.1 General

To evaluate the whether the scalp's microbiota and the morphological characteristics of the hair might interfere or not in the susceptibility to pediculosis.

1.1.2 Specific

- I.Identify the prevalence of *P. humanus capitis* in school children.
- II. Capillary analyzes of diameter, scales, type, color and length
- III. Isolate and identify the scalp's microbiota;

To organize the different topics covered, the thesis is presented in four chapters:

Chapter I - Summary of the bibliography;

Chapter II - Prevalence of P. humanus capitis;

Chapter III - Association between trichology with pediculosis;

Chapter IV - The relationship between pediculosis with the scalp's microbiota composition.

2 SUMMARY OF THE BIBLIOGRAPHY

2.1 INTRODUCTION

Lice have been human parasites for millions of years. In fact, the presence of this insect has been reported in mummies in the New World and even mentioned in the Bible as a plague. The dispersal of these insects, due to the migration of the populations and their strong association with humans, may explain why these ectoparasites are considered cosmopolitan (Araújo et al., 2000; Boutellis et al., 2014; Grimaldi and Engel, 2006; Mumcuoglu et al., 2003).

According to Barbosa and Pinto (2003) anyone can be infested by lice, regardless of age, sex, race or creed. Transmission of pediculosis occurs by direct contact, especially in children. It is believed that stimuli such as temperature, humidity and odor may interfere with the preference of lice by certain people (Burkhart and Burkhart, 2005; Catalá et al., 2005a; Greive and Barnes, 2012; Ko and Elston, 2004).

2.2 Pediculus humanus capitis

2.2.2 **Systematic Position**

The lice are ectoparasites belonging to the phylum Arthropoda, class Insecta, order Pthiraptera, suborder Anoplura, family Pediculidae or family Pthiridae. There are three species that parasitize humans, such as *Pediculus humanus capitis* (human scalp lice), human *Pediculus humanus humanus* (human body louse) and *Pthirus pubis* (pubic lice). *P. humanus capitis* parasites the scalp, whereas *P. humanus humanus* the body and *P. pubis* the pubic region (Ko and Elston, 2004).

2.2.2 Morphology

The insects of the Anoplura suborder are obligatorily ectoparasites, hematophagous at all evolutionary stages. They have an elongated and dorsoventral flattened body, being divided in head, thorax and abdomen. The head is small and ovoid, with a pair of lateral eyes, antennas with five articles. The thorax has three segments, each of which includes a pair of legs and a pair of breathing opening, called spiracles. Each leg is formed by a thigh, trochanter, femur, tibia and tarsus or tarsal claw. The tarsal claw enables the insect to be firmly attached to the fur. The abdomen consists of nine segments, the first two of which are fused and the last is rounded in males and bi-lobed in females. Laterally, each segment has a pair of spiracle plaques (Leung et al., 2005; Neves et al., 2004). As for size, males measure on average 2-3 mm, while females measure 3-4 mm (Leung et al., 2005).

The eggs, called nits of *P. humanus capitis*, have an elliptical shape, are yellowish-white, operculate and measure approximately 0.8 mm long by 0.3 mm wide (Ko and Elston, 2004; Leung et al., 2005; Nutanson et al., 2008). According to Burkhart and Burkhart (2005) the eggs of *P. humanus capitis* are fixed, on average 10 mm from the scalp and are firmly adhered to the hair, which its removal makes difficult. The viable nits are characterized by the capsule surrounding them, only nits located up to 25 mm from the base of the strand of hair may remain viable.

2.2.3 **Biology**

Insects of the Anoplura suborder present incomplete metamorphosis (hemimetaboly), that is, nymphs are morphologically similar to adults and pass through three ecdysis until they reach adulthood, which occurs approximately seven days after egg hatching (Nutanson et al., 2008; Tebruegge et al., 2011).

Adults and nits are obligatory hematophagous, so they feed several times a day every three to six hours (Centers for Disease Control and Prevention, 2015; Ko and Elston, 2004; Leung et al., 2005; Tebruegge et al., 2011). The nits are fixed at 1 mm near the scalp; they hatch between six and nine days releasing the first stage nymphs. As a result, the nits' shell becomes more yellowish, which facilitates visualization (Centers for Disease Control and Prevention, 2015).

The life span of a louse is approximately 30 days in the host and 1 or 2 days outside the host. During their adult life, females lay 3 to 6 eggs per day, a total of 50 to 150 eggs during adult life (Tebruegge et al., 2011).

2.2.4 Epidemiology

Pediculosis affects people in developed and developing countries. In recent years there has been an increase in prevalence rates, which may be related to the lice developing resistance to commonly used pediculicides, as well as improper application, as a routine treatment for "prevention" (Hazrati Tappeh et al., 2012; Vahabi et al., 2012). The high prevalence of *P. humanus capitis* is reported in countries that differ in the level of economic and cultural development (Bartosik et al., 2015).

Pediculus humanus capitis is more prevalent in schoolchildren and the insect's infestation rate can reach up to 80% among several countries around the world (REFERENCE). Epidemiological studies show that the prevalence of *P. humanus capitis* was 13.1% in Turkey, 29.7% in Argentina, 28.8% in England, 1.6% in Poland, 8.9% in Belgium, 15% in France, 9.4% in Spain, 78.6% in Libya, 55% in Israel, 8% in Lebanon, 26.6% in Jordan, 6.85% in Iran, 16.59% in India, 40% in Taiwan, 4.1% in Korea, 33.7% in Australia and 42.7% in Brazil (Borges et al., 2007; Borges and Mendes, 2002; Heukelbach et al., 2003).

2.2.5 **Control**

The control of this insect is mainly carried out through repeated or continuous applications of pyrethroids (permethrin), organophosphates (malathion), carbamate (carbaryl), organochlorine pesticides (lindane), and in some countries ivermectin, benzyl alcohol and spinosad (Barker and Altman, 2011; Dolianitis and Sinclair, 2002; Jones and English, 2003). The most used pediculicides in the world are permethrin, melation and lindane (Jones and English, 2003) and oral ivermectin (Chosidow et al., 2010; Jones and English,

2003). However, the repeated use of pediculicides caused lice resistance (Bohl et al., 2015).

Considering that the use of pediculicides is not indicated for all cases of infestation, since this class of medication acts in adults and nits, having no effect on nits, the main form of control of pediculosis is the frequent use of fine comb and visual inspection, but due to the lack of knowledge of the population about this parasitosis, and even for not considering it a disease, relatives do not spend the attention necessary to control the infestation since it is a work that demands time and patience (Smith and Goldman, 2012).

2.2.6 The skin microbiota's role in attracting insects

Studies reveal that odors produced by the host are the most important mechanism through which insects select host, each individual has a characteristic odor which is related to the bacterial and fungal flora resident on the skin. These micro-organisms are able to convert non-volatile compounds into volatiles, which attract or repel insects (Davis et al., 2013; Smallegange et al., 2011). Microbial skin communities play an important role in the production of odors in the human body, and studies have shown that the skin's microbiota composition affects the attractiveness of insects to humans. The body odor emitted by each individual is correlated with the presence of specific microorganisms (Knols et al., 1997; Rennie et al., 1991; Smallegange et al., 2011).

Certain bacterial genera such as *Staphylococcus*, *Leptotrichia* and *Delftia* are considered as strongly attractive to mosquitoes *Anopheles gambiae*, while the genera *Pseudomonas* and *Variovorax* were related as poorly attractive (Verhulst et al., 2011). As for fungi, it is suggested that the fatty acids in the skin, products of the degradation of triglycerides by *Malassezia* (resident fungus of the sebaceous glands), alter the metabolic activity of other microorganisms of the microbiota, which may explain the differential attractiveness to the *A. gambiae* (Knols et al., 1997).

The location of the host by females of hematophagous mosquitoes is mediated by physical and chemical factors, the main one being the scent. Among the compounds produced by the microbiota of the skin, carbon dioxide, ammonia, lactic acid and carboxylic acids, for example, play an important role in attracting these insects by humans (Mboera et al., 2007; Verhulst et al., 2016, 2010).

Although there are several studies that associate the attractiveness of hematophagous mosquitoes with odors produced by the microbiota of the skin (Smallegange et al., 2011), there are no reports of microorganisms identified as attractive or repellent related to *P. humanus capitis*.

2.2.7 Capillary Morphology

Pediculosis contagion is due to direct physical contact, the disease is more persistent in places with population clusters, such as in Early Childhood Centers (Borges and Mendes, 2002). Long hair provides greater contact between the heads of children, so girls are usually more affected, as they usually have longer hair as well as have social behaviors that physically approximate them (Borges et al., 2007; Cetinkaya et al., 2011; Gutiérrez et al., 2012).

The hair is composed of keratinized cells and presents three morphologically different concentric layers, being the cuticle (outermost), the cortex (intermediate) and the medulla (innermost) (Teerink, 1991). The cuticle consists of 6 to 10 layers of rectangular cells that overlap in the longitudinal direction of the fiber (Ogle and Fox, 1999). The fractions of cuticular cells that remain exposed in the hair thread compose scales organized in a specific pattern that allows the identification of mammals at the species level (Quadros and Montero-Filho, 2006).

There are five cuticle patterns: conoidal, smooth or ornate, foliaceous and angular, and the pattern of the human species is waved with ornate borders. The cuticular pattern of the human species is waved with ornate borders (Silveira, Sbalqueiro and Monteiro-Filho, 2013).

The cortex is composed of spindle cells with a thickness of 1 to 6 μ m and a length of 50-100 μ m, which are parallel to the axis of the fiber (Yang et al., 2014). It is also composed of the intermacrofibrillar matrix and the

membrane complex cell, within which the marrow is located. The color observed in the hair comes from the melanin present in the cord marrow, as well as air bubbles, and may vary greatly depending on the amount of pigment deposited (Quadros and Monteiro-Filho, 2006).

The molecular structure of the hair fiber, constituted of keratin, is reflected macroscopically in the shape of the hair, which are classified according to Ogle and Fox (1999) as: smooth, curved, wavy and curly, depending on the presence or not of curvatures. Smooth hair is defined as hair without curvature or with curvatures smaller than that of a circle with a diameter of 80 cm. In contrast, curvy hairs are those that have a slight curvature but do not exhibit waves and wavy hairs are considered to have curvatures that change direction by forming sinuous waves but do not bend over to form circles when placed on a flat surface. Curly hairs are those that, when placed under a flat surface, curl about themselves into circles and are subdivided into "Loose Curls" and "Tight Curls" according to the diameter and number of the curly hair formed circles. Such hair characteristics may be related to the preference of *P. humanus capitis*.

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CHAPTER II: PREVALENCE OF HEAD LICE IN CHILDREN FROM SOUTHERN BRAZIL

Juciliane Haidamak^a, Camila Yumi Oishi^c, Bruno Lustosa^d, Larissa Reifur^b, Maria Adela Valero^c, Débora do Rocio Klisiowicz^{a,b}

- a. Post-Graduation Program in Microbiology, Parasitology and Pathology, Basic Pathology Department, Federal University of Parana, Curitiba, Brazil.
- b. Basic Pathology Department, Federal University of Parana, Curitiba, Brazil.
- c. Faculty of Pharmacy, University of Valencia, Spain.
- c. Biomedical, Federal University of Parana, Curitiba, Brazil.
- d. Student in Biology, Federal University of Parana, Curitiba, Brazil

Corresponding author: Debora do Rocio Klisiowicz, Post-Graduation Program in Microbiology, Parasitology and Pathology, Basic Pathology Department, Federal University of Parana, Curitiba, Brazil. E-mail: klisiowicz@ufpr.br

ABSTRACT

Head lice infestation is an ancient problem observed in people of all ages and of any social-economical level or geographic part of the world. Pediculosis caused by Pediculus humanus capitis is considered a public health problem especially due to the adverse effects derived from scalp itching. Worldwide cases are reported yearly, however, there is not published prevalence information from Southern Brazil. Therefore, the aim of this study was to fulfill this gap, considering the importance of the disease and the urge for control. A total of 658 children (338 girls and 320 boys), 1 to 10 years old, were selected by random sampling, from 08 distinct public preschools and schools, from four including Almirante Tamandaré, Colombo, Curitiba, and Lapa cities. municipalities. The diagnosis was performed by visualization of live louse or nit, followed by its detection by suction and the use of a stainless-steel louse and nit comb. The overall prevalence of head louse infestation was 45.5%, the highest prevalence rate ever been published in the American continent. There was a significant association between pediculosis sex and age. Despite the fact that the prevalence was higher in girls (55.9%) than in boys (36.2%), the rate detected in boys in this study was the highest reported in boys in the American continent. On the contrary, when taking only the 2 and 3 year-old-groups, boys were more affected than girls. Overall, children of all ages presented

pediculosis, but the 6 year-old-group appeared to be the most infested. Regarding the distinct cities selected, children from Almirante Tamandaré showed the highest prevalence (53.7%) and, despite the fact that there was not a statistically significant difference between rates observed in children from rural versus urban areas, children from the rural schools in Almirante Tamandaré ans Colombo were more infested than children from urban locations. Nonetheless, these prevalence rates were very high, therefore, educational campaigns had been started to help control the disease.

Keywords: Head lice, *Pediculus humanus* capitis, diagnosis, Brazil

1 INTRODUCTION

Pediculosis, caused by *Pediculus humanus capitis* De Geer, 1778, has a cosmopolitan distribution and affects especially school-aged regardless of gender or socioeconomic background (Nutanson et al., 2008; Feldmeier, 2012; Smith; Goldman, 2012; Gulgun et al., 2013). The pruritus caused by head lice saliva during blood feeding leads to insomnia and arrested physical and intellectual development in children. In addition to the medical consequences, children with head lice can be bullied especially because it is wrongly associated with lack of hygiene (Williams et al., 2001; Catala et al., 2005). In some cases, the infestation can lead to anemia and secondary bacterial infections in scalp lesions (Robinson et al., 2003; Leung et al., 2005; Nutanson et al., 2008; Tappeh et al., 2012; Vahabi et al., 2012). Popular beliefs point to long hair as favoring the infestation in girls and social behavior, as of playing closely together, in addition to the lack of hair inspection by the parents favoring the infestation in children rather than in adults (Catala et al., 2004; Catala et al., 2005). Hair and scalp inspection including the search for lice eggs, ninphs, or adults is how the diagnosis is reached (Ko; Elston, 2004; Burkhart; Burkhart, 2005). Despite the fact that pediculosis infection is dated back to centuries ago, there are still many unanswered questions (Canyon et al., 2002; Weiss, 2009). There is a lack of information regarding the prevalence of pediculosis in Brazil (Borges; Mendes, 2002; Borges et al., 2007) and there are not studies regarding lice and its prevalence in Curitiba and surrounding boroughs. Contrary to other parasitosis, head lice is long and well diagnosed by the population, however, the lack of information and false popular beliefs, allied to louse quick propagation, complicate the understanding and control of the infestation. Determining the prevalence is the start point to epidemiological studies and to strategic control measures. Therefore, the aim of the present work was to determine the prevalence of *P. humanus capitis* in children from public schools of Curitiba and surrounding cities.

2 MATERIAL AND METHODS

A total of 658 children (338 girls and 320 boys) with aged 1 to 10 years were analyzed of four located (169 of Curitiba, 134 of Almirante Tamandaré, 95 of Lapa and 260 of Colombo). Curitiba is the state capital of Paraná located on the east side of the state and in the southern region of the country, with the coordinates of 25.4244° S and 49.2654° W (Fig. 1). The 2017 Census estimated a population of 1,900,000 inhabitants only in Curitiba, however, this work also included children from neighbor cities such Almirante Tamandaré, Colombo, and Lapa (Fig. 1). The population of the last three cities together sum up to 400,000 inhabitants. While the human development index for Curitiba is of 0,823, the other three cities show lower human development, between 0,7 and 0.4.

As part of this study, all results were anonymously released to the parents/tutors of the selected children and all of them, including neighbors, were invited to participate of educational activities towards head lice control. This study was conducted during 2015 and 2016 with the approval of an ethics committee from UFPR (CAAE 38757614.9.0000.0102).

2.1 HEAD LICE DIAGNOSIS

The diagnosis of head lice infestation was made, for one year, using three distinct procedures, always to find a live nymph or adult. A) Visual inspection of the head. In this case, the head was divided in four quadrants to search for eggs, nymphs, or adults. B) Use of a louse comb. A metal louse comb was used several times through the four head quadrants. C) Suction of the hair and scalp. A vacuum cleaner adapted to retain small arthropods was applied to the

individuals' scalp and hair to dislodge nits and lice for suction into a veil, which was replaced at each new examination. This vacuum cleaner was decorated with stickers and playfully called "empowerment machine" in the recreational activities used to perform the exam (Fig. 2). The applications of each method was made for five minutes. In this procedure, adults, nits, exuviae, other insects' parts, and viable eggs were recovered in the veil.

The diagnosis obtained from the first procedure, upon visualization, was annotated in a tab and later confirmed by the other procedures. The metal louse combs used in each of the children were stored in plastic zipper bags and the veils stored in plastic petri dish plates (47 x 10 mm). The contents from both, the comb and the veil in the petri dish, were analyzed under a stereoscopic microscope in the laboratory.

The data was analyzed using the chi-squared test with a 5% significance level using OpenEpi 3.01 (DEAN et al, 2013) and BioEstat 5.3 (AYRES et al, 2007).

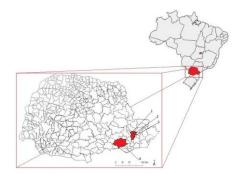


Figure 1-Brazilian map at the top highlighting in red the Paraná State. The cities within the Paraná state map include 1. Almirante Tamandaré; 2. Colombo; 3. Curitiba; 4. Lapa.

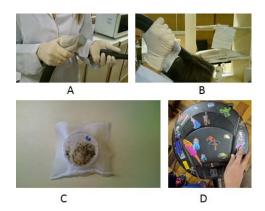


Figure 2-Suction of the hair procedure for head lice diagnosis. A) Position of the veil in the vacuum cleaner hose to act as a filter to capture the head lice. B) Suction of the hair and scalp. C) Storage of the veil and how it looked after the procedure was com conducted in one individual. D) Decorated vacuum cleaner, playfully used as an "power machine" to perform the procedure.

3 RESULTS AND DISCUSSION

In summary, the scalp and hair of 658 children (338 girls and 320 boys) were analyzed. From the state capital Curitiba and Lapa, 297 toddlers and children (1-5 years, from public preschools) were included and from Almirante Tamandaré, Colombo, and Lapa, other 361 children (6-10 years, from public schools) were included. The positive diagnosis was considered after the finding of any head louse life stage by any of the three procedures conducted (visual inspection, head louse comb, suction of the hair and scalp). The overall prevalence of 45.5%, showed in detail in Table 1, is the highest prevalence (Linardi et al., 1989; Borges; Mendes, 2002; Heukelbach et al., 2005; Borges et al., 2007; Borges-Maroni et al., 2011; Nunes et al., 2015; Costa et al., 2017; Mendes et al., 2017) ever reported in Brazil. Other equally high prevalence numbers detected in Brazil include 44.9% in Jundiaí (São Paulo state) (Rocha et al., 2012) and 44.8% in Manaus (Amazonas state) (Amazonas et al., 2015). In Manaus, the high prevalence rate can be explained because 57.9% of the children examined were from orphanages or shelters, which maintained an elevated number of individuals in the same area.

The global and Latin American prevalence of pediculosis is variable, because this disease is multifactorial. In the Bolivia, for example, the rates its varies from 34,3% to 52,2% (Devera, 2012). The prevalence in the present study is similar than the rates reported in other Latin American countries (Catalá et al., 2005; Milena et al., 2008; Ríos et al., 2008; Toloza et al., 2009; Manrique-Saide et al., 2011; Gutiérrez et al., 2012; Lesshafft et al., 2013; Gazmuri et al., 2014; Molina-Garza and Galaviz-Silva 2017).

The prevalence in girls is unanimous in contrast to the prevalence in boys in every study that discriminates gender, and this was also observed in this work, 54.1% of girls versus 36.8% of boys (chi square P>0.05) were diagnosed as positive. In the southeast Iran, the prevalence rate in girls was of 67.3% in a low-income area (Soleimani-Ahmadi et al., 2017). However, the prevalence in boys can exceed that in girls, as it was found (40.5%) in Poland (Bartosik et al., 2015). Regarding the prevalences from all four selected cities, children from Almirante Tamandaré city were found to be more infested than

from the other municipalities, however, the rates were not statistically significant (Table 1).

Table 1- *Pediculus humanus capitis* prevalence in preschool and school children from Curitiba, Almirante Tamandaré, Lapa and Colombo municipalities.

		Girls			Boys		
	N	Age (year)	Positive	Negative	Positive	Negative	%
Curitiba	169	1	2	2	0	2	42.6
		2	2	2	3	4	
		3	6	18	6	19	
		4	12	10	9	12	
		5	12	10	18	11	
		6	1	6	1	1	
Almirante Tamandaré		6	17	5	8	22	53.7
		7	19	5	5	16	
	134	8	7	2	5	2	
		9	6	1	0	3	
		10	3	2	2	4	
	95	2	2	6	8	9	48.4
		3	0	1	1	2	
Lapa		4	6	5	3	5	
_ 0.po.		5	7	5	6	4	
		7	8	0	0	6	
		8	3	2	2	4	
	260	5	8	10	3	8	42.7
		6	23	14	14	15	
Colombo		7	8	10	5	10	
		8	9	9	5	20	
		9	13	16	8	5	
		10	9	14	6	18	
Total	658		183	155	118	202	
		%	54.1	45,9	36,8	63,2	

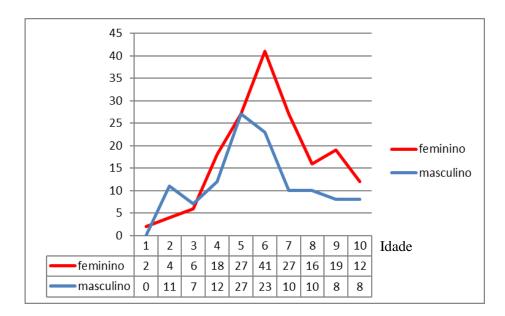


Figure 3- Number of children with positive results for *Pediculus humanus capitis* according to each age group.

It was observed that even one-year-old toddlers were found with head lice infestation (Fig.3). When they reached 2-3 years, boys appeared more infested then girls. This could be explained because at this age girls have shorter hair. At 5 years, boys and girls were equally infested but when older than 5 then girls were found to be more infested. While more girls seemed to be infested at 6 years, boys peaked earlier, at 5 years. After that peak, the rates decrease drastically but for girls it was observed another, rather small, peak of pediculosis when they reach 10 years (Figure 3). Considering all children from this study, ages of 1-10 years, the pediculosis prevalence was of 38.2%, much higher than other prevalence studies previously conducted in Brazil for the same age group (Linardi et al., 1989; Borges and Mendes, 2002; Rocha et al., 2012).

Table 2- Prevalence rates of head lice infestation among children living in rural and urban areas.

	Rural/urban	% Positive	% Negative
Almirante Tamandaré	urban	46.8	53.2
	rural	63.6	36.4
Colombo	urban	41.9	58.1
Colombo	rural	44	56

There is not an agreement pointing rural or urban areas as a predisposing factor to head lice in children. Some studies have found children from urban areas to be more infested (Riabi; Atarodi, 2012; Gazmuri et al., 2014) but at least one other study disagreed (Davarpanah et al., 2013). Some other data, including ours (Table 2), indicate no distinction between prevalence rates among children from rural or urban areas, like in Uberlândia (Borges and Mendes, 2002) and South Korea (Oh et al., 2010; Dehghanzadeh et al., 2015). Nonetheless, when contrasting the results of only two of the four cities selected for this work, there were more children with head lice from rural areas in Almirante Tamandaré than Colombo (P>0.05) (Table 2). The selected rural area within Almirante Tamandaré resembles that of slums, explaining why the number of infested children was higher than those living in urban areas. A small percentage of children from the selected rural area in Colombo were also living

in something similar to shantytowns, however, with lower population density and better life style, explaining the distinct head lice prevalence rates. A somewhat similar study found a higher prevalence rate among people living in a shantytown when contrasted to people living in a fisherman village, both in Fortaleza (Brazil) (Heukelbach et al., 2005).

Pediculus capitis infestation is dated from centuries ago (Canyon *et al.*, 2002; Weiss, 2009) and there is still a lack of scientific information regarding worldwide prevalences, especially in Brazil. Therefore, the present study is the first reporting prevalence rates in children from southern Brazil.

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CHAPTER III: ASPECTS OF TRICHOLOGY ASSOCIATED WITH HEAD PEDICULOSIS

Juciliane Haidamak^a, Thabata Caroline de Oliveira Santos^b, Isabelle Cristine Prüss^c, Amanda Bisson^d, Débora do Rocio Klisiowicz^{a,b}

- a. Post-Graduation Program in Microbiology, Parasitology and Pathology, Basic Pathology Department, Federal University of Parana, Curitiba, Brazil.
- b. Post-Graduation Program in Physiology, Basic Pathology Department, Federal University of Parana, Curitiba, Brazil.
- c. Biologist, Federal University of Parana, Curitiba, Brazil.
- d. Student in Biomedicine, Federal University of Parana, Curitiba, Brazil.

Corresponding author: Juciliane Haidamak. Post-Graduation Program in Microbiology, Parasitology and Pathology, Basic Pathology Department, Federal University of Parana, Curitiba, Brazil. E-mail: jucilianeha@gmail.com

ABSTRACT

Pediculosis caused by the arthropod *Pediculus humanus capitis*, commonly known as lice, is a disease that affects mainly children in the 3 to 11 years of age group. Several factors may be related to the occurrence of this disease, among them, age, gender, socioeconomic conditions and structural characteristics of the hair. However, it is not yet known exactly what factors favor certain children to be more susceptible to infestation by these insects than others, since many children have daily contact with other children with pediculosis and are never affected by the disease. Thus, the objective of this study was to analyze the characteristics of children with and without pediculosis such as: gender, capillary diameter, color, length, type and scales of hair strands, in order to understand whether these characteristics may be associated with disease susceptibility. The study was conducted with 154 children aged 4 to 11 years old from the municipalities of Almirante Tamandaré and Lapa, located in the State of Paraná, Brazil. Of the children analyzed, a higher risk for pediculosis was observed among girls with dark, long, curly hair, with thinner hair strands and narrow scales. It is known that certain morphological characteristics of hair and host hair, already described in the

literature, interfere with the predisposition to pediculosis. But what was not yet known was that a smaller diameter and narrow scales could also interfere with the preference of *P. humanus capitis*.

Keywords: Pediculus humanus capitis, hair thread, morphology

1 INTRODUCTION

Pediculosis caused by the arthropod *Pediculus humanus capitis*, commonly known as lice, is a disease that mainly affects children in the age group of 3 to 11 years old. The transmission of this disease is rapid and the most common form of contagion is direct head-head contact, through hair accessories or objects such as hairbrushes (Feldmeier, 2012; Ríos et al., 2008).

Although this pathology is associated with lack of hygiene, it should be noted that anyone can be infested with lice (Falagas et al., 2008). According to (Vahabi et al., 2012) the rate of infestation increases when the socioeconomic level decreases, and decreases as the parent's level of education of the parents increases, since they are believed to have greater knowledge about parasitosis. Several factors may be related to the occurrence of pediculosis, among them: age, sex, socioeconomic conditions and structural characteristics of the hair (Vahabi et al., 2012).

In relation to gender, female children are more affected. It is assumed that this is related to their preference for long hair, which facilitates contagion and makes it difficult to apply control measures, such as the use of fine combs. Hormonal factors may also influence, since it modifies the characteristics of the scalp and the behavior of the children (Barbosa and Pinto, 2003; Catala et al., 2004; Hazrati Tappeh et al., 2012; Ko and Elston, 2004).

However, it is considered that girls of school age are more affected by pediculosis than boys; this is due to the social behavior that physically approaches them in the games. Moreover, girls generally have longer hair, which favors contagion and at the same time makes it difficult to use control measures for lice (Borges et al., 2007; Catala et al., 2004; Cetinkaya et al., 2011; Gutiérrez et al., 2012; Manrique-Saide et al., 2011; Mohammed, 2012). According to Doroodgar et al., (2014) the majority of the infested population has long and straight hair.

Although hygiene conditions are considered one of the main factors associated with the occurrence of the disease, there are differences as to its influence on the infestation's development (Ríos et al., 2008; Vahabi et al., 2012).

All evolutionary stages of lice use the tarsal claws to anchor themselves to hair strands. The hair fiber's structure consists of keratin and is reflected macroscopically in the hair's shape which is classified according to (Ogle and Fox, 1999) as: straight, curved, wavy and curly, depending on the presence or not of curvatures. Hairs, like other hairs of mammals, are formed by three concentric layers of keratinized cells, the cuticle being more external; the cortex, the intermediate; and the medulla the innermost layer (Teerink, 1991).

The hair cuticle is formed by 6 to 10 layers of rectangular cells superimposed in the longitudinal direction of the fiber, so only 1/6 of the cells are exposed, forming the surface on which *P. humanus capitis* clings and deposits the nits (Scanavez de, 2001). The portion of cuticular cells exposed in the hair strand form scales arranged in a specific pattern through which it is possible to identify mammals at the species level (Silveira, Sbalqueiro and Monteiro-Filho, 2013) reports the existence of five cuticle patterns: conoidal, wavy straight or ornate, foliaceous and losangular, and the pattern of the human species is waved with ornate borders.

However, it is not yet known exactly what factors favor certain children to be more susceptible to *P. humanus capitis* infestation than others, since many children have daily contact with other children with pediculosis and are never affected by the disease. It is believed that morphological characteristics of hair strands may be related to pediculosis, since it is in the hair strands that the lice attach and lay their eggs. Thus, the objective of this study was to analyze the characteristics of children with and without pediculosis, such as gender, capillary diameter, color, length and external structure of hair strands, in order to understand if such characteristics may be associated with susceptibility to pediculosis.

2 MATERIALS AND METHODS

The study was conducted with 154 children aged 4 to 11 years in schools of the municipalities of Almirante Tamandaré, latitude: 25° 19 '29 "S and longitude: 49° 18' 36" 41 " and longitude 49° 40 '58 " and Lapa, latitude: 25° 46' 11 " S (Geografo) and longitude 49° 42 '57' 'W (Federal Government) located in the State of Paraná, Brazil. The research was approved the Ethical Committee of the Federal University of Parana with the number 38757614.9.0000.0102

Therefore, a questionnaire (appendix 1) was given to parents and guardians whose answers served as a basis for determining the risk of pediculosis among students. Thus, children were grouped into two groups according to the questionnaire responses, where the group with risk of pediculosis (group A) was formed by children who always had lice in the school period and the group without risk to pediculosis (group B) was formed by children who never had lice.

In addition to the questionnaire, all the heads of the children were inspected visually and the parameters color, length, hair strand diameter and shape were analyzed. Hair colors were classified empirically as light (blonde), red (red) and dark (light brown to black). The length was defined as short (near the scalp), medium (up to shoulder length) and long (below shoulder length).

For the determination of hair format, the work of Ogle and Fox (1999) was used as reference, being the hair classified as straight (those without curvatures), wavy / curvy (those that presented curvatures and / or waves, but which did not bend over itself to form circles-those which had ripples to the point of folding in circles.)

The collecting of hair strands was performed at the school, in which ten hairs were collected from the occipital region of each child. Five minutes before hair removal, 5% lidocaine ointment was applied to the occipital region of the children's head, which has anesthetic action. In order to conserve the entire capillary bulb, the hair strands were removed from the scalp with the aid of tweezers. After the collecting, the hair strands were stored in paper envelopes and sent to the Human Parasitology Laboratory at the Department of Basic Pathology of the Federal University of Paraná.

To measure the diameter, each hair strand was placed between a slide and a coverlet with a drop of water and observed by a Leitz Dialux white light microscope model 22/22 EB (at 400x magnification), the measurement was performed with a micrometric ruler. The process was done in triplicate for each hair strand analyzed and the diameter was calculated based on the arithmetic average of the values obtained for each hair thread.

The collected hair was submitted to cuticular impression analysis, which was performed using the hair microstructure observation technique proposed by Quadros and Montero-Filho (2006). The hair threads to be analyzed were washed in commercial alcohol and dried with absorbent paper to remove any residues that would impair microstructure observation. A thin layer of Colorama® colorless nail polish was applied onto a slide after drying for about 15-20 minutes. The hair tread (with the bulb) was then placed on the enamel layer and deposited on a slide. After this step, two more rectangular pieces of wood and Styrofoam (the same size of the slide) coated with transparent tape were placed outside the slides, forming a kind of "sandwich". This "sandwich" was pressed with the help of a walrus. After pressing and removing the pieces of wood and waited 30 minutes for the enamel to dry completely so that the thread could be removed with gentle movements of its distal end with the finger.

The impression left by the hair thread in the enamel layer was analyzed using a white light microscope (magnification of 400x) and then photographed with a 5.0 MegaPixels Bioptika digital color CMOS camera attached to the microscope. The scales of the hair strands were analyzed according to the parameters: cuticular distance (narrow, medium or wide), the shape of the scales (straight, wavy or smooth) and the continuity of the scales edges (complete or incomplete borders) (Quadros and Monteiro-Filho, 2006) (Figure 1).

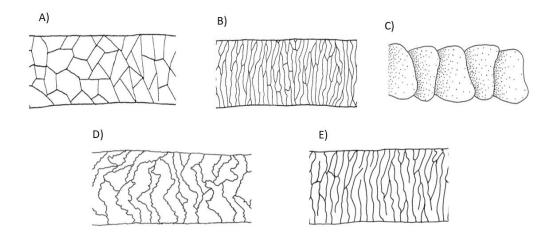


Figure 1 Morphological patterns of mammalian hairs. A) Straight; B) Waves; C) Smooth; D) Ornate with full edges; E) Ornamented with incomplete bords. Source: Adapted from Quadros and Monteiro, 2006

2.1. STATISTICAL ANALYSIS

The analysis of the hair diameters of the groups of boys and girls aged 5-8 and of 9 to 12 were made with the T student test. The relative risks of presence of pediculosis (RR) were estimated using multivariate logistic regression [in the context of risk factors, the Exp (B) result are estimates of RR] using SPSS v.22. Six models of multiple logistic regression were analyzed. The inclusion of two terms that represent the multiplicative interaction was useful to increase the adjustments (models 2, 4-6). The following models were used as independent variables: Model 1: female gender and geographical location (Lapa, Almirante Tamandaré); Model 2: female gender, age x female gender and dark hair; Model 3: long hair; Model 4: long hair, age x gender, dark hair and age and hair diameter; Model 5: female gender, dark hair, curly hair, narrow scale, narrow scale x hair diameter and curly x hair diameter and model 6: female gender, curly hair, curly hair x hair diameter and narrow scale x hair diameter. OpenEpi version 3.01 and BioEstat version 5.3 was performed to compare group the A with B. The results were considered statistically significant when P < 0.05.

3 RESULTS AND DISCUSSION

The variables analyzed for the risk of pediculosis in groups of boys and girls aged 5-8 and 9 to 12 are reflected in tables 2 (appendix).

Six multiple logistic regression models were used. The RR of pediculosis associated with the feminine gender from Lapa in model 1 (Table 1) showed RR of 3.310 and 0.009 respectively. The results of this multiple logistic regression analysis support that pediculosis is associated with female gender and geographical location. The RR of pediculosis associated with gender (Model 2) showed a remarkable RR increase (5,510) when taking age (age x gender) and hair color into account. The RR of pediculosis associated with long hair in model 3 was shown to RR of 2.701. The results of this multiple logistic regression analysis (model 4) show that pediculosis is associated with hair length (long), hair color (dark) and hair diameter, which is reflected in the relative risk of pediculosis associated with age x gender (RR: 10,411). The RR of pediculosis associated with gender (female), color hair (dark), curly hair, kind of hair-scale (narrow) and hair diameter (narrow hair-scale x diameter, curly x diameter) was analyzed through logistic regression in models 5 and 6. The color hair, the kind of hair-scale as hair diameters as covariates increases the risk of pediculosis in the dependent variable curly to 62.852 and 30.072 respectively, indicating that these variables partly explains this susceptibility. Of all variables analyzed, curly hair is the one that presents the highest pediculosis risk, even exceeding the variables gender, age or long hair.

In all the analyzed samples, it was observed that the scales were waved and their borders were ornamented (complete and incomplete), according to the literature (Deedrick and Koch, 2004) (Figure 2).

Table 1 Multivariate logistic regression analysis of different factors obtained in the study of pediculosis and regression coefficients [Exp (B) = RR = relative risk of presence of pediculosis] with significance in different models.

Characteristics		Model 1	Model 2	Model 3	Model 4	Model 5	Model 6
Gender	RR	3.310	5.510	-	-	3.170	2.909
	P Value	0.001	0.002	-	-	0.002	0.003
Lapa	RR	0.118	-	-	-		
	P Value	0.009	-	-	-		
*AT	RR	0.335	-	-	-		
	P Value	*NS	-	-	-		
Short hair	RR	-	-	2.701	0.316		
	P Value	-	-	0.00	*NS		
Age x Gender	RR	-	0.548	-	10.411		
J	P Value	-	*NS	-	0.039		
Dark Hair	RR	-	1.986	-	2.182	1.004	
2 4	P Value	-	*NS	-	*NS	*NS	
Age	RR	-		-	0.791		
9 -	P Value	-		-	*NS		
Diameter	RR	-		-	0.985		
	P Value	-		-	*NS		
Curly	RR	-		-	-	62.852	30.072
,	P Value	-		-	-	0.011	0.028
Straight Scale	RR	-		-	-	0.231	
	P Value	-		-	-	*NS	
Straight Scale x	RR	-		-	-	1.023	1.006
	P Value	-		-	-	*NS	*NS
Diameter						_	-
Curly x Diameter	RR	-		-	-	0.948	0.957
	P Value	-		-	-	0.009	0.024

Caption: **Model 1:** Female gender and geographical location. **Model 2:** Female gender, age X gender and dark hair. **Model 3:** Long hair. **Model 4:** Long hair, age X sex, dark hair, age and hair diameter. **Model 5:** Gender, dark hair, curly hair, narrow scale, narrow scale X hair diameter and curly X hair diameter. **Model 6:** Gender, Curly Hair, Curly X Hair Diameter and Narrow Scale X Hair Diameter. *AT: Almirante Tamandaré. *NS: Not statistic.

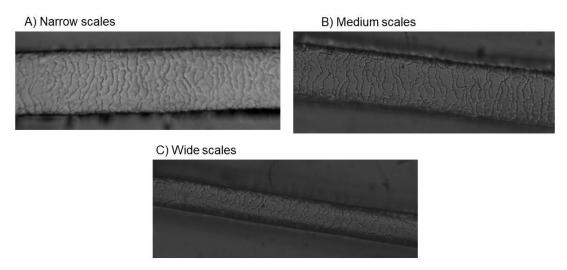


Figure 2 Photomicrographs of hair scale (400x magnification). A) Narrow scale; B) Medium scale; C) Wide scales.

Of the 154 children analyzed (Tables 2-3 in the appendix), it was observed that the risk for pediculosis was significantly higher for the girls in relation to the boys, corroborating with data already described in the literature (Abdulla, 2015; Bartosik et al., 2015; Tohit et al., 2017). According to (Mumcuoglu et al., 2001) the male gender is less susceptible to the disease in relation to the female gender, probably due to the behavioral characteristics because girls are usually closer to each other and tend to have a closer and prolonged head contact in small groups (Mohammed, 2012). In addition, in boys, prevalence declines with age, while in girls it remains high throughout the primary school period (Catalá et al., 2005b; Gulgun et al., 2013).

The risk of pediculosis, attributed to long-haired girls observed in this study, may be associated with a higher likelihood of infestation, since long hair increases the surface of interpersonal contact and is more difficult to clean. Long hair also reduces the effectiveness of ovicides of *P. humanus capitis* compared to shorter hairs (Barker and Altman, 2011). Short hairs are easy to diagnose and control lice (AlBashtawy and Hasna, 2012; Borges and Mendes, 2002; Gulgun et al., 2013; Gutiérrez et al., 2012).

In this study, it was observed that dark hair is also associated with the risk of pediculosis. It is believed that a higher percentage of dark-colored lice would facilitate the camouflage of dark-colored lice (Borges and Mendes, 2002; Soultana et al., 2009).

Although Gazmuri et al., (2014) report that hair type does not interfere with lice infestation. In this study, it was observed that curly hair presented a greater risk of pediculosis, corroborating with Doroodgar et al. (2014) and Mohammed (2012). However, it is believed that hormonal factors and skin characteristics influence the greater susceptibility to *P. humanus capitis* in girls (AlBashtawy and Hasna, 2012; Borges and Mendes, 2002; Gutiérrez et al., 2012).

Birkemoe et al. (2016) proposes that thick hair increases the risk of pediculosis, which contrasts with the present study in that it is possible to observe that children with hair smaller diameter are at higher risk for lice infestation. The smaller diameter may favor insect fixation in the hair due to the nymphal phases, because they have smaller tarsal claws, would be easier to attach to thinner hair. It is possible that thicker hair strands make it difficult for

the parasite to attach, facilitating its removal with the thin comb. This may explain why children are more affected by lice than adults, because the diameter of children's hair strands is smaller in relation to adult hair (Mansilla et al., 2011).

In this study, even though the scales analysis shows that there is great variability in the arrangement of the cuticles (wide and spaced cuticles or narrow and imbricated cuticles), it was observed that imbricated and narrow scales were statistically significant to the risk of pediculosis, suggesting that they aid in the permanence of the lice.

The results obtained in this study show a lower RR of pediculosis in the municipality of Lapa. Pediculosis is associated with the socioeconomic scenario (Cetinkaya et al., 2011; Yazar and Sahin, 2005) and social status is an important modifier of disease prevalence (Cetinkaya et al., 2011). Low educational levels, low family income, many siblings, and reduced number of bedrooms in the home favors predisposition to the illness (Gulgun et al., 2013). Residents of the city of Lapa presents rural characteristics where there is a greater distance between dwellings with a lower demographic density, and because they have a higher vocation for agriculture and livestock have better living conditions in contrast to the other groups analyzed in the regions of Almirante Tamandaré. It is believed that the children from Lapa are protected from the risk of pediculosis, this may be due to the socioeconomic characteristics and the habit of life that they present in relation to the other regions studied, because the geographical, ethnic, hygienic and climatic conditions may also play an important role in parasite distribution (Cetinkaya et al., 2011).

Thus, it is known that certain characteristics of the host, already described in the literature, such as gender, length, type and color of hair strands interfere in the predisposition to pediculosis. However, what has not yet been reported was that the diameter and scales of the hair can also interfere with the preference of *P. humanus capitis*.

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CHAPTER IV: SCALP MICROBIOTA AS POSSIBLE CAUSE TO PEDICULOSIS

Juciliane Haidamak^a, Germana Davila dos Santos^a, Bruna Jacomel Favoreto de Souza^a, Valéria Mendes^e, Raquel Vizzotto de Menezes^c, Amanda Bisson^c, Amanda Santos Talevi^d, Renata Rodrigues Gomes^a, Vânia Aparecida Vicente^{a,b}, Débora do Rocio Klisiowicz^{a,b}

- a. Post-Graduation Program in Microbiology, Parasitology and Pathology, Basic Pathology Department, Federal University of Parana, Curitiba, Brazil.
- b. Basic Pathology Department, Federal University of Parana, Curitiba, Brazil.
- c. Student in Biomedicine, Federal University of Parana, Curitiba, Brazil.
- d. Student in Biology, Federal University of Parana, Curitiba, Brazil.
- e. Biomedical, Federal University of Parana, Curitiba, Brazil.

Corresponding author: Juciliane Haidamak. Post-Graduation Program in Microbiology, Parasitology and Pathology, Basic Pathology Department, Federal University of Parana, Curitiba, Brazil. E-mail: jucilianeha@gmail.com

ABSTRACT

Pediculosis is a disease caused by the insect *Pediculus humanus capitis* that happens usually in childhood. According to popular knowledge, some individuals are more susceptible to this infestation than others. Therefore, the present study aims to isolate and identify the microbiota of the scalp surface in order to know whether those microbiota could infer on life infestation predisposition. The investigated groups were separated into children with pediculosis (group A) with 10 individuals and without pediculosis (group B) with 10 individuals. The samples were collected by swab with Stuart Transport Medium and were isolated in Sabouraud Dextrose Agar with tetracycline to fungal microbiota and Blood Agar to bacterial microbiota and then were sequenced using the regions 16S and ITS of the ribosomal DNA gene, for bacteria and fungi respectively. A total of 186 isolates were obtained from group A, with 35 bacteria and 40 fungi, and in group B 47 bacteria and 64 fungi were isolated. The results indicate that bacterial and fungal species could be involved in the susceptibility to pediculosis. In relation to bacterial microbiota, in group A, represented by infested individuals, Staphylococcus capitis was significantly different (p < 0.01), regarding the Staphylococcus epidermidis founded in higher

frequency in the individual from the group A. Among fungal isolates it was observed that Debaryomyces sp. was the most frequent (prevalent) fungus (15,6%) in group B and (2,5%) in group A (p < 0.01). In conclusion, the data suggests that microbiota scalp diversity could infer in the susceptibility to pediculosis.

Keywords: Pediculus humanus capitis; children; bacterial and fungal.

1 INTRODUCTION

Pediculosis, caused by the insect *Pediculus humanus capitis*, is considered a public health problem that affects millions of peoples worldwide, usually children ranging from ages from three to eleven years old, regardless of economic background (Falagas et al., 2008; Weiss, 2009). The main form of transmission occurs by head to head contact or by sharing objects such as hats, pillows, combs or hair clips that could serve as fomites in transmitting the parasite (Gulgun et al., 2013). The insect infestations cause serious health problems to the individual such as social embarrassment, discomfort, anxiety, lack of concentration and scratching the scalp could lead to secondary infections (Leung et al., 2005; Vahabi et al., 2012).

The main form of pediculosis control is the frequent use of lice combs and visual inspection. The use of pediculicides is not recommended for all cases of infestation especially because this class of medication acts only in adult insects and do not have any effect on the nits, besides the large poisoning risk (Hamad et al., 2016). In addition, the restricted knowledge of the population regarding this disease (Falagas et al., 2008; Smith and Goldman, 2012) associated with the absence of adequate programs of infestation monitoring, such as the lack of domestic and or governmental actions have neglected the disease and as a consequence, the risk of incidence and infestation has increased.

The global prevalence of pediculosis varies from 0% to 61.4% in countries such as the USA, Cuba, Argentina, Australia, Turkey, Korea, Egypt and Israel. In Brazil, infestation rates in children range from 7.7% to 43.3% (Borges et al., 2007; Borges and Mendes, 2002; Catala et al., 2004; Falagas et al., 2008; Heukelbach et al., 2003).

Pediculosis is a multifactorial disease. Factors such as gender, variations in hair shape and density are associated with the disease (Catalá et al., 2005b) but are not instructive to establish pediculosis susceptibility (Borges and Mendes, 2002). Moreover, several studies reported that the most important mechanism in insects to select a host are odors produced by their skin's microbiota. The microbiota develops a key role in the production of smell in the human body, as the microorganisms are capable to convert non-volatile compounds into volatile compounds that attract or not the vector (Davis et al., 2013; Smallegange et al., 2011; Verhulst et al., 2011). Some groups of bacteria such as genus *Staphylococcus*, *Leptotrichia* and *Delftia* and the fungi *Malassezia* were classified as strong attractives for insects, while the genus *Pseudomonas* and *Variovorax* were reported as having the opposite effect (Knols et al., 1997; Rennie et al., 1991; Verhulst et al., 2011).

The question of whether the scalp's microbiota interferes in the attraction of head lice remains to be evaluated as a cause for host susceptibility. Therefore, the purpose of this study was to isolate and identify fungi and bacteria from the scalp of children with different medical histories of pediculosis in order to elucidate the microbiota interference on the disease epidemiology.

2. MATERIALS AND METHODS

2.1. SAMPLES

The present study analyzed the bacterial and fungal microbiota from 20 children aged six to 10 years old, residents from Almirante Tamandaré city, Paraná state, Brazil. The selection of children was done through the observations of head lice infestation in the students by teachers during the school year. The children were separated into two groups: A (10 children that always have head lice) and B (10 children that never had head lice, sharing the same environment with children with head lice). The children chosen to participate in the study were instructed not to use shampoo, conditioners, comb cream, perfume, soap or similar products and also eat foods like onions, garlic and pepper in the period of 24 hours before collecting the samples (Qiu et al.,

2006; Verhulst et al., 2016). Children that had chronic dermatological disease or that had used antibiotic in the six months before the sampling were excluded.

The research was approved the Ethical Committee of the Federal University of Parana with the number 38757614.9.0000.0102.

2.2 BACTERIAL AND FUNGAL ISOLATION

The bacterial and fungal microbiotas were collected using swabs that were rubbed in the occipital, retroauricular and frontal regions of the scalp of each child. The four swabs, one for each area, were rubbed for 10 seconds and transported in Stuart medium. This region was chosen based on highest concentration of head lice, adapted from Fierer et al. (2010).

The bacterial isolation was done in Blood Agar (BA) followed by incubation under aerobic atmosphere at 37° C from 24 to 48 h. The fungal isolation was done in Sabouraud Dextrose Agar (SDA) with tetracycline 100 µg/mL, incubated at 28° C for 14 days.

2.3 MORPHOLOGY AND BIOCHEMICAL IDENTIFICATION

The bacterial isolates were submitted to Gram coloring to identify groups of Gram-positive cocci (CGP), Gram-negative bacilli (BGN), Gram-positive bacilli (BGP) and yeast. After separating the respective groups, the bacterial isolates were previously submitted to biochemical testing such as motility test, sugar utilization test, oxidase test, catalase test in order to identify the enzymatic activity of isolated organisms (Abdulkadir and Waliyu, 2012; Holding and Collee, 1971). Stock cultures were maintained in 40% glycerol at -20C.

The preliminary identification of fungal isolates was carried out based on macro and microscopic features of the colonies after slide culturing on Sabouraud Dextrose Agar (SDA) at 28°C for 14 days (Vicente et al., 2008). The microscopic observations were done in optical microscopy Leitz Dialux 22/22 EB model. Stock cultures were maintained on slants of Malt Extract Agar (MEA).

2.4 MOLECULAR CHARACTERIZATION AND SEQUENCING

DNA from bacterial and fungal isolates were extracted by physical maceration of the samples in a mixture of 80 mg of silica gel/celite (2:1) in 300 µL CTAB (cetyltrimethylammonium bromide) buffer. The isolated DNA was precipitated by CIA (acidic solution of chloroform-isoamyl alcohol). Cells were macerated automatically with a sterile pestle for approximately 2 min. posteriorly, 200 µL of CTAB buffer was added, and the mixture was vortexed and incubated for 10 min at 65 °C. After adding 500 µL of chloroform, the solution was mixed and centrifuged for 5 min at 12000 rpm and the supernatant transferred to a new tube with 2 vol. of ice cold 96 % ethanol. DNA was allowed to precipitate overnight at -20°C and then centrifuged again for 5 min at 12000 rpm. Subsequently the pellet was washed with cold 70 % ethanol. After drying at room temperature, it was resuspended in 100 µL of ultrapure water before storing at -20 °C. The process was adapted from Vicente et al. (2008).

Bacterial 16S Rrna gene sequences were amplified using the primer pair Y1 (5'-TGGCTCAGAACGAACGCTGGCGGC -3') **Y3** (5'and TACCTTGTTACGACTTCACCCCAGTC -3') and the reaction conditions of polymerase chain reaction (PCR) were adapted from Young et al. (1991) as follow: one cycle at 94 C for 2 min, followed by 35 cycles at (94 C for 20s, 52°C for 20s, 72°C for 20 s) and a final extension at 72°C for 7 min. To fungal, the nuclear ribosomal DNA (rDNA) internal transcribed spacer (ITS) region were amplified using the primer pair ITS1 (5'- TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'- TCCTCCGCTTATTGATATGC-3'). The PCR reaction conditions were adapted from Vicente et al. (2008) as follow: one cycle at 94°C for 2min. followed by 35 cycles at (94°C for 35s, 52°C for 30s, 72°C for 1 min) and final extension at 72°C for 1 min.

Reactions were purified with *Polyethylene glycol*-PEG 6000 (Sigma Chemical Co., St Louis, MO, USA) and the sequencing was done using ABI prism BigDyeT^M terminator cycle sequencing kit (Applied Biosystems) and analyzed on an ABI3500 sequencer.

2.5. PHYLOGENETIC ANALYSIS

Sequences were edited with the BioEdit software (Hall, 1999). The alignment was performed with the MAFFT (Katoh et al., 2005) and the visual inspection using MEGA 7 version software (Kumar et al., 2016). The best evolutionary model for each dataset was estimated using the software MEGA v.7. The phylogenetic trees were constructed with 1000 bootstrap replicates using the Maximum Likelihood Implemented in Mega v.7. software. New sequences generated in this study were deposited in the NCBI GenBank nucleotide database (www.ncbi.nlm.nih.gov) and the alignment and phylogenetic tree in TreeBASE (Data not showed during this submission will be able after acceptation). The sequence strains studied were deposited in Genbank (http://www.ncbi.nlm.nih.gov/genbank/) and the reference sequenced strains used are presented in the supplementary data (Table 2 and 3).

2.6 STATISTICAL ANALYSIS

All data were transformed to $x + 0.5 = \sqrt{x}$ (where x = number of bacteria and fungi isolated). Differences between groups (A and B) and the disease's gender susceptibility were analyzed by a two-way analysis of variance (ANOVA) followed by the Bonferroni post hoc test. Values were expressed as mean \pm standard error of mean (SEM). The level of significance was set at P \leq 0.05.

3 RESULTS

A total of 82 bacterial and 104 fungal isolates were obtained from 20 individuals. From the group considered susceptible to pediculosis (Group A) a total of 35 bacterial and 40 fungi isolates were obtained and from the group considered as non-susceptible to the disease (group B) a total of 47 and 64 bacterial and fungal isolates were obtained, respectively.

According to the morphology analysis, 67 isolates of bacteria were identified as Gram-positive cocci (CGP), 13 Gram-negative bacilli (BGN) and 02

Gram-positive bacilli (BGP) which were identified by biochemical tests and molecular analysis (Table 1). A set of 25 morphotypes were identified by sequencing and the phylogenetic tree (Figure 1) was built by Maximum Likelihood implemented in model Kimura 2 evolutionary parameters. A total of 1,416 sites were evaluated, and the empirical base frequencies were pi (A): 0.2535 pi(C): 0,2564, pi(G): 0.02564, pi(T): 0.2535, with 1000 bootstrap inferences. According to the topologies of the tree, the 25 bacterial morphotypes were distributed into 6 clades (Figure 1). The genus more frequently isolated was *Staphylococcus*, with 79.9% of isolation in group A and 78.4% in group B. However, the groups were differentiated in what concerns to the species and frequency of the isolates. In group A, S. epidermidis (65.7%), S. capitis (8.5%) and Staphylococcus sp. (5.7%) were observed, while in group B S. epidermidis (31.9%), S. capitis (38.2%), Staphylococcus sp. (6.3%) and S. hominis (2.1%) (Table1) were found.

Table 1- Bacterial and fungal isolates from children human scalp susceptible to pediculosis (group A) and non-susceptible to pediculosis (group B)

	Bacterial	Number of isolates	Frequency (%)	Fungal	Number of isolates	Frequency (%)
Group A	CGP			Aspergillus fumigatus	7	17.5
	Staphylococcus capitis	3	8.5	Aspergillus sp.	4	10
	Staphylococcus epidermidis	23	65.7	Aureobasidium sp.	1	2.5
	Staphylococcus sp.	2	5.7	Bipolaris sp.	1	2.5
	BGN			Candida parapsilosis	3	7.5
	Acinetobacter sp.	4	11.4	Chaetomium sp.	2	5
	Pseudomonas sp.	2	5.7	Cladosporium holotolerans	6	15
	Psychrobacter sp.	1	2.8	Cladosporium sp.	1	2.5
				Debaryomyces sp.	1	2.5
				Pestalotiopsis sp.	12	30
				Phoma sp.	2	5
	Total	35			40	
	CGP			Aspergillus fumigatus	5	7.8
	Klebsiella oxytoca	2	4.2	Aspergillus sp.	1	1.5
	Staphylococcus capitis	18	38.2	Aureobasidium sp.	2	3.1
	Staphylococcus epidermidis	15	31.9	Candida parapsilosis	5	7.8
	Staphylococcus hominis	1	2.1	Cladosporium holotolerans	10	15.6
	Sthaphylococcus sp.	3	6.3	Cladosporium sp.	3	4.6
Group	BGN			Cryptococcus sp.	1	1.5
В	Acinetobacter nasocomialis	1	2.1	<i>Curvularia</i> sp.	4	6.2
	Acinetobacter sp.	3	6.3	Debaryomyces sp.	10	15.6
	Pseudomonas stutzeri	2	4.2	Leptosphaerulina sp.	1	1.5
	BGP			Pestalotiopsis sp.	12	18.7
	Bacillus muralis	2	4.2	Phoma sp.	3	4.6
				Rhodotorula mucilaginosa	4	6.2
				Rhodotorula sp.	3	4.6
	Total	47			64	

Abbreviations used: Gram-positive cocci (CGP), Gram-negative bacilli (BGN) and Gram-positive bacilli (BGP).

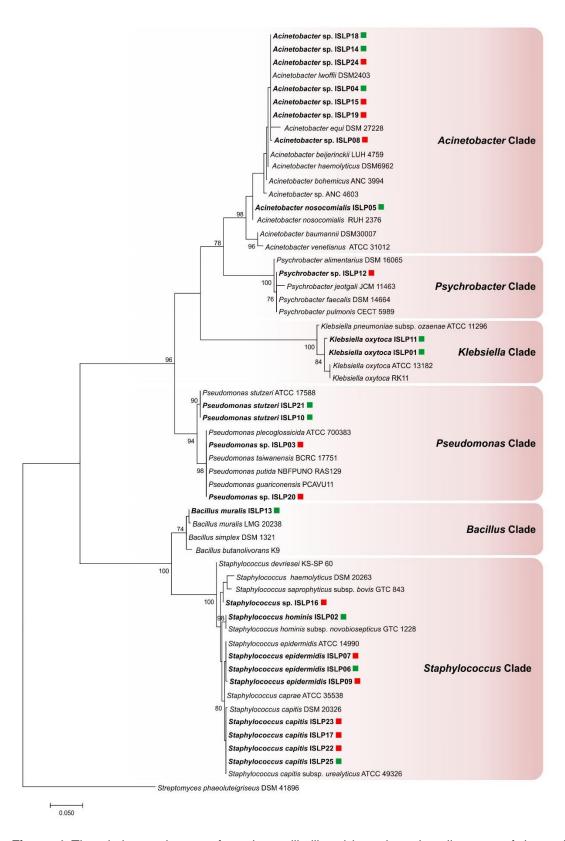


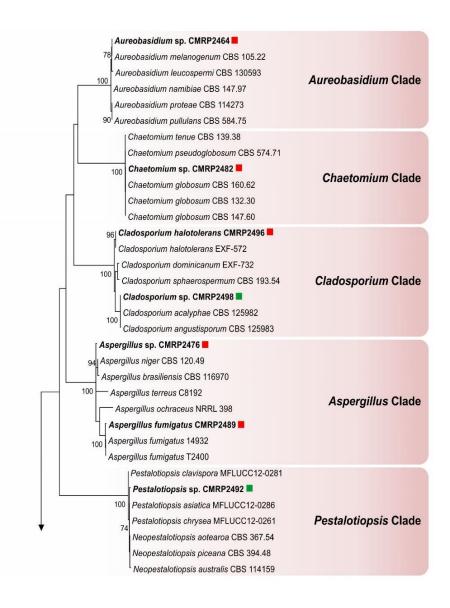
Figure 1 The phylogenetic tree of maximum likelihood based on the alignment of the entire region of 16S was built using 1000 bootstrap, using the evolutionary model Kimura 2 parameters with the software Mega version 7. *Streptomyces phaeoluteigriseus* DMS 41896 was used as an outgroup. The tree showed six clades of the genera *Acinetobacter*, *Psychrobacter*, *Klebsiella*, *Pseudomonas*, *Bacillus* and *Staphylococcus*, according to species diversity. Red square (group A); Green square (group B).

The statistical analysis demonstrated that there is no statistical difference between the microbiota of the two groups analyzed ($P \ge 0.05$). However, it was observed that *S. epidermidis* is numerically predominant in group A, while *S. capitis* in group B (Table 1). *Staphylococcus capitis* seems to be associated with protection to pediculosis as it was isolated in higher frequency in group B, with a significant difference (P < 0.01) in relation to group A ($P \ge 0.05$). In addition, in group B occurred in a higher diversity in relation to group A, demonstrated by the presence of bacteria such as *Staphylococcus hominis* (2.1%), *Klebsiella oxytoca* (4.2%), *Pseudomonas stutzeri* (4.2%), *Acinetobacter nosocomialis* (2.1%), and *Bacillus muralis* (4,2%), while in group A only *Pseudomonas* sp. (5.7%) and *Phychrobacter* sp. (2.8%) were found.

In relation to the fungal isolates, a total of 27 isolates were classified as yeast and 77 as filamentous. From these isolates, based on their morphological characteristics, these were initially classified into 16 groups (Table 1). A set of 16 morphotypes were identified by ITS sequences and compared with reference strains (Supplementary File 3). A selection of these strains was used to build a tree with Maximum Likelihood implemented in MEGA v. 7.0.4 using the Tamura + G substitution model. A total of 533 sites were evaluated, and the empirical base frequencies were pi (A): 0.2735 pi(C): 0.2264, pi (G): 0.02264, pi(T): 0.2735, with 1,000 bootstrap inferences (Figure 2). Among fungal isolates, it was observed that the fungus Debaryomyces sp. was more frequent in group B (15.6%) in relation to group A (2.5%). This isolate present significant difference to species and this difference also occur between the two groups analyzed (p < 0.01). Regarding diversity, the microorganisms that appeared only in group A were: Chaetomium sp. (5%), Bipolaris sp. (2.5%) and Aspergillus fumigatus (7.8%) and in group B, five different microorganisms were characterized: Curvularia sp. (6.2%), Rhodotorula mucilaginosa (6.2%), Cryptococcus sp. (1.5%), Rhodotorula sp. (4.6%), Leptosphaerulina sp. (1.5%). There were no statistical differences (P > 0.05) among these species amongst the groups studied.

In the gender analysis (males =10 and females = 10) of isolates from bacterial and fungal microbiota, it was verified that both groups analyzed presented similar numbers of microorganisms considering the fungi and bacteria species isolated (P > 0.05). However, group B presented a higher

diversity considering the total number of fungal and bacteria isolates (64 and 47,respectively).



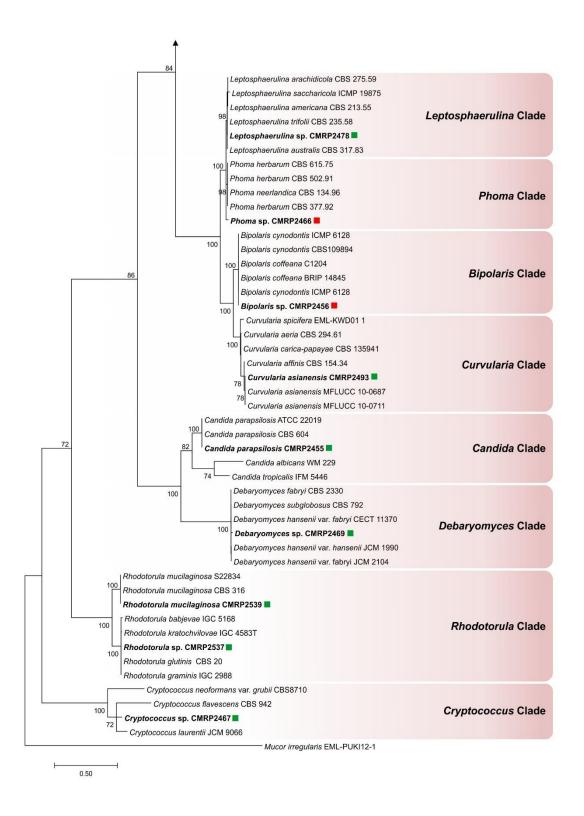


Figure 2 The phylogenetic tree of maximum likelihood based on the alignment of the entire region of ITS was built using 1000 bootstrap, using the evolutionary model Tamura + G substitution model with software Mega version 7. *Mucor irregularis* was used as an outgroup. The tree showed 13 clades of the genera *Aureobasidium*, *Chaetomium*, *Cladosporium*, *Aspergillus*, *Pestalotiopsis*, *Leptosphaerulina*, *Phoma*, *Bipolaris*, *Curvularia*, *Candida*, *Debaryomyces*, *Rhodotorula* and *Cryptococcus* according to the species diversity. Red square (group A); Green square (Group B).

4 DISCUSSION

This is the first study about the association of the microbiota scalp to the human pediculosis attraction. Previous studies have demonstrated that skin microbiota is important in the host-seeking behavior (Baviera et al., 2014; Verhulst et al., 2016, 2011). The attraction of insects occurs due the release of certain volatile compounds that are produced by bacteria; those compounds influence insect behavior. The combination of compounds such as carbon dioxide (CO2), ammonia, lactic acid and carboxylic acids are considered attractive to the insects (Okumu et al., 2010; Smallegange et al., 2009, 2005). In the opposite way, it is known that some bacterias cause metabolism inhibition of another bacterias and this may reduce the attraction of insects, such as in the malaria mosquitoes (Ara et al., 2006). This effect could be happening with the presence of S. capitis, isolated in major frequency in group B, where certain compounds such as short-chain fatty acids and aldehydes produced by this microorganism (Yamamoto et al., 1998) would be interfering with head lice infestations and, as a consequence, could be protecting the children against pediculosis.

According to the literature, *Staphylococcus* plays a role in the formation of fatty acids volatiles (James et al., 2004a, 2004b), which have a distinct sweaty odor. The *Staphylococcus* species can convert branched-chain amino acids to highly odorous short-chain fatty acids volatiles (James et al., 2004b) that play an important role in the host-seeking behavior of *Anopheles gambiae* (Verhulst et al., 2010). Therefore, more heterogenous microbiotas may include more bacterial species that produce volatiles attenuating the attraction, such as in group B which presented higher diversity of bacteria species.

Species such as *S. epidermidis* was isolated in both groups. However, it was predominant on the pediculosis scalps. This microorganism is closely related in the attraction of *Aedes* and *Anopheles* insects (Logan et al., 2008; Verhulst et al., 2010). Furthermore, species such as *Corynebacterium minutissimum*, *Brevebacterium epidermidis*, *Bacillus subtilis*, *Leptotrichia* sp., *Delftia* sp. and *Actinobacteria* sp., have also been considered attractive to mosquitoes (Smallegange et al., 2011; Verhulst et al., 2016, 2011, 2010).

Genre such as *Pseudomonas* sp., *P. aeruginosa* and *Variovorax* sp. have been able to attenuate the attraction of mosquitoes (Smallegange et al., 2011; Verhulst et al., 2010, 2011, 2016) due to emission signals to other bacteria in ways that it prevents them from emitting the attractive compounds, as reported by Verhulst et al. (2011). It suggested that *Pseudomonas stutzeri* isolates observed in group B could be involved in producing compounds that repel head lice or mask the effect of the attractive volatiles emanating from the human skin.

In addition, studies demonstrate that volatile organic compounds interfere in the oviposition of the insects such as in *A. gambiae* mosquitoes associated to the malaria disease (Davis et al., 2013). Therefore, it is possible that some compounds produced by group B microbiota could interfere in the oviposition of *P. humanus capitis* and thus, likewise, interfere in the susceptibility to pediculosis.

Regarding the fungal microbiota, in the group with predisposition to pediculosis (group A), among isolates (n=40) of different species (Table1) it was observed that *Bipolaris* sp. and *Chaetomium* sp. were specifically found in this group, while the species *Cryptococcus* sp., *Curvularia* sp., *Leptosphaerulina* sp., *Rhodotorula* sp., *R. mucilaginosa* were isolated only in group B assumed as with low predisposition to pediculosis.

Gao et al. (2010) developed quantitative PCRs to enumerate the total bacterial and fungal populations in skin. Apparently, the diversity and heterogeneity of fungal microbiota may be interfering in the individual preference of head lice as observed in the groups analyzed in our study. The literature revealed that methyl eugenol produced by plants is considered an effective compound in head lice control (Bagavan et al., 2011) and has been used in combination with other insecticides (Kaul et al., 2008). Furthermore, related to pediculicides, the most toxic compound to the female of *P. humanus capitis* is eugenol followed by methyl salicylate (Bagavan et al., 2011). This compound has already been observed being produced by the fungus *Alternaria* sp. (Kaul et al., 2008). The behavioral analysis of the body lice (*Pediculus humanus humanus*) in response to clove essential oil and its constituents revealed that eugenol has a strong repellent effect on body lice. Methyl eugenol may have a weak repellent effect on body lice, however eugenol alone has a

strong repellent effect in the species (Bagavan et al., 2011; Iwamatsu et al., 2016).

Isoeugenol is another compound that suppresses progeny development of the coleopterans *Tribolium castaneum* and *Sitophilus zeamais* (Ho et al., 1994). Eugenol also exhibited potent acaricidal activity against mite species such as *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* (Kim et al., 2003).

Therefore, the specific group of fungus found in group B needs to be investigated relating their activity to pediculosis control. Among the fungus isolated, only *Debaryomyces* sp. presented statistical difference between the two groups studied with higher frequency in group B. Although this fungus is isolated from human skin (Grice and Segre, 2011), air, soil, seawater, plants, insects and the guts of vertebrates (Suzuki et al., 2011), in this study, the results suggested that this microorganism could be related to the susceptibility to pediculosis with a potential of protecting host against *P. humanus capitis*.

Debaryomyces sp. has the ability to lipid-accumulate (Johnson and Echavarri-Erasun, 2011) and could generate ammonia and several volatile compounds of methyl group derivatives, volatile phenols, terpenes, alcohols, aldehydes, acetates, volatiles acids, ethyl esters, Sulphur compounds and hidrocarbons (Salgado et al., 2012; Satyanarayana and Kunze, 2009). Species such as Debaryomyces hansenii, Cryptococcus sp. and Rhodotorula mucilaginosa have been reported as assimilators of L-methionine as a sole nitrogen source and producers of volatile organic compounds (VOCs) such as ammonia, 2-methyl butanol, 3-methyl butanol, methanethiol, S-methyl thioacetate, dimethyl sulfide, dimethyl disulfide, dimethyl trisulfide, dihydro-2-methyl-3(2H)- thiophenone and 3-(methylthio)-1-propanol (MTP) (Buzzini et al., 2005). Therefore, we believe that these volatile compounds should be investigated against head lice.

The human skin has various microorganisms (Findley et al., 2013; Grice and Segre, 2011). Most of the fungal flora found in the two groups analyzed presents environmental occurrence and/or are related as opportunistic human infection (Bauters et al., 2002; Bensch et al., 2010; Fellner, 2013; Lagacé and Cellier, 2012; Manfredi et al., 2006; Schubert et al., 2007; Shankar et al., 2006) e.g., *Pestalotiopsis* sp. found in 30% in group A and 18.7% in group B which

have been observed as plant pathogens, saprobes and endophytic (Ji-Guang et al., 2007; Maharachchikumbura et al., 2014). Moreover, the isolates of *Candida parapsilosis*, *Phoma* sp., *Rhodotorula mucilaginosa*, *Aureobasidium* sp., *Cladosporium* sp., *Pestalotiopsis* sp., *Rhodotorula mucilaginosa*, *Candida parapsilosis* (Table 1) have already been mentioned in the literature as possible agents of seborrheic dermatitis (Tanaka et al., 2014) and *Aspergillus fumigatus* (Table 1) associated to aspergillosis (Balajee et al., 2005; Järv et al., 2004; Pfaller and Diekema, 2004). The diversity in fungal microbiota observed could have a positive ecological aspect concerning host susceptibility helping protection against pediculosis.

The composition of female and male microbiota was not significantly different when compared as a factor for pediculosis. Considering that susceptibility to pediculosis is multifactorial (Feldmeier, 2012), head lice prevalence has been associated with factors such as poor socioeconomic status and hair shapes (Nazari et al., 2016). The literature has reported that females are usually more infested by head lice due to hair length (Feldmeier, 2012; Mumcuoglu et al., 2001). In our study, gender seemed not to interfere in the composition of microbiota, however, our sampling had reduced numbers of individuals. Therefore, it is important to emphasize that the relation between gender and microbiota should be further investigated

In conclusion, this study, suggested that the diversity of microbiota represented, by bacterial species such as *Staphylococcus hominis*, *Klebisiella* sp., *Pseudomonas stutzeri*, *Acinetobacter nosocomialis*, and *Bacillus muralis* and fungal species such as *Cryptococcus* sp., *Curvularia* sp., *Leptosphaerulina* sp., *Rhodotorula* sp., *R. mucilaginosa* found only in individuals with low predisposition to pediculosis could be related to the protection of this disease. This evidence, in the features, can contribute to new alternatives to combat this insect, such as the production of specific products, by secondary metabolites that will promote the decrease of certain scalp microorganisms. Moreover, this study is pioneer to indicate the bacterial and fungal potential against pediculosis.

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GENERAL CONCLUSIONS AND PERSPECTIVES

Despite being a public health problem and affecting thousands of people in the world, the prevalence of pediculosis is the first time reported in children from Southern of Brazil. Although Curitiba and metropolitan region presents high socioeconomic index, it was reported a high prevalence of this parasitosis. These results will be sent to the health authorities in order to implement public policies in an effective program of pediculosis control in this region.

The results prove that gender, length, type and color of hair strands interfere in the predisposition to pediculosis. It is the first time the diameter and scales are indicated as conditions that can interfere in the predisposition to pediculosis. There is a technical difficulty to observe the capillary scales, but it is not difficult to observe the capillary diameter. This fact can be used to indicate which children are more susceptible to pediculosis. With this information it may be noticed that children with thin hair should have better prophylactic measures face to the pediculosis.

It is considered that microbiota scalp diversity could interfere in the susceptibility to pediculosis. It is possible to continue the study to suggest the inclusion of products in the capillary hygiene for the control of pediculosis.

This study is part of a larger extension project whose objective was to meet a demand from the school community (parents and teachers), which questioned the possible causes of pediculosis.

In conclusion, it is believed that with the results obtained in this study, could be used to help in the implementation of public policies for the control and control of lice in schoolchildren.

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APPENDIX

CHAPTER III

APPENDIX 1- QUESTIONNAIRE

The project titled "Different aspects of susceptibility to pediculosis" aims to investigate why some children are more affected by lice than others. Therefore, it is intended to study if certain characteristics of the hair can influence the predisposition to the disease. For this research to be carried out, it will be necessary to remove hair strands for analysis. Note: You may select more than one alternative to the questions below.

more than one alternative to the questions below.
Parents's or responsable full name
2. Child's full name:
3. Gender of the child: () Male () Female
4. School's name
5. Child's age
 6. Your child: () Never had lice, despite living with other children with head lice () Does not had lice even living with a family member who had or is having lice () Have lice at the moment () Had several times lice this year () Had several times lice in the previous year () Not have with lice at the moment, but has had
7. You rate your child's hair as: () Straight () Wavy () Curly
8. The color of your child's hair is:() Dark (light brown to black)() Light (blonde)() Red
9. Your child has hair:() Short (hair near the scalp)() Medium (hair up to shoulder height)() Long (hair below shoulder height

APPENDIX 2- Table 2- General sample description

		Prese	ence of			ence o		
		Pedic	ulosis		Ped	liculosis	3	
		n	%	IC	n	%	IC	Р
Gender	Boys	37	36,6	27,7-	34	64,1	50,6-	
C 0.1.0.0.	20,0	•	23,3	46,3		0 ., .	76,2	
	Girls	64	63,3	53,6-	19	35,8	54,5-	
			·	72,3			79,4	
Years	5-8	63	62,3	52,6-	36	67,9	54,5-	
				71,4			79,4	
	9-11	38	37,6	28,6-	17	32	20,1-	
				47,4			45,5	
Color hair	Dark	84	83,2	74,9-	44	83	71,1-	
				89,5			91,4	
	Light	15	14,8	8,9-	9	17	8,6-	
				22,8			28,9	
	Red	2	2	0,3-	0	0	0	
				6,4				
Length hair	Short	43	42,6	33,2- 52,4	35	66	52,6- 77,8	
riali								
	Medium	26	25,7	17,9- 34,9	5	9,4	3,5- 19,7	
	Lana	20	24.7		40	24.5		
	Long	32	31,7	23,2- 41,2	13	24,5	14,4- 37,4	
Hair type	Straight	59	58,4	48,6-	35	66	52,6-	
пан туре	Straight	39	56,4	46,0- 67,7	33	00	77,8	
	Wavy	24	23,8	16,2-	9	17	8,6-	
	,		_0,0	32,8	·		28,9	
	Curly	18	17,8	11,7-	9	17	8,6-	
	·			26,2			28,9	
Diameter		76			81			
hair								
Scales	Narrow	48	47,5	39,7-	22	41,5	28,9-	
				53,3			55,1	
	Medium	46	45,5	36,0-	29	54,7	41,2-	
				55,3			67,7	
	Long	7	6,9	3,1-	2	3,8	0,6-	

			13,2			11,9
Straight	0	0		0	0	
Wanted	101	100	97,1- 100	53	100	94,5- 100
Smooth	0	0		0	0	
Ornate	101	100	97,1- 100	53	100	94,5- 100
Full borders and incomplete borders	101	100	97,1- 100	53	100	94,5- 100

CI- confidence interval; p<0,05 for all.

APPENDIX 3- Table 3- Boys 5-8 years

		Presen	ce of Pe	ediculosis	Abse	Absence of Pediculosis			
		n	%	IC	n	%	IC	Р	
Color hair	Dark	23	95,8	81,1-99,8	18	78,2	58,2- 91,6		
	Light	0	0		5	21,7	8,4- 43,7		
	Red	1	4,1	0,2-18,9	0	0			
Length hair	Short	24	100	88,3-100	23	100	87,8- 100		
	Medium	0	0		0	0			
	Long	0	0		0	0			
Hair type	Straigh	20	83,3	64,5-94,5	21	91,3	74,1- 98,5		
	Wavy	1	4,1	0,2-18,9	0	0			
	Curly	3	12,5	3,3-30,4	2	8,6	1,5- 25,9		
Diameter hair		78,9			82,5				
Scales	Narrow	12	12,5	6,9-20,3	7	7,6	3,4- 14,5		
	Medium	10	10,4	5,4-17,8	15	16,3	9,8- 24,9		
	Long	2	2,0	0,3-6,7	1	1,0	0,05- 5,2		
	Straight	0	0		0	0			
	Wanted	24	25	17,1-34,4	23	25	16,9- 34,6		
	Smooth	0	0		0	0			
	Ornate	24	25	17,1-34,4	23	25	16,9- 34,6		
	Full borders and incomplete borders	24	25	17,1-34,4	23	25	16,9- 34,6		

APPENDIX 4- Table 4- Boys 9-11 years

		Prese	ence of	Pediculosis	Abse	nce of F	Pediculosis	
		n	%	IC	n	%	IC	Р
Color hair	Dark	12	92,3	67,5-99,6	11	100	76,2-100	
	Light	1	7,6	0,4-32,5	0	0		
	Red	0	0		0	0		
Length hair	Short	13	100	79,4-100	11	100	76,2-100	
	Medium	0	0		0	0		
	Long	0	0		0	0		
Hair type	Straigh	9	69,2	41,3-89,4	8	72,7	42,2-92,5	
	Wavy	2	15,3	2,7-42,2	1	9,0	0,4-37,3	
	Curly	2	15,3	2,7-42,2	2	18,1	3,2-48,3	
Diameter		80,8			86,6			
Scales	Narrow	7	13,4	6,1-24,8	5	11,3	4,3-23,4	
	Medium	6	11,5	4,8-22,5	6	13,6	5,7-26,2	
	Long	0	0		0	0		
	Straight	0	0		0	0		
	Wanted	13	25	14,7-38,0	11	25	13,9-39,3	
	Smooth	0	0		0	0		
	Ornate	13	25	14,7-38,0	11	25	13,9-39,3	
	Full borders and incomplete borders	13	25	14,7-38,0	11	25	13,9-39,3	

APPENDIX 4- Table 5- Girls 5-8 years

		Prese	nce of Pedic	ulosis	Abser	nce of Pec	liculosis	
		n	%	IC	n	%	IC	Р
Color hair	Dark	31	79,4	64,7-89,9	11	84,6	57,8-97,3	
	Light	7	17,9	8,2-32,3	2	15,3	2,7-42,2	
	Red	1	2,5	0,1-12	0	0		
Length hair	Short	4	10,2	3,3-22,9	0	0		
	Medium	15	38,4	24,3-54,3	3	23	6,2-50,9	
	Long	20	5,1	35,8-66,6	10	15,3	49,1-93,8	
Hair Type	Straigh	17	43,5	28,8-59,3	5	38,4	15,7-65,9	
	Wavy	11	28,2	15,8-43,7	5	38,4	15,7-65,9	
	Curly	11	28,2	15,8-43,7	3	23	6,2-50,9	
Diameter		71,2			74,8			
Scales	Narrow	20	12,8	8,2-18,8	6	11,5	4,8-22,5	
	Medium	16	10,2	6,2-15,8	6	11,5	4,8-22,5	
	Long	3	1,9	0,5-5,2	1	1,9	0,1-9,1	
	Straight	0	0		0	0		
	Wavy	39	25	18,7-32,2	13	25	34,8-61,8	
	Smooth	0	0		0	0		
	Ornate	39	25	18,7-32,2	13	25	34,8-61,8	
	Full borders and incomplete borders	39	25	18,7-32,2	13	25	34,8-61,8	

APPENDIX 5- Table 6 Girls 9-11 years

		Prese	ence of	Pediculosis	Absence of Pediculosis			
		n	%	IC	n	%	IC	Р
Color hair	Dark	18	72	52,3-86,8	4	66,6		
	Light	7	28	13,1-47,7	2	33,3		
	Red	0	0		0	0		
Length hair	Short	2	8	1,4-24	1	16,6		
	Medium	11	44	25,7-63,6	2	33,3		
	Long	12	48	29,2-67,2	3	50		
Tipo pelo	Straigh	13	52	32,7-70,8	1	16,6		
	Wavy	10	40	22,4-59,8	3	50		
	Curly	2	8	1,4-24	2	33,3		
Diameter		78,9			74,9			
Scales	Narrow	9	9	4,5-15,9	4	16,6	5,5- 35,5	
	Medium	14	14	8,2-21,9	2	8,3	1,4- 24,9	
	Long	2	2	0,3-6,4	0	0		
	Straight	0	0		0	0		
	Wavy	25	25	17,3-34,2	6	25	10,8- 44,9	
	Smooth	0	0		0	0		
	Ornate	25	25	17,3-34,2	6	25	10,8- 44,9	
	Full borders and incomplete borders	25	25	17,3-34,2	6	25	10,8- 44,9	

CHAPTER IV

APPENDIX 1- Table 1- List of reference strains GenBank and scalp bacterial isolates

Species	Strain no.	Isolate source	Country	GenBank no.
Acinetobacter baumannii	DSM 30007	Urine	Unknown	X81660
Acinetobacter beijerinckii	LUH 4759T	Human scalp	Unknown	AJ626712.1
Acinetobacter bohemicus	ANC 3994	Deciduous forest soil	Czech Republic	KF679797.1
Acinetobacter equi	114	Horse faeces	Germany	KC494698.1
Acinetobacter genomosp.	ANC 4603	Unknown	Unknown	KX548338.1
Acinetobacter haemolyticus	DSM 6962	Human scalp	Unknown	X81662.1
Acinetobacter Iwoffii	DSM2403	Unknown	Unknown	X81665.1
Acinetobacter nosocomialis	ISLP05	Human scalp	Brazil	MF125018
Acinetobacter nosocomialis	RUH 2376	Sputum of an inpatient	Rotterdam	HQ180192.1
Acinetobacter sp.	ISLP15	Human scalp	Brazil	MF125028
Acinetobacter sp.	ISLP04	Human scalp	Brazil	MF125017
Acinetobacter sp.	ISLP24	Human scalp	Brazil	MF125037
Acinetobacter sp.	ISLP18	Human scalp	Brazil	MF125031
Acinetobacter sp.	ISLP14	Human scalp	Brazil	MF125027
Acinetobacter sp.	ISLP08	Human scalp	Brazil	MF125021
Acinetobacter sp.	ISLP19	Human scalp	Brazil	MF125032
Acinetobacter venetianus	ATCC 31012	Tar on beach	Unknown	AJ295007.1
Alicyclobacillus herbarius	NBRC 100860	Dried flowers, Hibiscus	Unknown	NR_113949.1
Bacillus butanolivorans	K9	Soil	Lithuania	EF206294.1
Bacillus huizhouensis	GSS03	Paddy field soil	Unknown	KJ464756.1
Bacillus muralis	ISLP13	Human scalp	Brazil	MF125026
Bacillus muralis	LMG 20238	Biofilm covering a mural painting	Germany	AJ316309.1
Bacillus simplex	DSM 1321T	Unknown	Unknown	AJ439078.1

Klebsiella michiganensis	W14	Tooth brush holder	USA	JQ070300.1
Klebsiella oxytoca	ISLP11	Human scalp	Brazil	MF125024
Klebsiella oxytoca	ATCC 13182	Pharyngeal tonsil	unknow	AF129440.1
Klebsiella pneumoniae subsp				
ozaenae	ATCC 11296	Unknown	Unknown	JQ070300.1
Klebsiella sp.	ISLP01	Human scalp	Brazil	MF125014
		Host rhizospheric soil	Venezuela:El	
Pseudomonas guariconensis	PCAVU11	Host mizospheric soil	Guarico	HF674459.1
Pseudomonas plecoglossicida	ATCC 700383	Diseased ayu	Japan	AB009457
Pseudomonas putida	NBFPUNO_RAS129	Unknown	Unknown	KJ819572.1
Pseudomonas sp.	ISLP03	Human scalp	Brazil	MF125016
Pseudomonas sp.	ISLP21	Human scalp	Brazil	MF125034
Pseudomonas sp.	ISLP20	Human scalp	Brazil	MF125033
Pseudomonas stutzeri	ISLP10	Human scalp	Brazil	MF125023
Pseudomonas stutzeri	LMG 11199	Sewage water	Spain	AF094748.1
Pseudomonas taiwanensis	BCRC 17751	Soil	Taiwan	AB009457.1
Psychrobacter alimentarius	JG-100	Squid jeotgal	Korean	AY513645.1
Psychrobacter faecalis	Iso-46	Bioaerosol	Germany	AJ421528.1
		Jeotgal, traditional Korean		
Psychrobacter jeotgali	JCM 11463	fermented seafood	Unknown	AF441201.1
Psychrobacter pulmonis	CECT 5989T	Ovis aries	Spain	AJ437696.1
Psychrobacter sp.	ISLP12	Human scalp	Brazil	MF125025
Staphylococcus capitis	ISLP17	Human scalp	Brazil	MF125030
Staphylococcus capitis	ISLP22	Human scalp	Brazil	MF125035
Staphylococcus capitis	ISLP25	Human scalp	Brazil	MF125038
Staphylococcus capitis	DSM 20326	Human skin	Unknown	L37599.1
Staphylococcus capitis subsp.				
urealyticus	ATCC 49326	Human skin	Unknown	AB009937.1

Staphylococcus sp.	ISLP16	Human scalp	Brazil	813.0.1
				45813
bovis	GTC 843	Cow	Unknown	AB233327.1
Staphylococcus saprophyticus subsp) .			
Staphylococcus saccharolyticus	ATCC 14953	Plasma	Unknown	L37602.1
novobiosepticus	GTC 1228	Human skin	North Carolina	AB233326.1
Staphylococcus hominis subsp).	•		
Staphylococcus hominis	ISLP02	Human scalp	Brazil	MF125015
Staphylococcus haemolyticus	DSM 20263	Human skin	Unknown	X66100.1
Staphylococcus epidermidis	ATCC 14990	Nose	Unknown	D83363.1
Staphylococcus epidermidis	ISLP09	Human scalp	Brazil	MF125022
Staphylococcus epidermidis	ISLP07	Human scalp	Brazil	MF125020
Staphylococcus epidermidis	ISLP23	Human scalp	Brazil	MF125036
Staphylococcus epidermidis	ISLP06	Human scalp	Brazil	MF125019
Staphylococcus devriesei	KS-SP 60	Milk cow	Unknown	FJ389206.1
Staphylococcus caprae	ATCC 35538	Goat milk	France	AB009935.1

APPENDIX 2- Table 3- List of reference strains GenBank and scalp fungal isolates.

Species	Strain no.	Isolate source	Country	GenBank no.
Aureobasidium leucospermi	CBS 116970	Production plant	Netherlands	FJ629324.1
Aspergillus brasiliensis	14932	Clinical isolate	Unknown	AF078892.1
Aspergillus fumigatus	CMRP2489	Human scalp	Brazil	MF154606
Aspergillus fumigatus	T2400	Clinical isolate	Unknown	AF078891.1
Aspergillus fumigatus	CBS 120.49	Unknown	Unknown	FJ629338.1
Aspergillus niger	NRRL 398	Unknown	Unknown	NR_077150.1
Aspergillus ochraceus	CMRP2476	Human scalp	Brazil	MF154605
Aspergillus sp.	C8192	Environmental	Unknown	AF078896.1
Aspergillus terreus	CBS 105.22	Unknown	Unknown	KT693729.1
Aureobasidium melanogenum	CBS 147.97	Dolomitic marble	Namibia	KT693730.1
Aureobasidium namibiae	CBS 114273	Leaves of Protea	South Africa	KT693731.1
Aureobasidium proteae	CBS 584.75	<i>Vitis vinifera</i> , fruit	France	KT693733.1
Aureobasidium pullulans	CMRP2464	Human scalp	Brazil	MF154607
		Leaves of Leucospermun	า	
Aureobasidium sp.	CBS 130593	conocarpodendron	South Africa	KT693727.1
Bipolaris coffeana	BRIP 14845	Coffea arabica	Kenya	KJ415525.1
Bipolaris coffeana	C1204	Cynodon dactylon	USA	KM230385.1
Bipolaris cynodontis	CBS109894	Cynodon dactylon	Hungary	KJ909767.1
Bipolaris cynodontis voucher	ICMP 6128	Cynodon dactylon	Australia	JX256412.1
<i>Bipolaris</i> sp	CMRP2456	Human scalp	Brazil	MF154608
Bipolaris cynodontis voucher	ICMP 6128	Cynodon dactylon	Australia	JX256412.1
Candida parapsilosis	CBS 604	Patient with candidemia	Puerto Rico	FJ872016.1
Chaetomium globosum	CBS 147.60	Raincoat	USA	JN209909.1
Curvularia asianensis	MFLUCC 10-0711	Panicum sp.	Thailand	JX256424.1
Candida albicans	WM 229	ISHAM-ITS_ID MITS475	Unknown	EF567995.1

Candida parapsilosis	ATCC 22019	Patient with candidemia	Unknown	FJ872015.1
Candida parapsilosis	ATCC 22019	Unknown	Unknown	KF313185.1
Candida parapsilosis	CMRP2455	Human scalp	Brazil	MF154609
Candida tropicalis	IFM 5446	Patient with bronchomycosis	USA	AB437068.1
Chaetomium globosum	CBS 132.30	Clay soil	USA	KC109755.1
Chaetomium globosum	CBS 160.62	Compost	Germany	KT214565.1
Chaetomium pseudoglobosum	CBS 574.71	Unknown	Unknown	KT214573.1
Chaetomium sp.	CMRP2482	Human scalp	Brazil	MF154610
Chaetomium tenue	CBS 139.38	Unknown	Unknown	KT214568.1
Cladosporium acalyphae	CBS 125982	Acalypha australis	South Korea	HM147994.1
Cladosporium angustisporum	CBS 125983	Alloxylon wickhamii	Australia	HM147995.1
	EXF-732 CBS	Hypercaline water of calterns	Dominican	
Cladosporium dominicanum	119415	Hypersaline water of salterns	Republic	DQ780353.1
Cladosporium halotolerans	EXF-572	Hypersaline wate	Namibia	DQ780364.1
Cladosporium halotolerans	CMRP2496	Human scalp	Brazil	MF154611
Cladosporium sp.	CMRP2498	Human scalp	Brazil	MF154612
Cladosporium sphaerospermum	CBS 193.54	Man; nail	Netherlands	DQ780343.1
Cryptococcus flavescens	CBS 942	Air	Japan	AB035046.1
Cryptococcus gattii	AFLP6_CBS10514	Bronchial wash of male patient	Canada	JN943780.1
Cryptococcus laurentii	JCM 9066	Palmvine	Congo	AB035043.1
Cryptococcus neoformans var			_	
grubii	CBS8710	Clinical	USA	EF211144.1
Cryptococcus sp.	CMRP2467	Human scalp	Brazil	MF154613
Curvularia aeria	CBS 294.61	Air	Brazil	HF934910.1
Curvularia affinis	CBS 154.34	Unknown	Indonesia	HG778981.1
Curvularia affinis	CRMP2493	Human scalp	Brazil	MF154614
Curvularia asianensis	MFLUCC 10-0687	Oryza sativa	Thailand	JX256422.1

Curullaria parios nanovas	CBS 135941	Leaves of Carios papers	India	HG778984.1
Curvularia carica-papayae		Leaves of Carica papaya		
Curvularia spicifera	EML-KWD01	Triticum aestivum	Korea	KT351794.1
Debaryomyces fabryi	CBS:2330	Spoiled sake	Japan	KY103188.
Debaryomyces hansenii var. fabry		Interdigital mycotic lesion	Germany	AJ586530.1
Debaryomyces hansenii var. fabry	i M 5011 JCM 2104	Human mycotic lesion	Germany.	AB018042.1
Debaryomyces hansenii vai	=			
hansenii	M 5012 JCM 1990	Unknown	Unknown	AB018041.1
Debaryomyces sp.	CMRP2469	Human scalp	Brazil	MF154619
Debaryomyces subglobosus	CBS:792	Infected nail	Austria	FN675240.1
Leptosphaerulina americana	CBS 213.55	Trifolium pretense	Georgia	GU237799.1
Leptosphaerulina arachidicola	CBS 275.59	Arachis hypochea	Unknown	GU237820.1
Leptosphaerulina australis	CBS 317.83	Eugenia aromatica	Indonesia	GU237829.1
		Leaves of Saccharum officinarum		
Leptosphaerulina saccharicola	ICMP:19875	L.	Thailand	KF670717.1
Leptosphaerulina sp.	CR2478	Human scalp	Brazil	MF154615
Leptosphaerulina trifolii	CBS 235.58	Trifolium sp.	Netherlands	GU237806.1
Mucor irregularis	EML-PUKI12-1	Gut of soldier fly larvae	Korea	KY047151.1
Neopestalotiopsis australis	CBS 114159	Telopea sp.	Australia	KM199348.1
•	CBS 394.48	Picea sp.	UK	KM199368.1
	CBS 367.54	Canvas	New Zealand	KM199369.1
•	MFLUCC12-0286	Dead plant material	China	JX398983.1
•	MFLUCC12-0261	•	China	JX398985.1
• •	MFLUCC12-0281	•	China	JX398979.1
•	CMRP2492	•	Brazil	MF154616
		•		GU237874.1
		•		FJ427022.1
				KT389536.1
	ICMP:19875 CR2478 CBS 235.58 EML-PUKI12-1 CBS 114159 CBS 394.48 CBS 367.54 MFLUCC12-0286 MFLUCC12-0261 MFLUCC12-0281	Leaves of Saccharum officinarum L. Human scalp Trifolium sp. Gut of soldier fly larvae Telopea sp. Picea sp.	Thailand Brazil Netherlands Korea Australia UK New Zealand China China China	KF670717 MF154618 GU23780 KY047152 KM19936 KM19936 JX398983 JX398985 JX398979 MF154610 GU237878 FJ427022

Phoma neerlandica	CBS 134.96	<i>Delphinium</i> sp.	The Netherlands	KT389535.1
<i>Phoma</i> sp.	CMRP2466	Human scalp	Brazil	MF154620
Rhodotorula babjevae	IGC 5168T	Herbaceous plant	Russia	AF387774.1
Rhodotorula kratochvilovae	IGC 4583T	Unknown	Unknown	AF387783.1
Rhodotorula mucilaginosa	CRMP2539	Human scalp	Brazil	MF154618
Rhodotorula mucilaginosa	S22834	Water	Unknown	EU871493.1
Rhodotorula sp.	CMRP2537	Human scalp	Brazil	MF154617
Rhodotorula glutinis	CBS 20	Air	Unknown	AF387775.1
Rhodotorula graminis	IGC 2988	Unknown	Unknown	AF387779.1
Rhodotorula mucilaginosa	CBS 316	Unknown	Unknown	NR_073296.1