Pediculus humanus capitis as a potential vector for *Acinetobacter baumannii*

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Abstract

Introduction: The presence of bacterial pathogens in the human head louse, *Pediculus humanus capitis*, has been subject to intense research in the last decade. Of particular interest, investigations from several countries in Europe, Asia, and Africa coincide in reporting *Acinetobacter baumannii*, a known opportunistic bacterium causing frequent health-care associated outbreaks. No reports from countries in South America have been published and is important to confirm whether this pathogen is also present in head lice in this continent.

Objective: The aim of the present study was to determine whether *Acinetobacter baumannii* was present in head lice specimens collected from three countries in Latin America.

Methods: 123 vials containing 368 head lice collected from Argentina, Colombia, and two locations in Honduras (La Hicaca and San Buenaventura), were analyzed in pools using PCR to determine the presence of *A. baumannii* DNA.

Results: Two vials containing 3 insects halves each from La Hicaca, Honduras were positive for *A. baumannii*. Since lice were analyzed in pools, it is not possible to calculate an exact frequency of infection in lice. However, based on whether per each vial one, two or all three lice were positive for *A. baumannii,* a range can be determined between 1.83 - 5.50%. positivity. Genetic sequencing was used to verify our positive results. The remaining lice from Argentina, Colombia, and San Buenaventura; Honduras were identified as negative for the DNA of *A. baumannii* under the test conditions described.

Conclusion: This study is the first to report the presence of *Acinetobacter* DNA in human head lice from Latin America. Further investigations are required to elucidate the significance of this finding.

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KEY TERMS

Ectoparasite: a parasite that lives on their host

Hematophagous: consumes blood

Incomplete metamorphosis: a type of insect development which starts by being hatched from an egg and includes several nymphal stages before maturation into an adult form

Infestation: the presence of an organism regarded as a pest or the presence of an ectoparasite

Infection: invasion and multiplication of an infectious agent which could manifest in disease

Infectious agent: organisms that are capable of producing infection or infectious disease

Nosocomial: originating in a hospital setting; also referred to as healthcare-associated infections (HAI)

Nymph: in entomology, sexually immature form similar to the adult; an insect life stage during incomplete metamorphosis

Pathogen: an agent that may cause disease or illness

Pediculicide: A chemical agent for eradicating lice

Vector: an organism that acts as an intermediary host for an infectious agent or parasite

Vectorial competence: the ability of a vector to transmit a specific infectious agent

CHAPTER 1: INTRODUCTION

Understanding arthropods as vectors of etiologic agents of disease has been crucial for the survival of humans. To this day, their unique ability to harbor and transmit pathogens such as bacteria, fungi, parasites, and viruses pose an immense threat to the health and wellbeing of humans and animals alike. Still, there are arthropods being investigated for their potential vectorial competence, or their ability to become infected, maintain, and transmit disease (CDC, 2018a). The discovery of pathogens within an arthropod may have big implications, which prompts further investigation in order to bridge gaps in the literature. Among the many groups of arthropods affecting humans, lice —both body lice, causing pediculosis corporis and head lice, causing pediculosis capitis— are unique in that they have been traced as human companions for millennia. Both belong to the genus *Pediculus* and are hematophagous, obligate, wingless, ectoparasites that belong to Order *Phthiraptera*: *Anoplura*. They are members of the subgroup of sucking lice, as opposed to chewing lice that infest non-human animals. *Pediculus* spp. complete their entire life cycle on their human hosts— where they go through several stages of development (egg, nymphal stages 1-3, and adult male and female). The infestation with these ectoparasites is named pediculosis.

Pediculosis corporis is the infestation of a person with *Pediculus humanus humanus* (Linnaeus, 1758), known as the body louse, that lives and completes its life cycle on the clothing of humans. Infestation with this ectoparasite can cause pruritis, and subsequently skin lesions due to scratching, as well as occasionally rashes due to an inflammatory response by the host. Pediculosis corporis is more commonly found in people who live in crowded conditions and lack access to regular bathing and clean clothing; they are an important affliction of the homeless. Body lice are of great medical importance as they are biological vector of important pathogens: the etiologic agents of trench fever, louse-borne relapsing fever, and louse-borne typhus (CDC, 2017). The first evidence of *P. h. humanus* acting as a vector for louse-borne relapsing fever was described by F. Percival Mackie in 1907 (Mackie, 1907). In 1928 Charles Nicole received a Nobel Prize for discovering that *P. h. humanus* was the link missing in understanding the transmission of *Rickettsia prowazekii*, the etiologic agent for Epidemic typhus, or louse-borne typhus (Schultz, 2009). The role *P. h. humanus* has in the transmission of *Bartonella quintana*, the etiologic agent of trench fever, was first described in the British Medical Journal by Pappenheimer and his colleagues in 1917, and later confirmed by Arkwright and Duncan in 1918 (Arkwright & Duncan, 1918; Pappenheimer et al., 1917).

Pediculosis capitis is the infestation of the head (scalp and hair) with head lice. This infestation occurs in a broader demographic range although it is predominant in children, especially those attending day-cares and schools. Those afflicted with pediculosis capitis may experience symptoms such as scalp itching, irritability, difficulty concentrating, sleep disturbances, and potentially secondary bacterial infections as a result of the abrasion caused by constant scratching (CDC, 2017). Moreover, head lice infestations are known to cause stigma and discrimination, thus affecting mental health and overall well-being of those infested. Among children, the infestation tends to be predominant in girls due to several factors including

behavioral norms, gender construct roles, and the length of their hair. School-age girls are in general more frequently in close contact with their friends at school, and have longer hair than boys —which provides an ideal environment for transmission (Mumcuoglu et al., 2021). At the institutional level, the identification with a child with head lice can be very disruptive, especially in schools with the outdated "no nit" policy (Mumcuoglu et al., 2021).

Due to their long-ranging distribution across continents and socio-economic strata, head lice are a global public health concern. Although socioeconomic status (SES) can increase the odds of infestation by increasing risk of exposure (for instance, residing in a crowded environment and/or sharing household items such as clothing and hairbrushes), it is not a determining factor (Falagas et al., 2008).

In addition to their direct medical effects, it has been suggested that due to their blood feeding habits, head lice have the potential to act as vectors of infectious agents (Amanzougaghene et al., 2019). This point of view is not yet supported by direct evidence, but rather by multiple reports informing of the presence of pathogens or their DNA in head and body lice. Although many factors play a role in vectorial competence, it is generally assumed that if pathogenic bacteria can survive in the midgut of lice, they may be transmitted through the lice's bite or feces. A 2006 study that facilitated an experimental infection of *P. h. humanus* specimens with *A. baumannii* displayed that body lice are able to transmit viable bacteria in their excrement, however, they were unable to support the hypothesis that body lice are able to transmit the pathogen when using a rabbit model (Houhamdi & Raoult, 2006).

Researchers have conducted experimental studies to assess if, like its close relative *P. h. humanus, P. h. capitis* could transmit pathogenic bacteria such as *B. quintana and R. prowazekii*. Despite resounding success under laboratory conditions (Kim et al., 2017; Murray & Torrey, 1975), uncertainty still exists as to whether head lice can accomplish transmission under natural circumstances. Research data, however, continues to point at biological plausibility as DNA from several pathogenic bacteria has been identified in infected head lice from Africa, Asia, Europe, and the United States of America. The majority of studies confirm the detection of *Acinetobacter spp., B. quintana,* and *B. recurrentis*, or a combination of the three (Amanzougaghene et al., 2019). In the U. S., Bonilla et al., 2009, 2014, identified *B. quintana* in head lice collected from homeless persons in California, whereas Eremeeva et al., 2017 reported significant findings of *A. baumannii* DNA present in 80.8% of head lice collected from schoolchildren in Georgia. It is important to note that *A. baumannii* is multi-drug resistant pathogen that causes a multitude of opportunistic infections. Of particular concern is that *A. baumannii* frequently causes health-care associated infections and is subject to increasing infection control surveillance.

Regardless of global interest, the current literature indicates that assessing the presence of bacterial pathogens in head lice is an area of research that remains unexplored in Latin America. Head lice are a global public health issue, and with the current beliefs shifting towards the plausibility of their vectorial competence, this is a gap in the literature that needs to be bridged.

The present thesis aimed to fill this knowledge gap by examining head lice for the presence of *Acinetobacter baumannii*. Insect specimens were collected from three countries in Latin America: Argentina, Colombia, and Honduras, and were tested for *A. baumannii* DNA through a polymerase chain reaction (PCR). This study contributes to the growing body of work documenting infectious agents naturally occurring in head lice and lends support to the hypothesis that *A. baumannii* can utilize arthropod vectors for its transmission.

CHAPTER 2: LITERATURE REVIEW

2.1 Arthropods as Vectors of Infectious Agents

Phylum Arthropoda is by far the largest phylum in the animal kingdom and diverges into several subphyla, classes, and orders. It encompasses a vast number of invertebrate animals, and diverges into two monophyletic groups: *Chelicerata* and *Mandibulata* (Giribet & Edgecombe, 2019). Animals in group *Chelicerata* are identified by the presence of chelicerae, whereas animals in group *Mandibulata* have mandibles. Included in group *Chelicerata* is class *Insecta*, which accounts for over 1 million species (Zhang, 2013). Understanding the role arthropods play in the transmission of diseases, microorganisms, pathogens, and parasites has been crucial for the survival of the human species throughout history and in present day. Identifying a species as a potential vector for infectious agents could not only mitigate the spread of the infectious agent, but also elicit change by increasing awareness and result in actions taken by the government to help those at risk of infection. Some notable arthropods that have been deemed of clinical importance to humans are body lice, fleas, flies, mosquitos, ticks, and triatomines among others outlined in Table 1. Although not all clinically significant arthropods have been identified as having the ability to transmit agents of disease, they are important to recognize as they still impose negative implications on human health.

Table 1. Notable arthropods of clinical significance to humans (Mehlhorn, 2012; Portillo et al., 2018)

Class	Order	Genus	Common name	Main disease associated
Insecta	Siphonaptera	*Pulex* *Xenopsylla,*	Flea	Plague, Flea-borne typhus
	Phthiraptera	*Pediculus* *Pthirus*	Body louse Head louse Crab louse	Trench fever, Louse-borne relapsing fever Louse-borne typhus None reported None reported
	Hemiptera	*Cimex* *Rhodnius* *Triatoma*	Bed Bug Kissing Bug	None reported Trypanosomiasis (Chagas disease)
	Diptera	*Aedes* *Anopheles* *Glossina*	Mosquito Tsetse fly	Chikungunya Dengue Yellow fever Zika Malaria Trypanosomiasis (Sleeping sickness)

		Lutzomyia	Sand fly	Leishmaniasis
Arachnida	Ixodida	*Ixodes*	Tick	Lyme disease

The first arthropods appeared in the Cambrian Period over 500 million years ago (MYA) (Edgecombe, 2014) and have had an unparagoned role in the transmission of agents of disease including but not limited to bacteria, fungi, parasites, and viruses. It would be naïve to believe that after all that time Phylum Arthropoda has remained static, as it is continually evolving and thus elicits the need for continued research and investigation into arthropods and their role in the transmission of pathogens and etiologic agents of disease.

Ectoparasites such as primate lice are of particular interest as they are species specific. Transmission requires direct physical contact of their hosts which has impacted their evolutionary divergence. The divergence of *Pedicinus* (lice specific to Old World monkeys, i.e. primates belonging to the *Cercopithcidae* family) into *Pediculus* (lice specific to humans and chimpanzees) and *Pthirus* (lice specific to humans and gorillas) can be traced back to an estimated 22.65 MYA (Reed et al., 2004). As it relates to humans, only *Pediculus humanus humanus*, commonly known as the body louse, has been associated with the ability to transmit infectious agents.

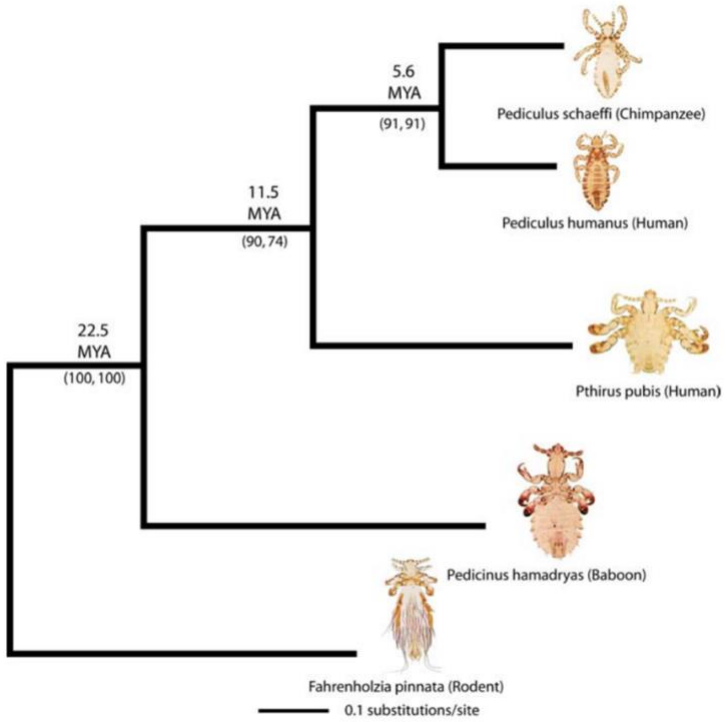


Figure 1. Phylogenetic tree depicting species of primate lice and when they diverged (Reed et al., 2004)

2.1.1 – Human Specific Lice

There are three different types of lice that infest humans: body lice, head lice, and pubic lice. All stemming from Class *Insecta*, Order *Phthiraptera*: *Anoplura*. Body and head lice (*Pediculus humanus humanu*s (De Geer, 1758) and *Pediculus humanus capitis* (De Geer, 1767), respectively) diverge into Family *Pediuclae*, whereas pubic lice (*Pthirus pubis* (Linneaus, 1758)) diverged into the *Pthiradae* Family around 10-13 MYA according to molecular evidence (Reed et al., 2007). Although both head lice and pubic lice have been investigated, neither of them have yet been positively identified as a vector of pathogens (Raoult & Roux, 1999). First theorized in 1915, later phylogenetic studies have shown that sucking lice (Anoplura) are monophyletic and have evolved from hematophagous chewing lice (Mallophaga) (Boutellis et al., 2014; Cumming, 1915) (Cruickshank et al., 2001; Johnson et al., 2004).

Lice are hematophagous permanent ectoparasites- i.e., they only consume blood and complete their entire life cycle on the host. Due to the close associations with their hosts, lice express limited abilities to move from host to host which have resulted in cospeciation. It is theorized that the ecotype *P. h. capitis* came before *P. h. humanus*, as humans evolved, they lost body hair and began wearing clothing (Reed, 2015) (Rogers et al., 2004). Researchers have identified parallels between the evolution of lice and their ecotypes alongside of the evolution of humans and have used the divergence of *P. h. humanus* and *P. h. capitis* as a way of dating the loss of body hair, the origin of clothing use, and even the migration of humans (Reed, 2015).

To our knowledge, infestation with head lice can date back to 8000 B.C., as researchers discovered a nit on a human hair from an archaeological site in Northeastern Brazil dating back to this period (Araujo et al., 2000). Lice have also been discovered in other archeological sites, as well as on mummies from Chile and Mexico (Araujo et al., 2000; Arriaza et al., 2012). Due to the long history between humans and their respective infesting lice, their cospeciation has been analyzed and used as a model to better understand cophylogenetic parasitic relationships (Boutellis et al., 2014). Mitochondrial DNA has aided in the understanding of the divergence of lice geographically throughout the world, via identifying the mitochondrial clade of head and body lice. Head lice have been found to belong to clades A-F, and body lice have been found to belong to clades A and D. This finding corresponds to the belief that body lice indeed diverged from head lice (Amanzougaghene et al., 2019; Boutellis et al., 2014; Light et al., 2008).

Table 2. Mitochondrial clades of lice and their distribution (Amanzougaghene et al., 2019; Boutellis et al., 2014; Light et al., 2008)

Clade	Geographic location
A	Worldwide
B	Africa: Algeria, Saudi Arabia, South Africa Australia Europe: Israel
C	Africa: Ethiopia, Republic of Congo Asia: Nepal, Pakistan, Thailand
D	Africa: Democratic Republic of Congo, Ethiopia, Republic of Congo, and Zimbabwe
E	Africa: Mali and Senegal
F	Africa: French Guyana Latin America: Argentina and Mexico

Pediculus humanus humanus and *Pediculus humanus capitis* are morphologically similar—both are obligate, similar in body length, and color. There are a few defining features that can help one distinguish the two species from one another, as seen in Table 3. *Pthirus pubis* specimens are more easily identified than the other two species of human specific lice, as they are much rounder, flatter, and shorter. All lice are wingless, and have three pairs of jointed legs which are equipped with tarsal claws- which facilitate climbing and movement (Durden, 2019). Human specific lice are most commonly identified by where they are found on the body, as all three species differ in feeding location. Head lice are very similar to body lice morphologically and genetically, which intrigues researchers and incites questions about their disparate vectorial competence.

Table 3. Morphological and behavioral differences between *P. humanus humanus*, *P. humanus capitis*, and *Pthirus pubis* (Bonilla et al., 2013; CDC, 2017, 2018b; Ko & Elston, 2004; Nuttall, 1918)

Characteristic	*P. humanus humanus*	*P. humanus capitis*	*Pthirus pubis*
Feeding location (on humans)	Body	Head	Pubic region
Meals/day	1-5 meals/day	4-10 meals/day	4-5 meals/day
Shape	Obligate	Obligate	Round, flattened
Female body length (mm)	2.4-3.6	2.4-3.3	1.5-2.0
Male body length (mm)	2.3-3.0	2.1-2.6	0.8-1.2
Color	Light-medium brown	Medium-dark brown	Tan-greyish white
Number of eggs laid by females	8-12/day	4-5/day	Up to 3 eggs/day

Longevity	Up to 60 days	Up to 27 days	3-4 weeks

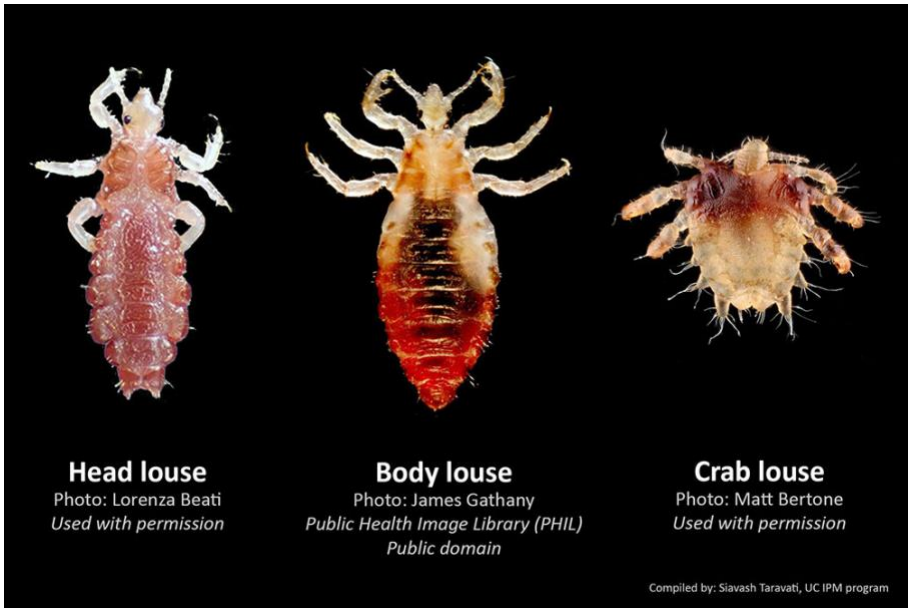


Figure 2. Comparison of morphology of the three ecotypes of human specific lice. Left to right: *Pediculus humanus capitis*, *Pediculus humanus humanus*, *Pthirus pubis* (Taravati, 2019)

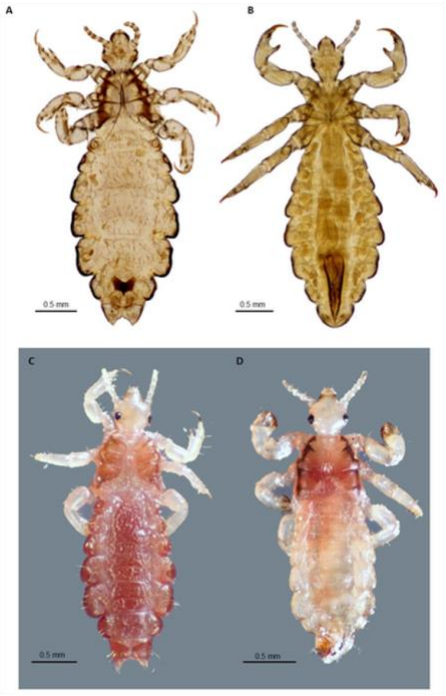


Figure 3. Image of Adult *P. humanus capitis* (A, C, D) and *P. humanus humanus* (B) taken with a Visionary Digital K2/SC long-distance microscope (Bonilla et al., 2013)

Life Cycle

Lice are strict ectoparasites, that is, their entire life cycle occurs on the host. All human-infesting lice go through similar life cycles and begin as eggs laid by an adult female louse, as lice are oviparous. They then undergo an incomplete metamorphosis in which nymphs hatch from the eggs and subsequently molt three times before becoming adult lice (Burgess & Cowan, 1998).

Eggs laid by *P. h. capitis* and *P. pubis* are connected to the hair shaft near the scalp of the infested person or the pubic region, respectively. The eggs are small (0.8-0.3mm) and can be commonly mistaken for dandruff (in the case of *P. h. capitis)*. Viable eggs, if undisturbed, will hatch and release a nymph after 6-9 days (CDC, 2017) (CDC, 2018b). The time in which it takes the nymphs to hatch is dependent on the temperature of the eggs.

Eggs laid by *P. h. humanus* differ, as adult females lay their eggs and attach them to fibers and seams in clothing as well as bedding that are close in contact to the skin of the human host. After 5-7 days the eggs hatch and a stage 1 nymph will emerge, going through three molts and eventually maturing into an adult louse (Powers & Badri, 2021) (CDC, 2017). The time it takes the nymphs to hatch is also dependent on the temperature of the eggs.

Although slightly different for each type of louse, nymphs of all three types start feeding immediately after hatching, as lice are strictly hematophagous and solely consume blood meals for the duration of their life. The amount of time it takes for lice to mature from hatching to becoming an adult varies, however, across all types the egg stage normally lasts 4-15 days and each nymphal stage can last anywhere from 3-8 days (Durden, 2019).

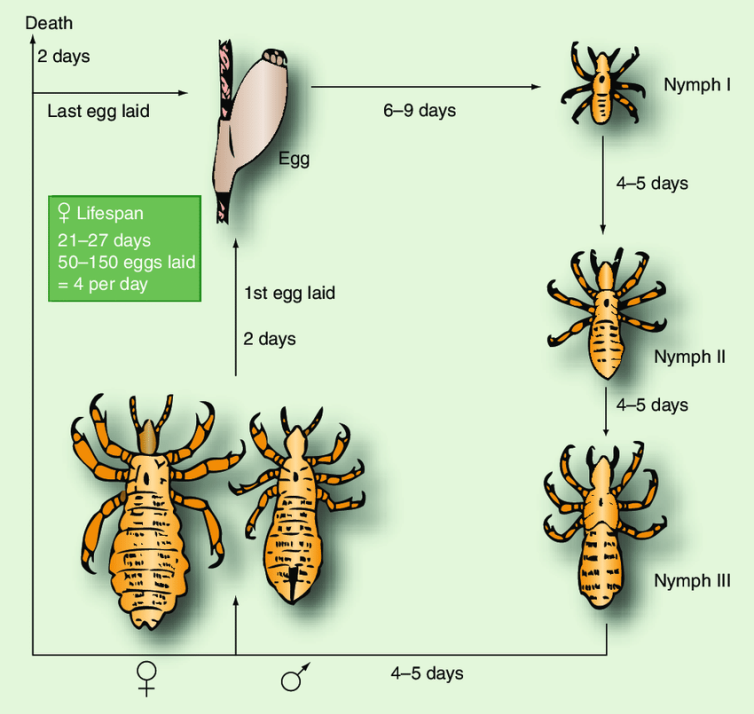


Figure 4. Life cycle of *Pediculus humanus capitis* (Mumcuoglu, 2009)

Figure 5. *Pediculus humanus capitis* at different stages of the life cycle including an egg, nymphal stages 1-3, and adults (male and female) as observed under a stereoscope at 15x magnification.

Transmission

The location and means of transmission for each type of louse varies as they are region specific and display slightly different behavioral patterns as outlined in Table 4. The front legs of lice are equipped with claws that enable them to crawl and climb on hair and fibers of textiles, which they use to travel from infested hosts to new hosts directly, or to an item which a potential host will use and then become infested.

Globally there is a negative connotation and stigma associated with carrying head lice. Current public beliefs often associate people with lice as unsanitary or of low income, however this is not the case. Socioeconomic status (SES) can have an impact on the rate of transmission, as if more people are sharing common areas, bedding, clothing, and hairbrushes, they are at higher risk of infestation, however, lice infest people worldwide regardless of SES and it is not a determining factor as to why people become infested (CDC, 2017, 2018b).

Table 4. The three types of lice that infest humans and their differences in affected cohorts, places, and means of transmission (CDC, 2017, 2018b; Chosidow, 2000; Raoult & Roux, 1999)

	P. humanus humanus	*P. humanus capitis*	*Pthirus pubis*
Feeding location (on humans)	Body	Head	Pubic region
Cohort most affected	Persons who live in crowded conditions with lack of access to regular bathing and clean clothing (the homeless, refugees)	Schoolchildren: females more frequently than males	Adults: can affect all racial, ethnic, and SES groups
Common place of transmission	Places of crowded living, unclean living environments	School, home, sports activities, slumber parties	Home
Common means of transmission	Direct contact with an infested person, or through contact with infested clothing, bed linens, and towels	Head-to-head contact, during play	Sexual contact
Uncommon means of transmission	None reported	Wearing clothing infested by another person, using infested combs, brushes, towels,	Close personal contact with clothing, bed linens, and towels of an infested person

bedding (pillow, bed, couch)

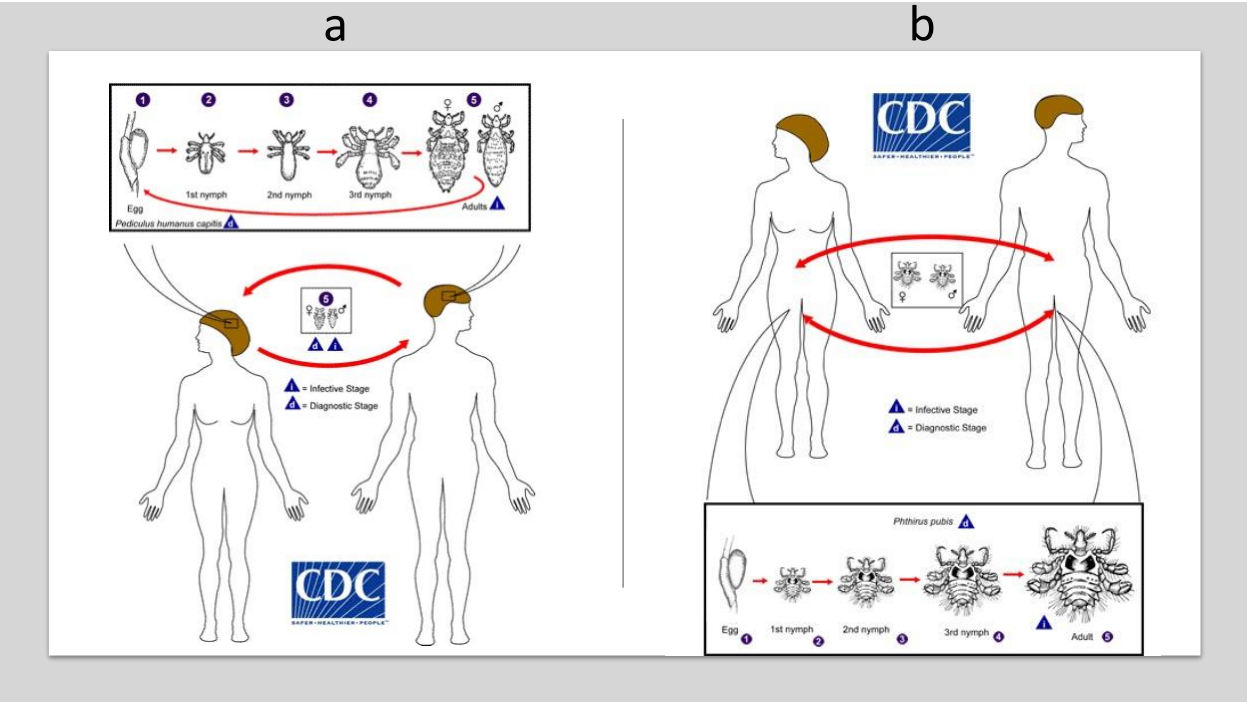


Figure 6. Life cycles and transmission of *P. humanus capitis* (a) and *P. pubis* (b) (CDC, 2017, 2018b)

Treatment and Resistance

There are many modalities for treatment for pediculosis capitis, including mechanical methods (such as combing) and treatment with chemical or plant-derived insecticides (Bonilla et al., 2013; Feldmeier, 2014; Mumcuoglu, 1999). Chemical insecticides work through either one of two modes of action: neurotoxic or physical. Pediculicides that employ physical modes of action work to coat and block louse spiracles —effectively hindering their respiration abilities. New treatments that act by dissolving the exoskeleton of lice, leading to dehydration and subsequently death of the lice, have also been approved for use by Health Canada (Cummings et al., 2018). Pediculicides that impose neurotoxic effects on lice work though several different mechanisms. The most popular treatment in this category, permethrin, works by binding to voltage sensitive sodium channels, which results in a hyper influx of sodium, leading to lice paralysis and death (Gellatly et al., 2016; Lee et al., 2003).

The short life cycle of head lice in combination with overuse of commercially available pediculicides have provided a perfect storm for evolutionary resistance. Interestingly, only pediculicides with a neurotoxic mode of action have been associated with resistant lice (Burkhart, 2004). Pediculicides that have been documented as having resistant lice populations include: carbaryl, DDT (dichlorodiphenyltrichloroethane), ivermectin, and permethrin. Head lice

resistant to pyrethroids have been documented in Argentina, Czech Republic, France, Israel, United Kingdom, and the United States of America (Cummings et al., 2018; Larkin et al., 2020).The resistance mechanism associated with the usage of pyrethroids, the most commonly used chemical insecticide for pediculosis capitis, is via the knockdown resistance mechanism (kdr). This mechanism is caused by single nucleotide polymorphisms or SNPs, located in the voltage-gated sodium channel gene (α subunit). Specifically, SNPs T917I, M815I, and L920F have been identified in relation to pyrethroid resistance, being shown to significantly reduce lice susceptibility (Firooziyan et al., 2017; Hodgdon et al., 2010; Lee et al., 2003; Yoon et al., 2003).

In a collaboration with the research team working under Dr. Ariel Toloza at the Department of Plagas y Insecticidas, (CONICET), we were able to publish a study describing the first evidence of mutations associated with pyrethroid resistant lice from two localities in Honduras (La Hicaca and San Buenaventura) (Larkin et al., 2020). This study was a novel addition to the literature as it is the only published study looking at head lice from Honduras. Insecticide resistance is an emerging issue that needs to be investigated further—adding data from an underrepresented country to the scientific knowledge is a step in the right direction.

New treatment options including plant-derived insecticides are now being investigated as more reports of pediculicide resistant lice populations are emerging. Most plant-derived pediculicides being studied include the use of fixed oils and essential oils, which have been shown to be an effective against head lice (Burgess et al., 2010; Mumcuoglu et al., 2002; Toloza, Lucia, et al., 2010; Toloza, Zygadlo, et al., 2010).

Table 5. Neurotoxic pediculicides associated with head lice resistance

Pediculicides with resistant head lice populations	
Neurotoxic mode of action	DDT (dichlorodiphenyltrichloroethane)
	Carbaryl
	Ivermectin
	Permethrin

2.1.2 – Clinical significance of head lice and potential to harbor pathogens

Although the transmission of *P. h. capitis* has been investigated thoroughly, the data in the literature is not comprehensive. Most available data point to residential and school environments as the primary locations of head lice transmission, however, recent studies have indicated the presence and transmission of head lice in clinical settings as well.

Pediculosis capitis was reported in 2.8% of children at a public hospital in Brazil in 2015-2017, and 6.1% of children analyzed in a later study at that same hospital in 2021 (Cadima et al., 2021; Marinho et al., 2018). Similarly, an observational study from India also documented the

prevalence of pediculosis capitis of children in a tertiary care hospital where they found 71.1% and 28.8% positivity rates in females and males, respectively (Saraswat et al., 2020). Recent case reports of patients infested with head lice have also been published from India and the USA (Challa et al., 2020; Pietri et al., 2020). A case report published by Challa et al. 2020, details the transmission of head lice from a mother to her newborn baby in a hospital. Furthermore, a case report from Turkey outlines the nosocomial transmission of head lice from a patient to a pediatric resident working in a hospital, and subsequently to her coworker (Yasar Durmus et al., 2020). These studies corroborate the plausibility of transmission of head lice in a clinical setting which illuminates a gap in the literature: can lice harbor pathogenic bacteria, and do they have the potential to be a vector? If the answer to the previous questions are yes, what implications would the transmission of a multi-drug resistant bacteria pose if it were transmissible by head lice?

Body lice have been shown to be capable of acquiring and transmitting bacterial pathogens, however, head lice have yet to be identified as able to do so (Raoult & Roux, 1999). There have been several studies in which head lice have been found to harbor pathogenic bacteria, yet the scope of their vector competency has yet to be discovered. While very similar to the human body louse, head lice differ morphologically, behaviorally, and as suggested in a study by Kim et al. 2017, head lice may have a more developed anti-microbial innate immunity when compared to body lice, which could indicate why head lice have yet to be identified as a successful vector. As a part of its global vector control response program aimed to decrease the impact of vector-borne disease, in 2017 the World Health Organization (WHO) identified lice as one of the known vectors to humans (WHO, 2017). The presence of these bacteria in head lice raise questions in the scientific community about the potential of head lice as vectors, and the dangers this would pose on a global scale.

Several species of the gram-negative bacteria *Acinetobacter* have been discovered in both body and head lice from several continents as outlined in Table 7. The most frequently identified species of this bacteria in lice is *Acinetobacter baumannii*, an opportunistic bacterial pathogen that can be found in nosocomial settings.

Table 6. Summary of human-infesting lice and the pathogens found to be carried by them (Amanzougaghene et al., 2020; Eremeeva et al., 2017; Mana et al., 2017; Mehlhorn, 2012; Raoult & Parola, 2007)

Species of lice	Infectious agent	Health outcome associated
Pediculus humanus humanus	*Acinetobacter spp.*	Bacteremia, infection, sepsis
	Anaplasmosis phagocytophilum	Anaplasmosis
	Bartonella quintana	Trench fever
	Borrelia recurrentis	Louse-borne relapsing fever
	Coxiella burnetti	Q fever
	Rickettsia prowazekii	Louse-borne typhus
	Yersinia pestis	Plague
Pediculus humanus capitis	*Acinetobacter spp.*	Bacteremia, infection, sepsis
	Anaplasmosis phagocytophilum	Anaplasmosis
	Bartonella quintana	Trench fever
	Borrelia recurrentis	Louse-borne relapsing fever
	Coxiella burnetti	Q fever
	Ehrlichia spp.	Ehrlichiosis
	Rickettsia aeschlimannii	Spotted fever - Rickettsiosis
	Rickettsia prowazekii	Louse-borne typhus
	Yersinia pestis	Plague
Pthirus Pubis	*Acinetobacter johnsonii*	Bacteremia, infection, sepsis
	Bartonella quintana	Trench fever

Table 7. Recent studies that found pathogenic bacteria in head lice (Amanzougaghene et al., 2019)

Bacteria	Location(s)	Population	References
Acinetobacter spp.	Algeria*	School children, refugees from Niger	(Mana et al., 2017) (Louni et al., 2018)
	Ethiopia*	Individuals from 7 different altitudes in the Southwest	(Kempf et al., 2012)
	France*	School children Homeless individuals Hospital patients	(Bouvresse et al., 2011) (Amanzougaghene et al., 2020)
	Republic of Congo*	Pygmy populations living in poor conditions	(Amanzougaghene et al., 2016)
	Russia*	Homeless individuals	(Eremeeva et al., 2017)
	USA*	School children	(Eremeeva et al., 2017)
A. phagocytophilum	Mali	Rural villagers living in poor conditions	(Amanzougaghene et al., 2017)
B. quintana	Democratic Republic of Congo	People living in a plague-endemic area	(Piarroux et al., 2013) (Drali et al., 2015)
	Ethiopia	Populations living at different altitudes Street beggars Patients with louse-borne relapsing fever	(Angelakis, Diatta, et al., 2011) (Cutler et al., 2012) (Boutellis et al., 2013)
	France	Homeless individuals	(Angelakis, Rolain, et al., 2011)
	Madagascar	Rural community, low income	(Sangare et al., 2014)
	Mali	Rural villagers living in poor conditions	(Amanzougaghene et al., 2017)
	Nepal	The homeless and school children	(Sasaki et al., 2006)
	Russia	Homeless individuals	(Eremeeva et al., 2017)
	Senegal	Rural villagers living in poor communities	(Boutellis et al., 2012) (Sangare et al., 2014) (Diatta et al., 2014)
	USA	School children Homeless individuals	(Eremeeva et al., 2017) (Bonilla et al., 2014; Bonilla et al., 2009)

B. recurrentis	Ethiopia	Patients with louse-borne relapsing fever	(Boutellis et al., 2013)
	Republic of Congo	Pygmy populations living in poor conditions	(Amanzougaghene et al., 2016)
C. burnetti	Algeria	Refugees from Niger	(Louni et al., 2018)
	Mali	Rural villagers living in poor conditions	(Amanzougaghene et al., 2017)
Ehrlichia spp.	Mali	Rural villagers living in poor conditions	(Amanzougaghene et al., 2017)
R. aeshlmannii	Mali	Rural villagers living in poor conditions	(Amanzougaghene et al., 2017)
Y. pestis	Democratic Republic of Congo	People living in a plague-endemic area	(Piarroux et al., 2013) (Drali et al., 2015)

*is indicative of presence of *A. baumannii*

2.2 Acinetobacter baumannii

Acinetobacter spp. is the fifth most common gram-negative bacteria that is associated with nosocomial infections (Vincent et al., 2009). *A. baumannii* is one of many multi-drug resistant (MDR) bacterial pathogens belonging to this genus, and as such, is very difficult to treat. The Center for Disease Control and Prevention (CDC) tracks *A. baumannii* infections as part of its Emerging Infections Program, as infection with *A. baumannii* can be catastrophic in vulnerable patients (CDC, 2019). Additionally, in 2019 the World Health Organization (WHO) published "Antibacterial Agents in Preclinical Development" where they placed *Acinetobacter baumannii* under priority 1, listing the bacteria as critical on their priority pathogens list (WHO, 2019). Reports of *A. baumannii* outbreaks been identified in different hospital wards (most notably intensive care units) across several countries as outlined in Table 8.

Table 8. Countries with recorded Acinetobacter spp. outbreak or infection (Kurihara et al., 2020; Merino et al., 2014; Morfin-Otero et al., 2013; Peleg et al., 2008).

Continent	Countries
Africa	South Africa
Asia and Middle East	China, Hong Kong, India, Israel, South Korea, Thailand
Australia, French Polynesia	Australia, French Polynesia
Europe	Belgium, Czech Republic, Denmark, England, France, Germany, Greece, Italy, Lebanon the Netherlands, Spain, Turkey
Latin America	Argentina, Brazil, Chile, Colombia
North America	Canada, Mexico, United States of America

The prevalence of *A. baumannii* in intensive care units presents additional risks as patients in ICUs can often be immunocompromised, undergoing invasive procedures, and in the hospital

for prolonged durations (Chusri et al., 2015; Henig et al., 2015). Due to its ability to produce biofilms, *A. baumannii* can also survive for up to four weeks on surfaces. This can increase its transmission as it can colonize medical equipment and other surfaces (such as curtains) if they are not properly sanitized (Pakharukova et al., 2018). Risk factors for infection with *A. baumannii* in hospitals have been outlined in Table 9.

Table 9. Risk factors associated with *Acinetobacter baumannii* infection (Ballouz et al., 2017; Guo et al., 2016; Jang et al., 2009; Kurihara et al., 2020; Nutman et al., 2014; Romanelli et al., 2013)

Risk Factors	
Medical equipment	Central venous catheters Curtains Drains Gastrectomy tubes Respirators
Susceptible populations/procedures	Chemotherapy Chronic disease Comorbidities Hematological disease Hospital duration >7 days Invasive procedures Newborn babies, increased risk if pre-term or underweight Organ transplant patients Presence of tumors Previous antibiotic use Recent bacteremia Recurrent hospitalizations Respiratory and organ failure Surgical procedures Transfer from another hospital

Recently, significant nosocomial outbreaks of *A. baumannii* are emerging along with a host of negative implications. Blood stream infections (BSI) with *A. baumannii* have been positively associated with sepsis, increased mortality rates, higher risk of organ failure, pneumonia, and longer stays in ICUs (Ballouz et al., 2017; Eberle et al., 2010; Kurihara et al., 2020). It has been

estimated that an average of 10.6% of patients die as a result of infections caused by multi-drug resistant *A. baumannii* (Kurihara et al., 2020). A report of an *A. baumannii* outbreak at a hospital in Israel found that patients with carbapenem-resistant *Acinetobacter baumannii* (CRAB) bacteriemia as a result of *A. baumannii* infection resulted in an 82% mortality rate over 72 hours and a 100% rate over a 30-day period (Ben-Chetrit et al., 2018). A retrospective study from China studied 102 patients positive for *A. baumannii* infection and found an overall mortality rate of 29.4% (Shi et al., 2020). A seven-year prospective cohort study in Lebanon found that 84% of infections with *A. baumannii* were nosocomial and found an overall mortality rate of 52-66% for those with multi-drug resistant *A. baumannii* infections (Kanafani et al., 2018). Across all studies it has been shown that there are worse health outcomes for those who are immunocompromised or have comorbidities prior to acquiring an *A. baumannii* infection.

Aside from negative health outcomes, outbreaks of *A. baumannii* also have detrimental financial implications. In the United States alone it is estimated that a single infection with multi-drug resistant *A. baumannii* can cost anywhere from $33,510 to $129,917 per-infection (Nelson et al., 2016). According to Nelson et al., 2016, there are more than 10,000 cases of MDR *A. baumannii* infections per year in the US, resulting in over 1,000 deaths, which accumulates to a total cost of up to $1.6 billion. *A. baumannii* outbreaks have been recorded in several countries aside from the United States and have a devastating impact wherever they emerge- finding new routes of transmission could reduce the immense health and financial burdens that this bacterium causes.

2.2.1 – Relationship between *A. baumannii* and *P. h. capitis*

The link between *A. baumannii* and head lice is under current investigation. The vectorial competence of head lice is still unknown; however, the current scientific belief is shifting towards that of biological plausibility. Due to increased innate immunity when compared to body lice, head lice may be less effective as vectors of bacterial pathogens, however, that does not eliminate the plausibility (Amanzougaghene et al., 2019; Kim et al., 2017). The DNA of several bacterial pathogens, including *A. baumannii*, has been discovered in head lice populations around the world. It is known that head lice can be present and transmitted in hospital settings, and *A. baumannii* is primarily an opportunistic nosocomial pathogen. For example, there are two studies that presented data regarding the presence and transmission of head lice in a hospital in Uberlândia, Brazil (Cadima et al., 2021; Marinho et al., 2018). In the same hospital there are multiple reports of *A. baumannii* outbreak and infection, albeit in different years (Brito et al., 2010; Dal-Bo et al., 2012; Rocha Lde et al., 2008; von Dolinger de Brito et al., 2005). Nosocomial transmission of head lice has been reported, and although it is uncommon, the presence of head lice are taken seriously in clinical settings. For instance, cancelling elective surgery of an individual infested with head lice is seen as the preferred practice, as health care professionals recognize the implications of infestation and secondary bacterial infections that may arise (Conner, 2000).

Although *P. h. capitis* has yet to be recognized as a competent vector, the evidence in the literature seems to point toward the biological and epidemiological plausibility of the transmission of *A. baumannii* via head lice.

2.3 Research Goal and Specific Objectives

The primary objective of this study is to identify the presence or absence of *Acinetobacter baumannii* DNA in head lice samples collected from three countries in Latin America: Argentina, Colombia, and Honduras.

CHAPTER 3: METHODOLOGY

3.1 Study design

The present study is based on secondary analysis of biological samples.

Several steps were taken in order to verify that the arthropods collected from the children in this study were in fact *Pediculus humanus capitis* specimens prior to proceeding with the analyzation for *A. baumannii* DNA. Measures that were taken to ensure the positivity of the specimens include: samples were only collected from the heads of participants, all specimens were observed individually using a Ken-a-vision stereomicroscope and verified due to their morphology, and DNA was extracted from all specimens used and analyzed using PCR. The addition of this control was taken to support a sound methodology and to ensure that our conclusions can be supported by our data. The presence of positive pediculosis capitis infections are often over-diagnosed (Pollack et al., 2000), and as such it was important to include these measures in our study.

3.1 Specimen collection

Pediculus humanus capitis samples were collected from schoolchildren from three different countries in Latin America: Argentina, Colombia and Honduras.

Argentina

Specimens from Argentina were a generous donation from Dr. Ariel Toloza and his research laboratory at the Department of Plagas y Insecticidas, (CONICET). These lice were collected in 2017 from schoolchildren enrolled in primary schools in Buenos Aires, Argentina through various studies, by means of traditional head lice combs and using stainless steel tweezers. The head lice specimens have been preserved in 70% ethanol for further analysis.

Colombia

Specimens from Medellín, Colombia, were collected in the Summer of 2018 by Oscar Palacio, an undergraduate student at Brock University under the supervision of Dr. Ana Sanchez. Lice were collected using a V-comb, which is a stainless-steel comb that utilizes suction power and collects all specimens and artifacts in small nets. The study had received ethics approval at Brock University (File 17-383, May 23, 2018). Lice were preserved in 70% ethanol for further analysis.

Honduras

Lice specimens were collected in two sites: (i) in La Hicaca, department of Yoro in 2016 for a qualitative study conducted by Shabana Jamani, undergraduate student at Brock University and published in 2019 (Jamani et al., 2019), and (ii) San Buenaventura, Francisco Morazán, in 2019, during a community service visit by Carol Rodriguez and Maria Mercedes Rueda, researchers at the National Autonomous University of Honduras (UNAH). Specimens from La Hicaca were collected using a V-comb, which is a stainless-steel comb that utilizes suction power and collects all specimens and artifacts in small nets; whereas lice from San Buenaventura were

collected by using traditional lice combs and stainless-steel tweezers. Lice from both sites were preserved in 70% ethanol for further analysis.

3.2 Sample size and preparation of head lice

Insect specimens contained in the vials were inspected to confirm they were in fact head lice, and if so, they were counted, and their life stage (egg, nymph or adult) recorded. Altogether, a total of 123 vials were obtained for analysis: 50 from Argentina, 25 from Colombia, 41 from La Hicaca, Honduras, and 7 from San Buenaventura, Honduras. The distribution per life stage is shown in Table 10. A variable number of lice was contained in each vial, but altogether, 368 insects were available for analysis.

Table 10. Life stages of specimens analyzed

	Argentina	Colombia	Honduras – La Hicaca	Honduras – San Buenaventura	**Total**
Nymphs	12	34	25	21	92
Adults	176	13	84	3	276
Total	188	47	109	24	**368**

As mentioned, insect specimens were kept in 1.5 mL Eppendorf tubes containing a 70% ethanol solution at 4 °C. Due to the varied nature of their collection, the contents of each vial were analyzed individually using a Ken-A-Vision™ Vision Scope 2 Stereo Microscope. Lice were then transferred to 100 x 15 mL petri dishes and separated from hair and debris (Figure 7a) (or V-Comb nets if applicable) using stainless steel tweezers. Hair, debris, molts, and nits were discarded. The sex and life stage of the lice were identified by morphology at 20x magnification (Figure 7b), and when in doubt, identification keys for morphological characteristics were used (Al-Marjan, 2015).

From each of the 123 vials, between 1 to 5 lice were selected, depending on their size, for a total of 368 insects. These were then cut longitudinally by hand using a sterile stainless-steel scalpel, with a carbon steel #22 blade (Figure 7c) in order to create a corresponding number of vials in duplicate, which were kept at -20 °C. One set was used for the present study, while the other was saved for future studies.

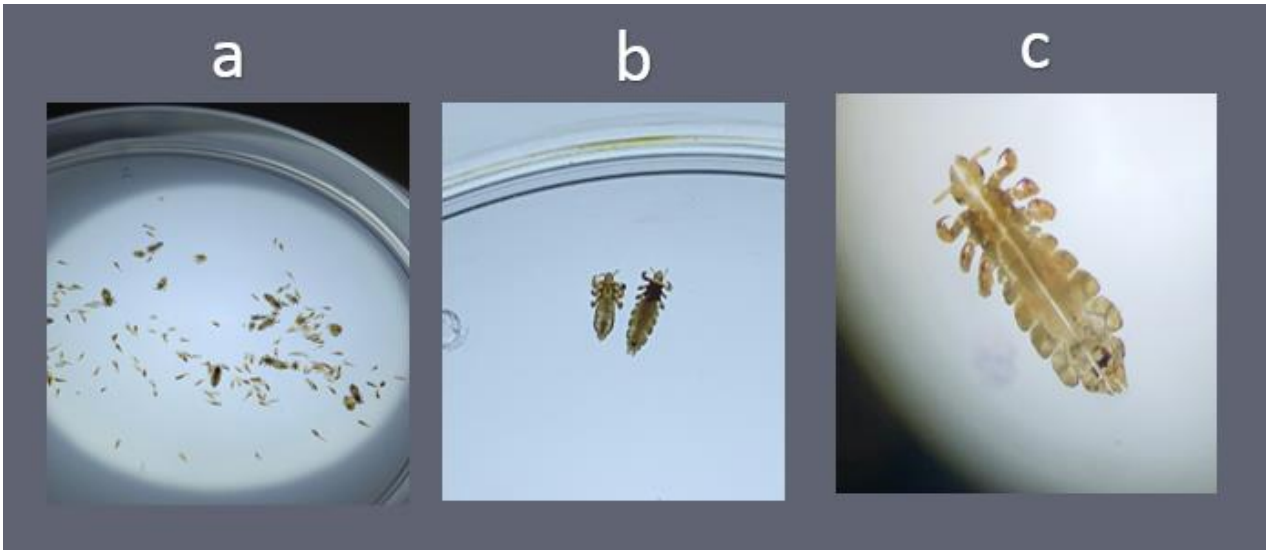


Figure 7. *P. h. capitis* specimens seen under the stereoscope. Panel a: specimens at 4x, prior to separation and identification; Panel b: Adult male and female respectively at 15x magnification; Panel c: Adult female cut longitudinally prior to DNA extraction at 20x magnification.

Detection of *Acinetobacter baumannii* DNA in head lice

In order to optimize our results, several measures were taken in order to establish a pure and positive control of *A. baumannii*, as well as determine the lowest possible number of *A. baumannii* bacteria that could possibly be detected by our PCR. Prior to analyzing the *P. h. capitis* DNA for the presence of *A. baumannii*, we cultured *A. baumannii* from a reference strain (ATCC 19606) over several weeks, positively identified the bacteria by using the gram-staining technique, observing it under a microscope, positively identifying its morphology, and by using PCR. We then created serial dilutions of the bacteria in a nutrient broth medium, analyzed its optical density (OD), and tested each of the serial dilutions using PCR to establish sensitivity.

3.3 *Acinetobacter baumannii* bacterial culture

Reference material of *A*. *baumannii* strain (ATCC 19606) was purchased from Cedarlane Labs (Burlington, Ontario). An aliquot of the reference strain was inoculated using the freeze-dried pellet reference material resuspended in 1 mL of sterile nutrient broth. Once combined, it was added to 50 mL of nutrient broth and mixed well. Using 10 µL of the suspension, duplicate nutrient agar plates were completed using the spread plate technique to test for strain purity before use. The nutrient broth containing the bacterial suspension and the two nutrient agar plates were then incubated at 37 °C for 24 hours in aerobic conditions.

Bacterial colonies from the incubated plates were collected (Figure 8a) then stained using the Gram staining technique (Beveridge, 2001) and observed under a microscope to ensure there was no contamination, determined by colony and cell morphology. It is important to note that *A. baumannii* is notoriously difficult to de-stain, and as such may have varied appearances when stained, which would explain why although it is a gram-negative bacterium, it is purple in appearance (Figure 8b) (Howard et al., 2012). In a nutrient agar setting, *A. baumannii* colonies appear smooth, pale yellow to white, and about 1-2 mm in diameter. Under a microscope, *A. baumannii* when in the log phase of growth, appear as short gram-negative rods, however in the stationary phase of growth are coccoid in appearance (Percival, 2014).

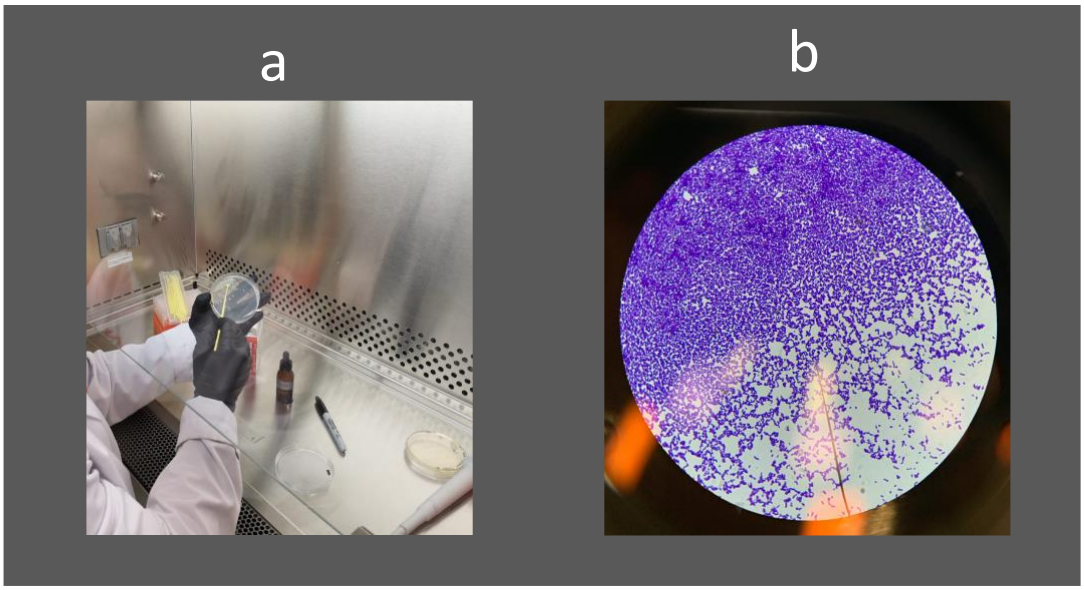


Figure 8. Ensuring *A. baumannii* culture purity. Panel a: Collecting a colony of *A. baumannii* with a sterile loop to analyze with the Gram staining technique; Panel b: *A. baumannii* smear stained with the Gram staining technique (40x magnification) under a microscope.

Once purity of the *A. baumannii* culture was verified, 10 mL of the incubated suspension was transferred to a sterile 15 mL centrifuge tube. It was then centrifuged at 4000 rpm for 10 minutes. After centrifugation, the supernatant was discarded, the pellet resuspended with 10 mL of sterile saline solution, and vortexed. That process was then repeated once more. This was followed by 10-fold serial dilutions of the suspension in 0.9% sterile saline.

Using the spread plate technique, 30 µL of each dilution was cultured three times, in order to establish an average amount of colony forming units (CFU) for each dilution (Figure 9a and 9b). Additionally, the optical density of each serial dilution was analyzed using a microplate reader at 600 nm. This was done in order to establish a correlation between optical density at 600 nm and number of colony forming units (CFU) / mL. The serial dilutions were then analyzed using PCR in order to establish the lowest possible quantifiable number of bacteria that could potentially be present in the extracted DNA from *P. h. capitis* samples.

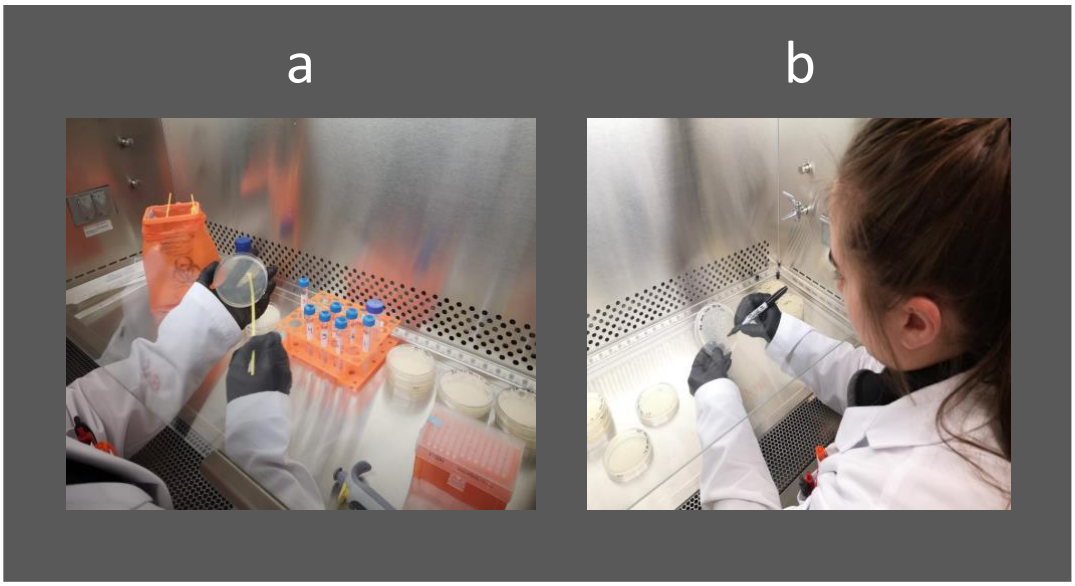


Figure 9. Culturing of *A. baumannii* and recording average CFU. Panel a: Spread plate technique used for each of the serial dilutions; Panel b: Counting CFU for each of the three plates cultured with the same dilution to determine average CFU.

3.4 DNA extraction

DNA from *P. h. capitis* and *A. baumannii* were both extracted using QIAmp DNA mini kits by QIAGEN cat# 51304, (Hilden, Germany) with the following adjustments to the original protocol: for *P. h. capitis*: Three 2-mm stainless steel beads were added to each sample before incubation in order to break up the specimens during vortex stirring. During the last step of extraction, 100 µL of buffer AE was added instead of 200 µL.

3.5 Polymerase Chain Reaction

Polymerase chain reaction (PCR) was used to verify the DNA of *P. humanus*, and further to analyze the potential DNA of *A. baumannii* within the lice samples. During PCR optimization, we used two sets of primers for both *Pediculus humanus capitis* and *Acinetobacter baumannii* to see which would provide the clearest results.

Selecting primers for *Pediculus humanus capitis*
The first set of primers we used for *P. humanus* are the following: Forward: GAG CGA CTG TAA TTA CTA ATC; Reverse: CAA CAA AAT TAT CCG GGT CC as reported in a study published by Louni et al., 2018. The second set of primers we used to test for *P. humanus* were published by Raoult et al., 2006, and are as follows: (PHND4F1) Forward: TTG TTG TGC TTT TGA CTT CTTG; (PHND4R1) Reverse: CCC TGA TTT GAA GTA TTA AAG AAA CTC, targeting NADH dehydrogenase subunit (NAD4) gene (GenBank accession number AY316847). The product size for the two reactions were 348 base pairs and 216 base pairs, respectively. Upon completion of running the gel electrophoresis, both primer sets produced positive results for our *P. humanus* positive

control; however, the bands were much clearer when using the primers described by Raoult et al., 2006. Therefore, we proceeded to use those primers for our study.

It is important to note that the NAD4 gene is not specific to *P. h. capitis,* as it is also present in *P. h. humanus.* We did not confirm that all specimens collected were of the *P. h. capitis* ecotype through biological methods. Due to the nature of specimen collection (all specimens collected from heads of participants which is the correct biotope for *P. h. capitis*) in conjunction with the absence of reports of *P. h. humanus* outbreaks in the areas of collection, we can presume that the specimens we analyzed were *P. h. capitis*.

Selecting primers for *Acinetobacter baumannii*
As well, two sets of primers were tested for *A. baumannii.* The first set includes 3 primers (2 forward, one reverse) and was published in 2007 by Higgins et al. while the second was determined by doing a search in the National Library of Medicine's BLAST (Basic Local Alignment Search Tool) database (BLAST, 2022).

The primers describer by Higgins et al. 2007, were as follows:
Forward: CAC GCC GTA AGA GTG CAT TA and GTT CCT GAT CCG AAA TTC TCG; Reverse: AAC GGA GCT TGT CAG GGT TA (Higgins et al., 2007). According to this publication "in a PCR with all three primers, *both A. baumannii* and genomic sp. 13TU would yield an amplicon of 294 bp (sp4F to sp4R) but only *A. baumannii* would yield a second amplicon of 490 bp (sp2F to sp4R)".

The second set of primers from the BLAST database were as follows: Forward: ACA TCG CAT TGG GGA TTG GT; Reverse: GAC GCG CTT GTA GAC CCA TA. These primers target the *rec*A gene. The amplicon size when using these primers was 366 base pairs.

When testing our positive control for *A. baumannii*, we found that the set of primers we identified from the BLAST database provided clearer bands when compared to those designed by Higgins et al., 2007 study. Therefore, we decided to use the second set of primers when analyzing our samples.

Running the PCR assay
The first set of PCR was done to confirm that all insect specimens were indeed *P. h. capitis*. All reactions contained 2.5 µL of 10X PCR buffer (Mg2+), 0.5 µl of dNTPs (10mM), 0.25 µL of Taq DNA polymerase, 1.5 µL (10 µM) of each primer (forward: TTG TTG TGC TTT TGA CTT CTT G , reverse: CCC TGA TTT GAA GTA TTA AAG AAA CTC), 17.75 µL of nuclease free water (NFW), and 1 µL (40 ng/µL) of DNA for a total volume of 25 µL (Raoult et al., 2006). The PCR reaction was programmed as follows: 95 °C for 3 min; 44 cycles of 95°C for 30s, 58°C for 30s, 72°C for 90s; and a final extension step at 72°C for 7 min.

The next experiment consisted of running a PCR to test the DNA from the *A. baumannii* cultured sample. The reaction contained 12.5 µL of Froggabio 2X Taq Froggamix, 1.5 µL of each primer- targeting RNA polymerase subunit B (rpoB) gene obtained using the BLAST searching tool (forward: ACA TCG CAT TGG GGA TTG GT, reverse: GAC GCG CTT GTA GAC CCA TA), 8.5 µL

of NFW, and 1 µL of DNA. The PCR reaction was programmed as follows: 94 °C for 3 min; 25 cycles of 94°C for 60s, 60°C for 30s, 72°C for 60s; and a final extension step at 72°C for 10 min. This protocol was used to test the insect specimens for the presence of *A. baumannii* DNA, using a 5 µL (40 ng/µL) of DNA template of DNA and 3.5 µL of NFW.

All PCR gels were made with 1.5% Agarose gel in Sodium Borate buffer, and 5 µL of Ethidium Bromide, and were run with the same electrophoresis conditions: 120 volts for 45 minutes, then analyzed using a BIO RAD ChemiDoc imaging system.

3.6 Ethics Statement

Specimens from Colombia and La Hicaca, Honduras were collected from children by researchers under ethical clearance from Brock University (Files BREB 15-248 and BREB 17-383, respectively). Informed consent was obtained from parents before all children participated in the studies.

Lice specimens from schoolchildren in San Buenaventura, Honduras were collected by schoolteachers during intervention programs and later donated to the researchers. As such ethical clearance was not required.

Head lice from Argentinian children were obtained at governmental, non-fee-paying schools. Only pupils whose parents had given informed consent for participation were requested to assent for head lice examination. The freedom to refuse to participate in the research was clearly established in each case. The protocol for lice collection was approved by an *ad hoc* committee of the Centro de Investigaciones de Plagas e Insecticidas (CONICET-UNIDEF, Buenos Aires, Argentina), and archived in the laboratory (#BA20061995ARG, June 1995), as reported previously (Picollo et al., 1998).

As per the Canadian Council on Animal Care, further ethics clearances to analyze collected insects were not required as *P. humanus capitis* (class Insecta) and *Acinetobacter baumannii* (class Gammaproteobacteria), are below class Cephalopoda (CCAC, 2020).

CHAPTER 4: RESULTS

4.1 Infection frequency

In total, 123 vials containing 368 head lice were analyzed in pools, using PCR to determine the presence of *A. baumannii* DNA. Two vials containing 3 insects halves each were positive for *A. baumannii*; from La Hicaca, Honduras. Since lice were analyzed in pools, not individually, it is not possible to calculate an exact frequency of infection in lice. However, based on whether per each vial one, two or all three lice were positive for *A. baumannii,* a range can be determined between 1.83 - 5.50%. positivity.

The remaining lice from Argentina, Colombia, and San Buenaventura; Honduras were identified as negative for the DNA of *A. baumannii* under the test conditions described.

4.2 Polymerase Chain Reaction

Identifying *Pediculus humanus*

We first used PCR to verify that all samples were positive for *P. humanus* DNA. This was done to ensure that our methodology for the collection and DNA extraction of head lice was successful prior to proceeding with our second round of PCR to detect *A. baumannii* DNA.

The target PCR product size was 216 base pairs. All samples tested yielded positive results for *P. h. capitis* DNA. All gel images were captured with a BioRad ChemiDoc imaging system.

P. h. capitis specimens collected in Argentina returned positive for *P. humanus* DNA as seen in Figure 10-11.

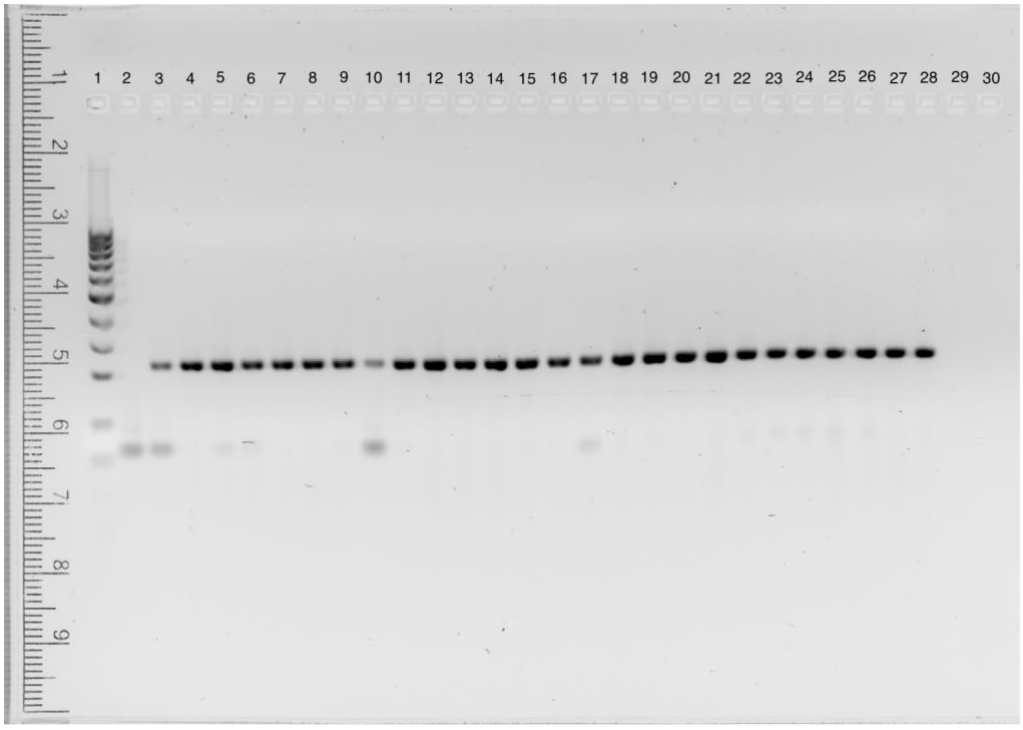


Figure 10. Argentinian samples 1-25 yielding positive results for *P. humanus* DNA.
Wells: 1: Ladder; 2: negative control (-) NFW; 3: *P. h. capitis* (+); 4-28: verified positive for head lice DNA; 29-30: empty.

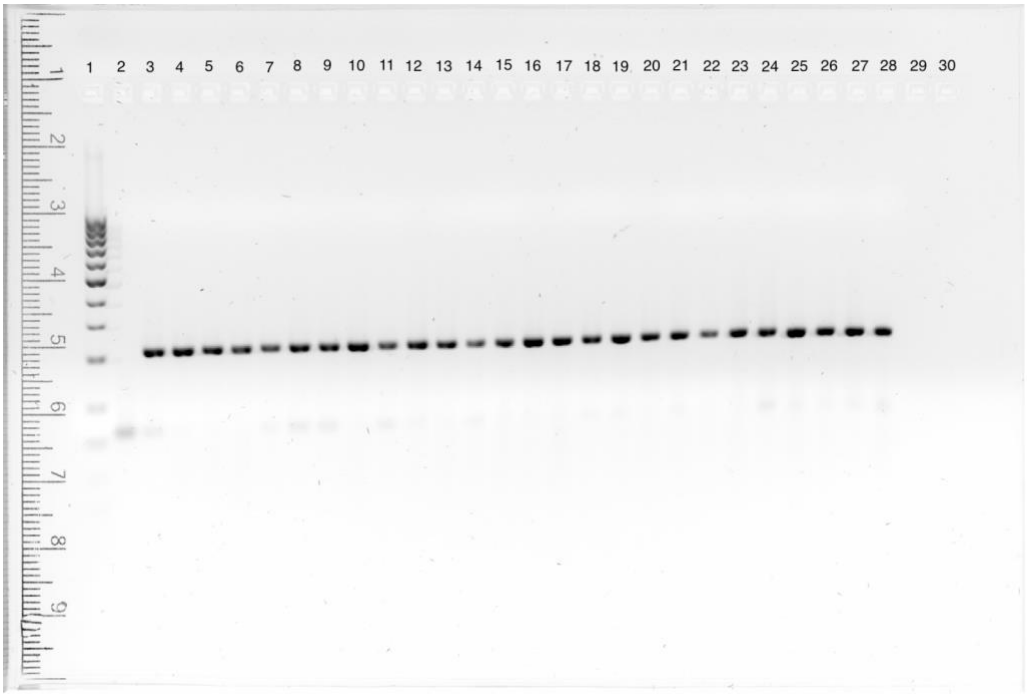


Figure 11. Argentinian samples 26-50 yielding positive results for *P. humanus* DNA.
Wells: 1: Ladder; 2: negative control (-) NFW; 3: *P. h. capitis* (+); 4-28: verified positive for head lice DNA; 29-30: empty.

Colombia

All insect specimens from Colombia returned positive for *P. humanus* DNA as seen in Figure 12.

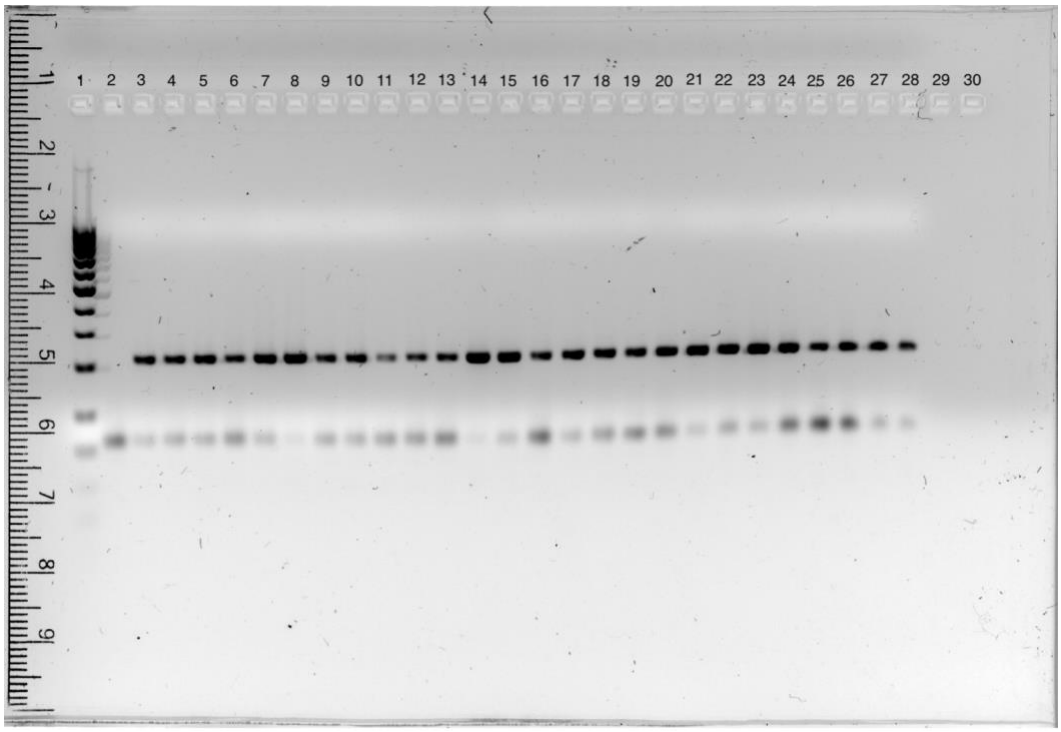


Figure 12. Colombian samples 1-25 yielding positive results for *P. humanus* DNA.
Wells: 1: Ladder; 2: negative control (-) NFW; 3: *P. h. capitis* (+); 4-28: verified positive for head lice DNA; 29-30: empty.

Honduras

La Hicaca

All insect specimens from Honduras returned **positive for** *P. humanus* **DNA** as seen in Figures 13-14.

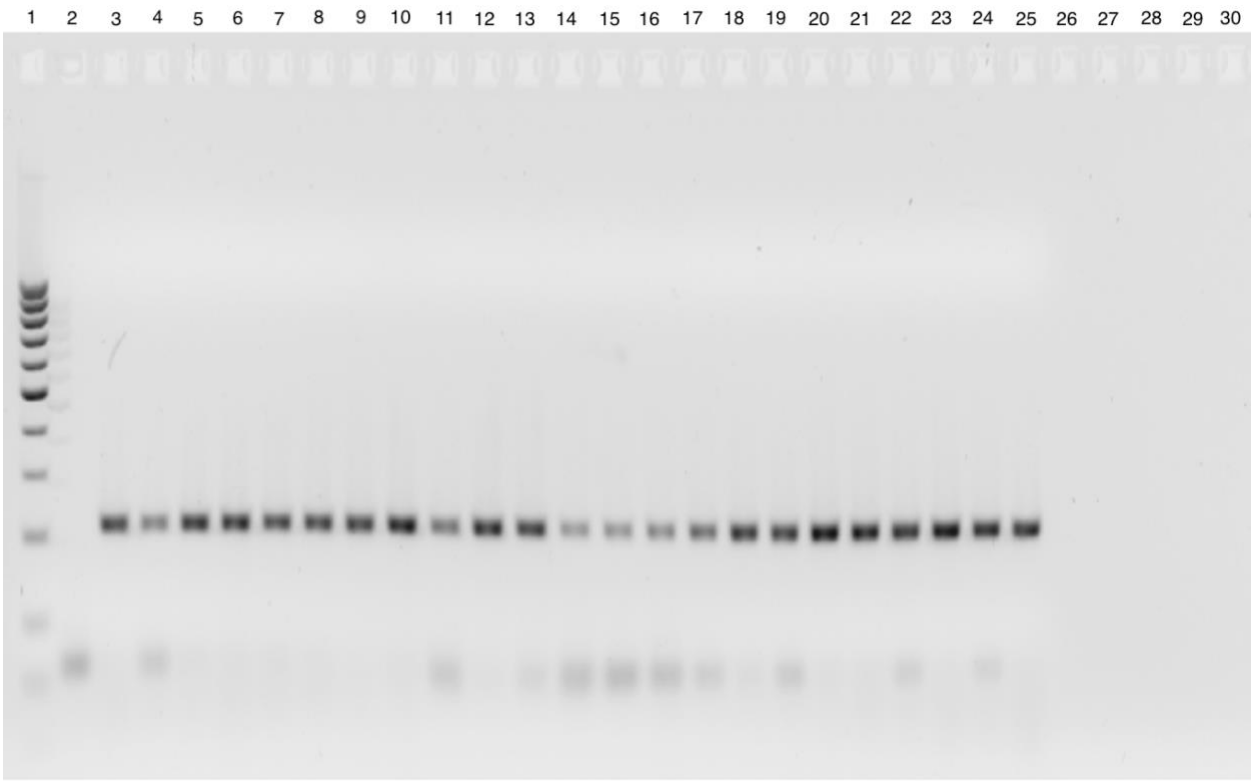


Figure 13. La Hicaca samples 1-22 yielding positive results for *P. humanus* DNA.
Wells: 1: Ladder; 2: negative control (-) NFW; 3: *P. h. capitis* (+); 4-25: verified positive for head lice DNA; 26-30: empty.

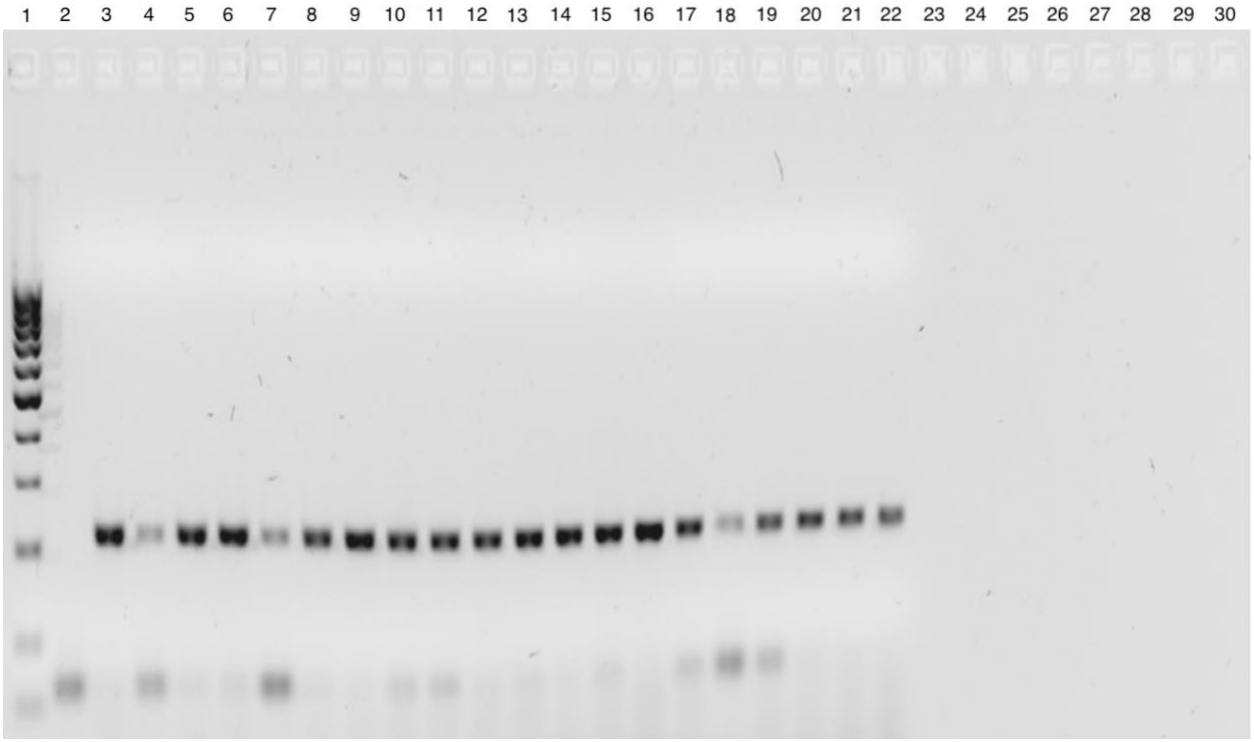


Figure 14. La Hicaca samples 23-41 yielding positive results for *P. humanus* DNA.
Wells: 1: Ladder; 2: negative control (-) NFW; 3: *P. h. capitis* (+); 4-22: verified positive for head lice DNA; 23-30: empty.

San Buenaventura

All insect specimens from San Buenaventura returned positive for *P. humanus* DNA as seen in Figure 15.

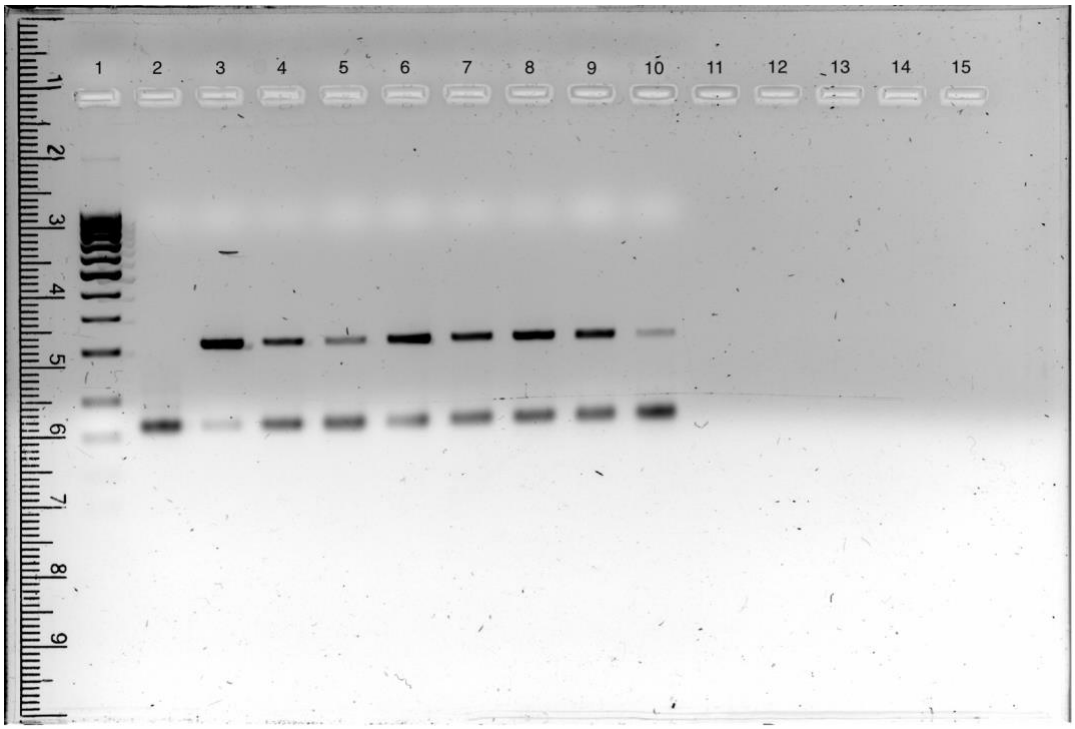


Figure 15. San Buenaventura samples 1-7 yielding positive results for *P. humanus* DNA.
Wells: 1: Ladder; 2: negative control (-) NFW; 3: *P. h. capitis* (+); 4-10: verified positive for head lice DNA; 11-15: empty.

Acinetobacter baumannii

Once all PCR results for *P. h. capitis* DNA came back positive, we proceeded to run PCRs in order to detect potential *A. baumannii* DNA from the head lice samples. The target PCR product size was 366 base pairs. All gel images were captured with a BioRad ChemiDoc imaging system.

Argentina

All insect specimens from Argentina returned negative results for *A. baumannii* DNA as seen in Figure 16-18.

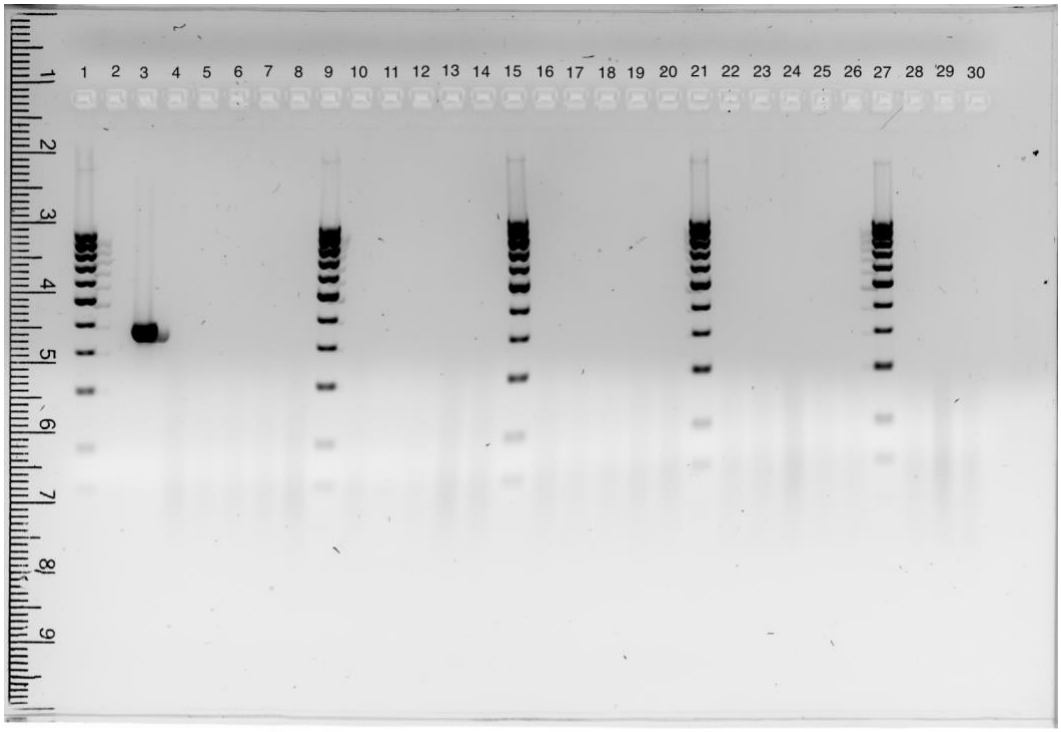


Figure 16. Argentinian pooled specimens 1-23 yielding negative results for A. baumannii DNA. Wells: 1, 9, 15, 21, 27: Ladder; 2: negative control (-) NFW; 3: A. baumannii (+); 4-8, 10-14, 16-20, 22-26, 28-30: negative for A. baumannii DNA.

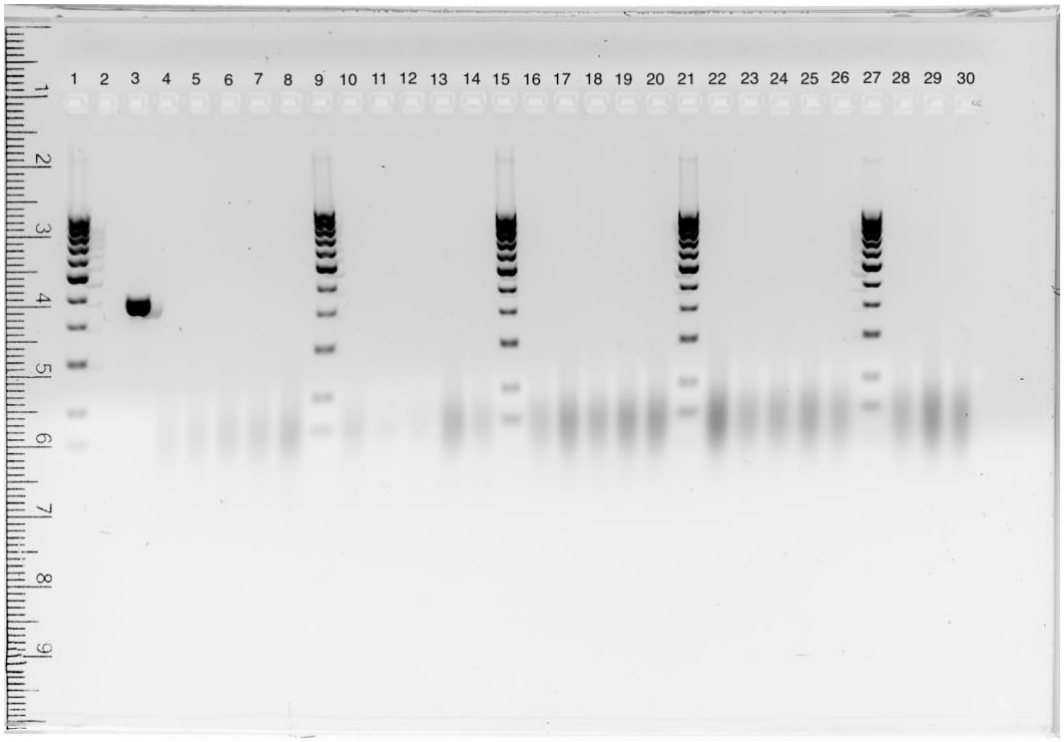


Figure 17. Argentinian pooled specimens 24-46 yielding negative results for *A. baumannii* DNA. Wells: 1, 9, 15, 21, 27: Ladder; 2: negative control (-) NFW; 3: *A. baumannii* (+); 4-8, 10-14,16-20, 22-26, 28-30: negative for *A. baumannii* DNA.

Figure 18. Argentinian pooled specimens 47-50 yielding negative results for *A. baumannii* DNA. Wells: 1: Ladder; 2: negative control (-) NFW; 3: *A. baumannii* (+); 4-7: negative for *A. baumannii* DNA; 8-30: empty.

Colombia

All insect specimens returned negative results for *A. baumannii* DNA as seen in Figure 19-20.

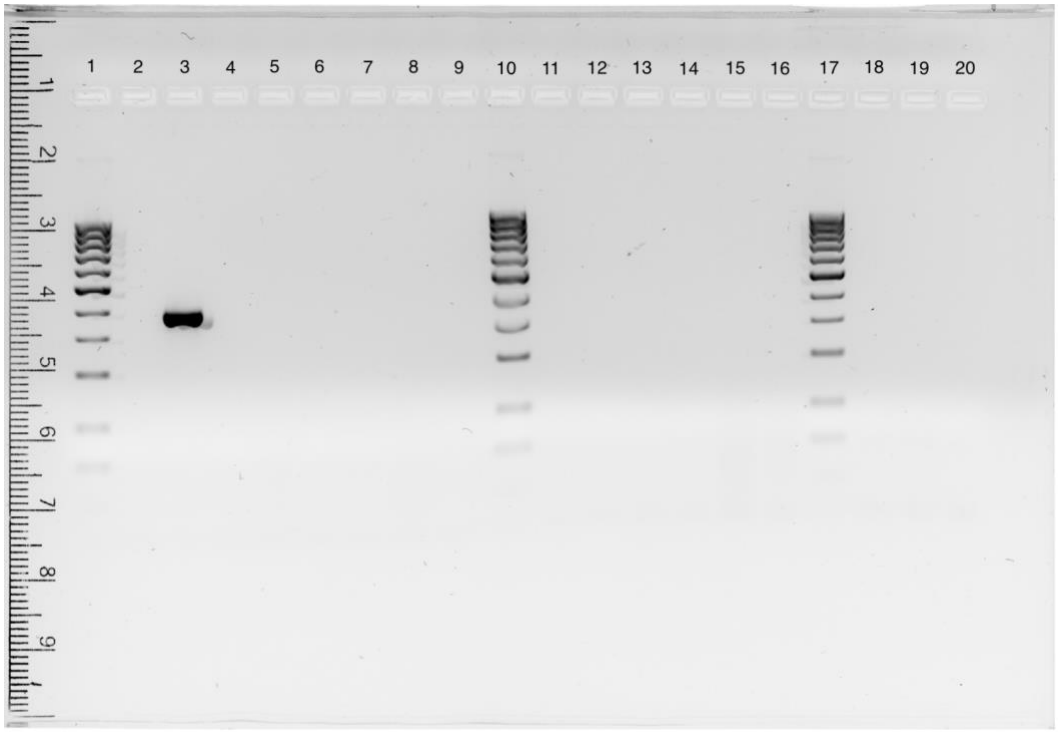


Figure 19. Colombian pooled specimens 1-15 yielding negative results for *A. baumannii* DNA. Wells: 1, 10, 17: Ladder; 2: negative control (-) NFW; 3: *A. baumannii* (+); 4-9,11-16,18-20: negative for *A. baumannii* DNA.

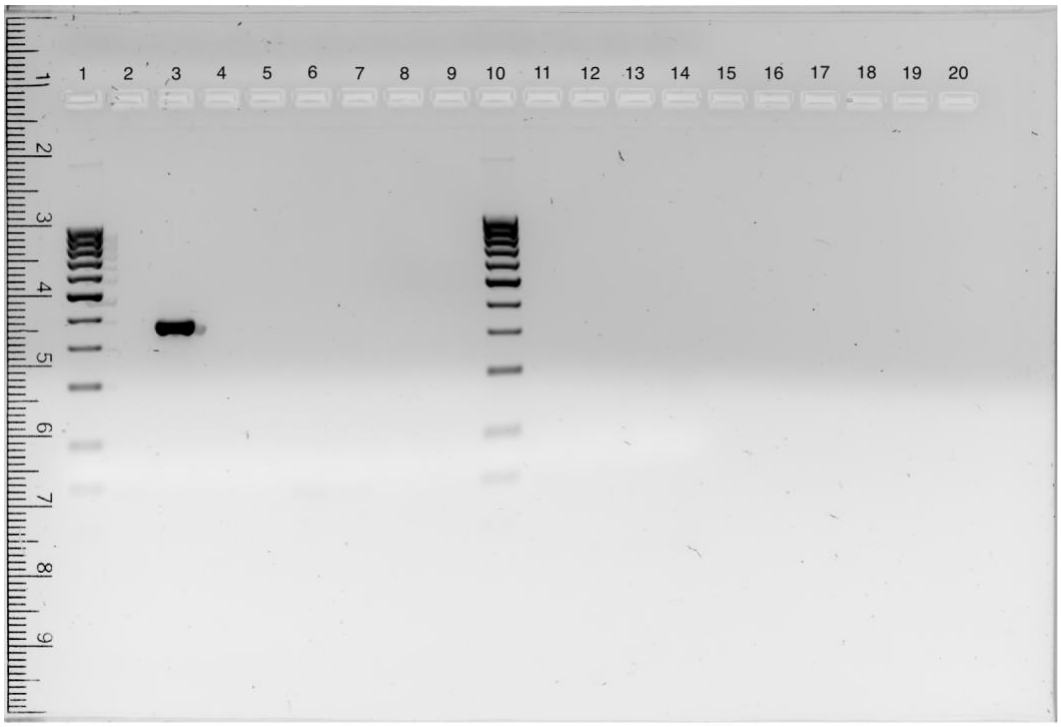


Figure 20. Colombian pooled specimens 16-25 yielding negative results for *A. baumannii* DNA. Wells: 1, 10: Ladder; 2: negative control (-) NFW; 3: *A. baumannii* (+); 4-9, 11-14: negative for *A. baumannii* DNA; 15-20: empty.

Honduras

La Hicaca

Two vials containing pooled specimens returned positive results for *A. baumannii* DNA (wells 7 and 8 in Figure 22), and the rest were found to be negative as shown in Figures 21-22.

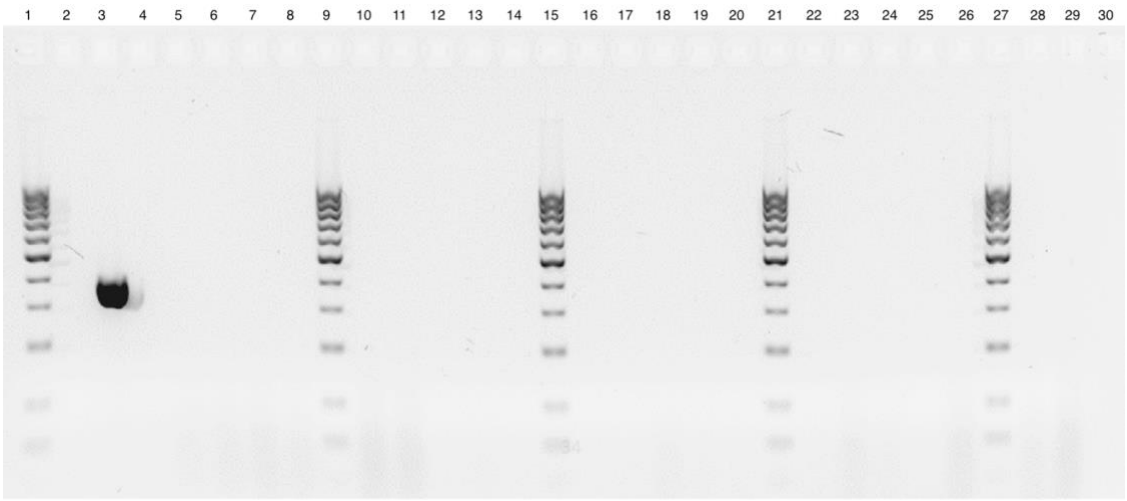


Figure 21. La Hicaca pooled specimens 1-22 yielding negative results for *A. baumannii* DNA. Wells: 1, 9, 15, 21, 27, Ladder; 2: negative control (-) NFW; 3: *A. baumannii* (+); 4-8, 10-14, 16-20, 22-26, 28-30: negative for *A. baumannii* DNA.

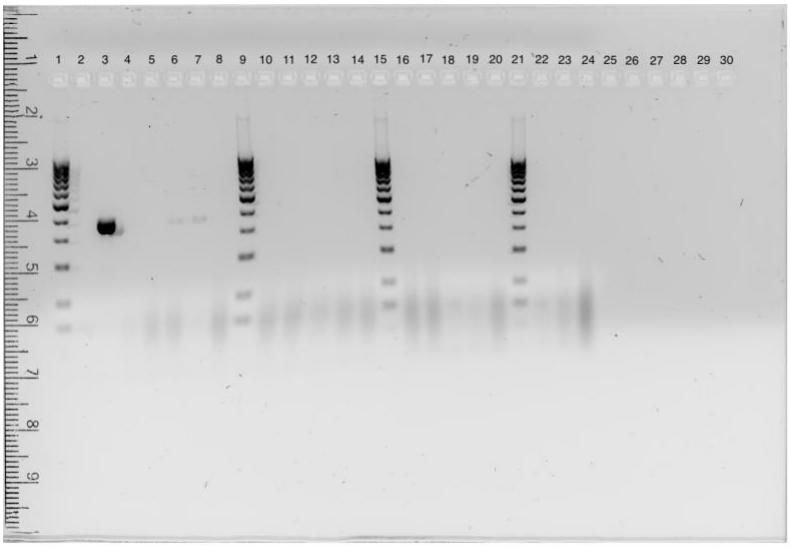


Figure 22. La Hicaca pooled specimens 25 and 26 returned **positive for *A. baumannii* DNA,** while samples 23-24, 27-41 were found to be negative for *A. baumannii* DNA.

Wells: 1, 10, 16, 22: Ladder; 2: negative control (-) NFW; 3: *A. baumannii* (+); 4-6, 9, 11-15, 17-21, 23-26: negative for *A. baumannii* DNA; 7-8: positive for *A. baumannii* DNA; 27-30: empty.

San Buenaventura

All pooled specimens returned negative results for *A. baumannii* DNA as seen in Figure 23.

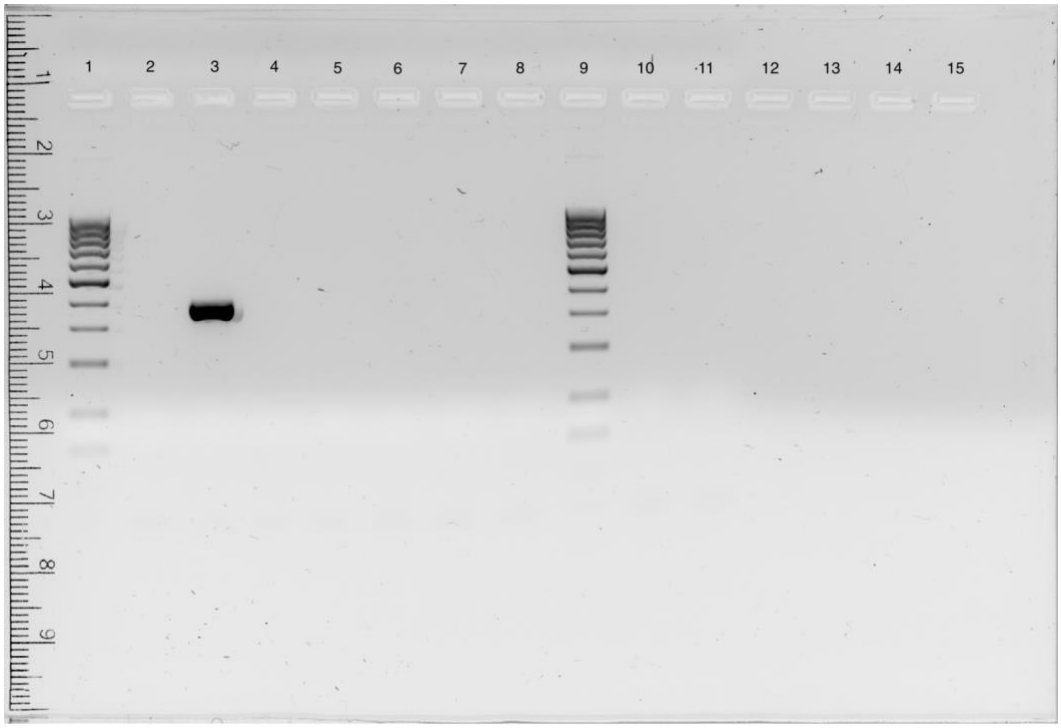


Figure 23. San Buenaventuran pooled specimens 1-7 yielding negative results for *A. baumannii* DNA.
Wells: 1, 9: Ladder; 2: negative control (-) NFW; 3: *A. baumannii* (+); 4-8, 10-11: negative for *A. baumannii* DNA; 12-15: empty.

Confirmation of A. baumannii by sequencing

PCR assay of all Honduran samples was run in duplicate at Brock U and at University National Autonomous of Honduras (UNAH), with identical results as shown in Figure 24 below. The amplification products were sequenced on both strands using the same PCR primers by Psomagen® (www.psomagen.com, Maryland, USA). The obtained sequences were analyzed with the GenBank BLAST tool to confirm their identity as *A. baumannii*.

The sequences were trimmed and edited with the Geneious® 9.1.7 software and deposited into the NCBI GenBank under accession number ON564437.

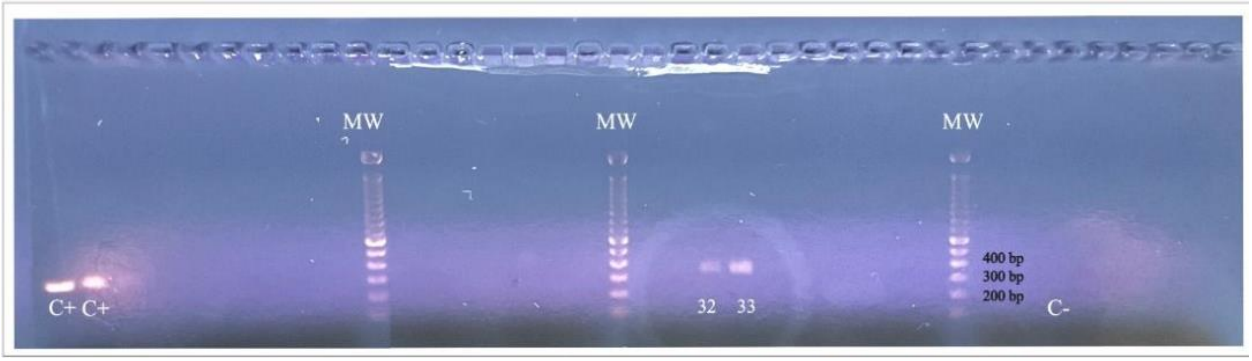


Figure 24. Honduran pool specimens from vials 32 and 33 are positive for A. baumannii. Bands of 366 bp are shown. (Photo by Dr.Gustavo Fontecha, UNAH. Honduras).

CHAPTER 5: DISCUSSION

Head lice are a prevalent ectoparasites and affect communities worldwide. Historically viewed as a small burden or an unavoidable part of attending primary school, pediculosis capitis is now capturing the attention of the scientific community as the understanding of these common arthropods deepen. Now that it has been accepted that head lice and body lice are two separate ecotypes of *Pediculus humanus*, more questions have arisen when considering the differences in vectorial capacity between the two. Many infectious agents, including most notably *Acinetobacter baumannii*, have been discovered in head lice specimens by various research groups around the world. This has scientists wondering if head lice have the ability to harbor and transmit pathogens. While this thesis is not exploring vectorial capacity of head lice, it does investigate the presence of one of the most concerning bacterial species found harbored by head lice. The present work adds to the literature in this respect, by contributing data from three countries in Latin America, a region without recorded data until now.

In the present study, pooled *P. h. capitis* samples from Argentina, Colombia, and Honduras were analyzed for the presence of *A. baumannii* DNA. The general socio-economic characteristics of the study sites were varied. Specimens from Argentina were collected from children attending five urban primary schools in different barrios of Buenos Aires, the large capital city which has a population over 15 million people when including the surrounding metropolitan areas (Macrotrends, 2022). This is by far the most industrialized and urban location from which we analyzed specimens. Samples from Colombia were collected from Children in Medellín, the Colombia's second largest city in the country comprising a mix of rural and metropolitan areas. Data shows that 70.8% of the population is living below middle class (Colombia Reports, 2021). Samples from Honduras were collected from school children in two separate locations: La Hicaca and San Buenaventura. La Hicaca is a small rural village, 393 km North of Tegucigalpa, the capital city of Honduras. San Buenaventura is a small peri-urban city, 22 km South of Tegucigalpa. As a whole, Honduras is categorized as a low-income country, with extreme disparities through its territory. La Hicaca is considered a remote community and has a higher percentage of population living in poverty when compared to San Buenaventura (Andréu, 2012). In addition to barriers to accessing health care, residents of La Hicaca also deal with geographical barriers as La Hicaca is located in the mountainous Northern region of Honduras, 55 km away from the closest hospital (Pearson et al., 2012).

Through polymerase chain reactions (PCR), we were able to interrogate the presence and absence of *Acinetobacter baumannii* DNA from head lice samples collected in those three countries. After verifying all samples were *P. h. capitis* by visualization and through PCR, we established a positive and negative control of *A. baumannii*. All sample images that displayed a distinct band at the 366 base pair mark were determined to be positive for *A. baumannii* DNA; whereas the absence of such band indicates that *A. baumannii* nucleic acids were either not present or not detected.

Our results indicated that only head lice from La Hicaca, Honduras were positive for the bacterial DNA of interest at a range of 1.83 - 5.50%. Overall positivity for *A. baumannii* DNA in

head lice was found to be between 0.54 - 1.63%, The reasons for finding *A. baumannii* only in specimens from la Hicaca may be related to the community's degree of extreme poverty and lack of sanitary infrastructure, most significantly lack of household running water, personal hygiene products and anti-pediculosis treatment (Jamani et al., 2019). These conditions may contribute to children's skin and scalp colonization by bacteria, which in turn will be picked up by head lice during blood meals.

Although similar studies in other countries do not analyze the socioeconomic status of children infested with head lice positive for *A. baumannii*, an Israeli study by Henig et al. 2015 concluded that the cohort of participants who were of low socioeconomic status were at almost twice the risk of being colonized with carbapenem resistant *A. baumannii* (CRAB), as compared to the higher socioeconomic status counterpart. Our findings coincide in that *A. baumannii-positive* head lice were only found in the location with the lowest level of socioeconomic status when compared to all other locations in our study.

Conversely, negative findings in lice specimens from the other study sites may also be explained by their better standards of living as compared to those in la Hicaca. For example, children in Argentina, Colombia, and San Buenaventura, are more likely to have received in the recent past, a range of pharmacological treatments including pediculicides, antibiotics, and anti-parasitic treatments. For instance, some antiparasitic medications such as Furazolidone and Metronidazole have both antiparasitic and antibiotic properties (Abbo et al., 2005; Campbell & Soman-Faulkner, 2021; Karaiskos et al., 2019). More specifically, Furazolidone belongs to the nitrofuran drug class and has been found to be extremely effective against multi-drug resistant gram-negative bacteria including *A. baumannii* and it also can inhibit biofilm formation (Bhando et al., 2020). Because the present study was not epidemiological in nature, specific data on social determinants and other confounders were not collected. Certainly, this information can prove valuable in future studies.

To ensure data reliability, we cultured *A. baumannii* reference material over a span of several weeks. After using the Gram staining technique, we visualized the bacteria under a microscope to ensure purity of our positive control in different stages of growth. In terms of head-lice, all specimens were collected from the heads of participants and observed individually using a stereomicroscope to verify that they were head lice using morphological keys. To confirm that our specimens were head lice and that we successfully cultured *A. baumannii*, DNA was extracted from both the bacteria and all head lice samples, after which PCR was completed to ensure that all DNA was extracted properly from specimens. During the optimization of our protocol, we tested several sets of primers to ensure the clearest gel readings possible. Using serial dilutions of *A. baumannii* in a nutrient broth medium, we ran several PCRs to try to establish the lowest quantifiable amount of bacterial DNA that our PCR and imaging system could record. The bands that appeared for our positive samples are faint but distinct, which correspond with a sound methodology. During our PCR sensitivity analysis, we discovered that a small amount of *A. baumannii* DNA (1 µL) returned with an oversaturated band. If our positive results were a false positive, surely the bands would be much darker and more heavily saturated. Even so, after completing optimization and data collection of our results, we re-

extracted DNA from the *A. baumannii* positive head lice samples and repeated the PCR for those samples to ensure that they were free of contamination and were true positive results.

Our positive findings add data to the scientific literature by again identifying *Acinetobacter spp.* in *P. h. capitis* samples from yet another continent. However, several unknowns regarding this relationship remain. How do head lice get infected with *Acinetobacter bacteria and specifically with A. baumannii*? And, are they able to transmit these bacteria?

In order to understand the potential transmission of pathogenic bacteria, it is important to understand the basics of head lice transmission. Head lice transmission between children predominantly occurs from head-to-head contact or from sharing infested items such as hairbrushes and bedding. Aside from the obvious routes of transmission, there is controversy in the scientific community surrounding the potential for head lice to be transmitted from fomites —inanimate objects that act as a passive vector when contaminated with pathogens such as bacteria, parasites, and viruses (Burkhart, 2003).

So far, it is unknown how head lice acquire *A. baumannii* but given that these insects are permanent ectoparasites with no part of their life cycle spent away from the host, it is suggested that they may acquire the bacteria from children's scalp.

According to a study by Haidamak et al., 2019, the presence of head lice significantly alters the microbiota of children's scalps, which is vital in understanding the origin of *A. baumannii* in head lice populations. Head lice ingest *A. baumannii* by piercing through colonized skin and proceed to excrete viable *A. baumannii* in their feces, which is left on the skin of their host. Transmission of *A. baumannii* from one person to another occurs by host scratching an infected area, and then touching other people or items in which the bacteria can proliferate and create biofilms. Alternatively, head lice travel from host to host and can spread *A. baumannii* through their feces.

A study published by Houhamdi and Raoult, 2006, aimed at capturing the transmission of *A. baumannii* via body lice using a rabbit model. Researchers used several techniques in order to identify where the bacteria was present and consequently what the most likely means of transmission would be. They used PCR and an immunofluorescence assay (IFA) to positively detect *A. baumannii* in the feces of lice, as well as confocal microscopy of immunofluorescence-stained sections of infected body lice to display the distribution of *A. baumannii* in the body of the infected lice. Their findings strongly suggest that viable *A. baumannii* can be found within infected lice, the remains of lice post-mortem, as well as in their feces. Their data did not support the transmission of *A. baumannii* through feeding. This conclusion supports the theory that the mechanism of transmission occurs through *A. baumannii* infected lice feces or crushed lice entering scratching lesions.

A. baumannii is mostly reported in health care settings. Its ability to survive for long periods of time on surfaces, as well as its ability to create biofilms and successfully proliferate on human skin may facilitate transmission and infection with the bacteria in both community and health

care environments. *A. baumannii* is a pathogenic, opportunistic bacteria that can proliferate in water, soil, and on human skin, create biofilms, and live on surfaces for up to four weeks (Pakharukova et al., 2018). Regardless of where it is found, in the scientific community it is widely accepted that human skin acts as a reservoir for many variations of *Acinetobacter spp.* and can be present as part of the microflora on human skin. The first report of human skin being an important reservoir for *Acinetobacter spp.* was published in 1963 (Taplin & Zaias, 1963). A report published in the Journal of Clinical Microbiology in 1997 compared the distribution of *Acinetobacter spp.* on hospital patients as well as healthy individuals and found over 6 microorganisms belonging to genus *Acinetobacter* from various locations on the participants (Seifert et al., 1997). Researchers have also identified *A. baumannii* from swabs taken from human scalps. A Thai study took swabs from psoriasis patients as well as healthy controls, and found that *A. baumannii* had colonized on the scalps, nares, and psoriasis lesions of some participants (Chularojanamontri et al., 2016). Another study from Saudi Arabia collected swabs from participant feet, hands, and scalps also identified the presence of *A. baumannii* as part of the skin flora. They also explained that close contact can be a viable way of transmitting the bacteria (Shami et al., 2019). These findings suggest that *A. baumannii* can colonize on human scalps and contribute to the microflora of certain populations. Furthermore, a Brazilian team of researchers has recently published a study that investigated scalp microbiota changes in children with pediculosis capitis. Not only did they find *A. baumannii* present on the scalps of children in both of their study groups (those susceptible to head lice and those not susceptible), but they also found that children with pediculosis capitis had significantly higher variation in their overall scalp microbiota including bacteria and fungi (Haidamak et al., 2019).

Gram-negative aerobic bacilli such as *A. baumannii* have enzymes which allow them to break down organic materials, which create volatile by-products and subsequently an odor, especially when colonized in chronic wounds (Bowler et al., 1999). This is significant because a recent study investigated head lice ability to distinguish volatile human odor compounds and found that they gravitate towards scalp environment odors when compared to human forearm or foot odor compounds (Galassi et al., 2019). If *A. baumannii* can be part of the human scalp microbiome, it is possible that head lice are able to detect gram-negative aerobic bacilli odor compounds and potentially prefer *A. baumannii* colonized hosts. The microbiome of head lice and body lice are also currently under investigation, as researchers believe the differences in their microbiota may help confirm the differentiation of the two ecotypes of lice, however, *A. baumannii* has yet to be identified as part of the microbiome of either organism (Agany et al., 2020).

In turn, children may acquire the bacteria from fomites, as *A. baumannii* is notorious for its ability to survive for extended periods of time and produce biofilms on surfaces and fomites (Burkhart & Burkhart, 2007). A study published in PLOS One in 2018 analyzed the virulence of different *A. baumannii* strains and found that the bacterium is able to maintain its virulence for up to 60 days after long term starvation on dry surfaces, such as fomites (Chapartegui-Gonzalez et al., 2018). This information suggests that the transmission of *A. baumannii* can occur in both community and health care environments. When factored together, the presence of *A.*

baumannii with its drug resistant properties, and its presence in head lice in both urban and rural areas, all point to the biological plausibility of bacterial transmission through head lice. Upon finding *A. baumannii* (or any other pathogen) in head lice, the next step is to determine whether these insects are endowed with vectorial competence. This question is natural since the ecotype *Pediculus humanus humanus* is a competent vector for many bacterial species.

A comprehensive review done by Amanzougaghene *et al*., 2019, outlines the different bacteria identified from both body and head lice. Understanding the role head lice could hold in the transmission of pathogenic bacteria is significant because of the impact these pathogens may impose on the communities suffering from their presence.

Due to the cross-sectional nature of the present study and the sampling methodology being one of convenience, it is not possible to draw more complex conclusions. We cannot confidently confirm how the lice we analyzed became infected with *A. baumannii*, nor can we attribute our findings to anything other than the differences in sanitization, level of poverty, and living environments of the communities we obtained samples from. Notwithstanding, finding *A. baumannii* DNA in head lice from children living in a poor, remote village in Honduras has important implications. Not only is this the first study to analyze head lice for pathogens in Latin America, but it also shows that the importance of *A. baumannii* may go beyond the health care settings. The pathogen colonizes skin and scalp but may cause considerable morbidity if is able to translocate to tissues or blood stream due to abrasions of injuries. This will certainly become a major medical emergency to people residing in villages such as La Hicaca, far from any major cities, hospitals, and modernized infrastructure.

Recently, the potential for acquiring an *A. baumannii* infection or becoming infested with infected head lice has been exacerbated by the COVID-19 pandemic. Hospitals and intensive care units have been at or above capacity since the beginning of the pandemic. Overcrowded hospital populations in conjunction with an exponential increase in respirator use make *A. baumannii* infection more probable. A recent study published by researchers in Argentina indicate that the COVID-19 pandemic has reduced the incidence of pediculosis capitis in Buenos Aires, however, this study did not investigate potential lice infections in clinical settings (Galassi et al., 2021). Despite increased social distancing and many people opting to remain at home unless necessary, the massive influx of patients in clinical settings could create opportunities for the infection and transmission of not only *A. baumannii*, but head lice as well.

Regardless of the origin of *A. baumannii* stemming from clinical settings, schools, other people, or naturally occurring colonized scalps, its ability to survive for extended periods of time in combination with potentially being spread by a widely distributed and transmitted ectoparasite is concerning.

In comparison to other studies, our *A. baumannii* positivity rates are low. Studies that have comparable positivity rates to our findings include studies from France and Thailand which reported 2.7% and 1.45% of analyzed head lice to be positive for *A. baumannii*, respectively (Candy et al., 2018; Sunantaraporn et al., 2015). The study in France analyzed lice from patients

(including two homeless individuals) in a hospital in Bobigny, France, which is an urban city 3 km North of Paris. The study in Thailand analyzed head lice from school children in 15 regions of Thailand and found *A. baumannii* positive lice in the Northern, Northeastern, and Southern regions. When compared to other regions of Thailand, the Northeastern region has the highest population, as well as the highest percentage of its population living below the poverty line (18%), and the lowest income per capita of any region. The Northern region is also poor with 16% of its population below the poverty line, whereas the Southern region has the second highest income per capita of any region in Thailand, and only 10% of its population below the poverty line (Knoema, 2012). The researchers involved proclaim that in future studies they would like to add epidemiological data to better interpret their findings.

In contrast, high frequencies of *A. baumannii*-positive lice have been reported from studies based in urban areas in Algeria (47%), and France (33%), as well as in more rural and less affluent areas located in Ethiopia (47%), Republic of Congo (31%), and the United States (81%) (Amanzougaghene et al., 2016; Bouvresse et al., 2011; Candy et al., 2018; Eremeeva et al., 2017; Kempf et al., 2012; Louni et al., 2018). To provide more context, the study in Algeria reported head lice positive for *A. baumannii* from both Nigerian refugees as well as local school children in Eastern Algiers, North Algeria. Algiers is the large urban capital city of Algeria and has a population of almost three million people (Louni et al., 2018; World Population Review, 2021a). The study that identified 33% of head lice positive for *A. baumannii* in France were collected from primary school children living in Paris, France which is an urban city in North Central France with a population of over 11 million people (Bouvresse et al., 2011; World Population Review, 2021b).

In comparison, there are also reports of high prevalence of *A. baumannii* infected head lice from several locations that collected *P. h. capitis* samples from people living in areas of lower income- including a study from Ethiopia, where they collected lice from healthy individuals in seven different locations throughout the Southern Nations, Nationalities, and Peoples' Region – which is a rural agricultural province with 86% of its inhabitants living in poverty (Data Africa, 2015; Kempf et al., 2012). In the Republic of Congo, 31% of head lice tested were reported to be positive for *A. baumannii*. Lice were collected from healthy indigenous (pygmy) individuals living in three separate villages: Thanry-Ipendja and Pokola in the North, and Béné Gamboma in the Eastern/Central region. All villages were poor and had limited access to basic resources (Amanzougaghene et al., 2016). Additionally, a study from Georgia, USA reported 81% of the head lice they analyzed to be positive for *A. baumannii*. They collected lice from 19 schools and found positives in three counties: Bullock, Bryan, and Jenkins, which have 21.9, 7.8, and 29% of their populations living below the poverty line, respectively (United States Census, 2021).

CHAPTER 6: CONCLUSIONS AND FUTURE RESEARCH

The primary objective of this study was to analyze *P. h. capitis* samples from three countries in Latin America for the presence or absence of *A. baumannii*. The present study provides solid evidence that *A. baumannii* was indeed harbored by head lice specimens from Honduras.

The present study would have been enhanced by associating our findings epidemiological data. This may provide insight as to why certain head lice populations are positive for *A. baumannii*. Including data such as age, gender, health status, as well as any previous exposure to pediculicides and/or antibiotics may be key in understanding our findings.

Another interesting avenue to explore is the source of head lice infection with *Acinetobacter* spp *and A. baumannii.* It wouldn't be unfeasible to collect take swabs of participant's scalps, and analyze them for the presence of *A. baumannii* and other potential pathogens. Preliminary results from a recent study analyzing the presence of *A. baumannii* on human skin and body lice found that 60% of individuals had at least one louse positive for *A. baumannii* DNA, and 26.7% had *A. baumannii* DNA identified in both their skin and lice samples. Interestingly, none of their participants had *A. baumannii* present in their blood (Ly et al., 2019). Although this study is investigating *A. baumannii* in body lice, their methodology was comprehensive as it included collecting lice samples, skin swabs from four separate locations, as well as blood samples from their participants. This study not only included more physiological data, but epidemiological data as well (including age, birthplace, body mass index, clinical presentations, presence of chronic disease, alcohol and tobacco use, migratory status, sex, and place of residence). Ly et al (2019) suggested that future studies should investigate further by comparing the *A. baumannii* isolates present from the lice and skin to see if they are the same. Transposing this methodology to fit one that is *P. h. capitis*-centric not only fits our recommendations for future studies, but also aligns with our overarching mission to understand the ability of head lice to harbor and transmit pathogenic bacteria. To ensure that our specimens are indeed *P. h. capitis* and not *P. h. humanus*, another addition that could be included in future studies could be to use multiplex real-time PCR to target the Phum_PHUM540560 gene to distinguish between the two ecotypes of lice as outlined in a PLOS One publication (Drali et al., 2013).

In summary, human infesting lice have been the focus of scientific investigation for over a hundred years, and for good reason. Our study is the first of its kind regarding the detection of pathogenic bacteria in lice from Latin America and while filling an important gap in the literature, brings about new research questions that should be addressed in the near future.

References

Abbo, A., Navon-Venezia, S., Hammer-Muntz, O., Krichali, T., Siegman-Igra, Y., & Carmeli, Y. (2005, Jan). Multidrug-resistant Acinetobacter baumannii. *Emerg Infect Dis, 11*(1), 22-29. https://doi.org/10.3201/eid1101.040001

Agany, D. D. M., Potts, R., Hernandez, J. L. G., Gnimpieba, E. Z., & Pietri, J. E. (2020, Feb). Microbiome Differences Between Human Head and Body Lice Ecotypes Revealed by 16S RRNA Gene Amplicon Sequencing. *J Parasitol, 106*(1), 14-24. https://www.ncbi.nlm.nih.gov/pubmed/31958374

Al-Marjan, K. K., Qaraman; Abdullah,Shamall. (2015, 4/23/2015). In Vitro Study On The Morphological Development Of Eggs (Nits) And Other Stages Of Head Lice Pediculus humanus capitisDe Geer, 1767. *Zanco Journal of Pure and Applied Sciences, 27*(3), 35-40. http://www.zancojournals.su.edu.krd/index.php/JPAS/article/view/162

Amanzougaghene, N., Akiana, J., Mongo Ndombe, G., Davoust, B., Nsana, N. S., Parra, H. J., Fenollar, F., Raoult, D., & Mediannikov, O. (2016, Dec). Head Lice of Pygmies Reveal the Presence of Relapsing Fever Borreliae in the Republic of Congo. *PLoS Negl Trop Dis, 10*(12), e0005142. https://doi.org/10.1371/journal.pntd.0005142

Amanzougaghene, N., Fenollar, F., Raoult, D., & Mediannikov, O. (2019). Where Are We With Human Lice? A Review of the Current State of Knowledge. *Front Cell Infect Microbiol, 9*, 474. https://doi.org/10.3389/fcimb.2019.00474

Amanzougaghene, N., Fenollar, F., Sangare, A. K., Sissoko, M. S., Doumbo, O. K., Raoult, D., & Mediannikov, O. (2017). Detection of bacterial pathogens including potential new species in human head lice from Mali. *PLoS One, 12*(9), e0184621. https://doi.org/10.1371/journal.pone.0184621

Amanzougaghene, N., Mediannikov, O., Ly, T. D. A., Gautret, P., Davoust, B., Fenollar, F., & Izri, A. (2020, Apr 7). Molecular investigation and genetic diversity of Pediculus and Pthirus lice in France. *Parasit Vectors, 13*(1), 177. https://doi.org/10.1186/s13071-020-04036-y

Andréu, C. (2012). Rural poverty in Honduras: Despite progress, an ongoing challenge. *Global Majority E-Journal, 3*(2), 100-109. https://www.american.edu/cas/economics/ejournal/upload/andre-u_accessible.pdf

Angelakis, E., Diatta, G., Abdissa, A., Trape, J. F., Mediannikov, O., Richet, H., & Raoult, D. (2011, Dec). Altitude-dependent Bartonella quintana genotype C in head lice, Ethiopia. *Emerg Infect Dis, 17*(12), 2357-2359. https://doi.org/10.3201/eid1712.110453

Angelakis, E., Rolain, J. M., Raoult, D., & Brouqui, P. (2011, Jul). Bartonella quintana in head louse nits. *FEMS Immunol Med Microbiol, 62*(2), 244-246. https://doi.org/10.1111/j.1574-695X.2011.00804.x

Araujo, A., Ferreira, L. F., Guidon, N., Maues Da Serra Freire, N., Reinhard, K. J., & Dittmar, K. (2000, Jul). Ten thousand years of head lice infection. *Parasitol Today, 16*(7), 269. https://doi.org/10.1016/s0169-4758(00)01694-x

Arkwright, J. A., & Duncan, F. M. (1918, Sep 21). Preliminary Note on the Association of Rickettsia Bodies in Lice with Trench Fever. *Br Med J, 2*(3012), 307-309. https://doi.org/10.1136/bmj.2.3012.307

Arriaza, B., Orellana, N. C., Barbosa, H. S., Menna-Barreto, R. F., Araujo, A., & Standen, V. (2012, Apr). Severe head lice infestation in an Andean mummy of Arica, Chile. *J Parasitol, 98*(2), 433-436. https://doi.org/10.1645/GE-2903.1

Ballouz, T., Aridi, J., Afif, C., Irani, J., Lakis, C., Nasreddine, R., & Azar, E. (2017). Risk Factors, Clinical Presentation, and Outcome of Acinetobacter baumannii Bacteremia. *Front Cell Infect Microbiol, 7*, 156. https://doi.org/10.3389/fcimb.2017.00156

Ben-Chetrit, E., Wiener-Well, Y., Lesho, E., Kopuit, P., Broyer, C., Bier, L., Assous, M. V., Benenson, S., Cohen, M. J., McGann, P. T., Snesrud, E., & Levin, P. D. (2018, Nov 21). An intervention to control an ICU outbreak of carbapenem-resistant Acinetobacter baumannii: long-term impact for the ICU and hospital. *Crit Care, 22*(1), 319. https://doi.org/10.1186/s13054-018-2247-y

Beveridge, T. J. (2001, May). Use of the gram stain in microbiology. *Biotech Histochem, 76*(3), 111-118. https://www.ncbi.nlm.nih.gov/pubmed/11475313

Bhando, T., Bhattacharyya, T., Gaurav, A., Akhter, J., Saini, M., Gupta, V. K., Srivastava, S. K., Sen, H., Navani, N. K., Gupta, V., Biswas, D., Chaudhry, R., & Pathania, R. (2020, Feb 1). Antibacterial properties and in vivo efficacy of a novel nitrofuran, IITR06144, against MDR pathogens. *J Antimicrob Chemother, 75*(2), 418-428. https://doi.org/10.1093/jac/dkz428

BLAST. (2022). *Basic Local Alignment Search Tool*. United States Government. https://blast.ncbi.nlm.nih.gov/Blast.cgi

Bonilla, D. L., Cole-Porse, C., Kjemtrup, A., Osikowicz, L., & Kosoy, M. (2014, Oct). Risk factors for human lice and bartonellosis among the homeless, San Francisco, California, USA. *Emerg Infect Dis, 20*(10), 1645-1651. https://doi.org/10.3201/eid2010.131655

Bonilla, D. L., Durden, L. A., Eremeeva, M. E., & Dasch, G. A. (2013). The biology and taxonomy of head and body lice--implications for louse-borne disease prevention. *PLoS Pathog, 9*(11), e1003724. https://doi.org/10.1371/journal.ppat.1003724

Bonilla, D. L., Kabeya, H., Henn, J., Kramer, V. L., & Kosoy, M. Y. (2009, Jun). Bartonella quintana in body lice and head lice from homeless persons, San Francisco, California, USA. *Emerg Infect Dis, 15*(6), 912-915. https://doi.org/10.3201/eid1506.090054

Boutellis, A., Abi-Rached, L., & Raoult, D. (2014, Apr). The origin and distribution of human lice in the world. *Infect Genet Evol, 23*, 209-217. https://doi.org/10.1016/j.meegid.2014.01.017

Boutellis, A., Mediannikov, O., Bilcha, K. D., Ali, J., Campelo, D., Barker, S. C., & Raoult, D. (2013, May). Borrelia recurrentis in head lice, Ethiopia. *Emerg Infect Dis, 19*(5), 796-798. https://doi.org/10.3201/eid1905.121480

Boutellis, A., Veracx, A., Angelakis, E., Diatta, G., Mediannikov, O., Trape, J. F., & Raoult, D. (2012, Jul). Bartonella quintana in head lice from Senegal. *Vector Borne Zoonotic Dis, 12*(7), 564-567. https://doi.org/10.1089/vbz.2011.0845

Bouvresse, S., Socolovshi, C., Berdjane, Z., Durand, R., Izri, A., Raoult, D., Chosidow, O., & Brouqui, P. (2011, Dec). No evidence of Bartonella quintana but detection of Acinetobacter baumannii in head lice from elementary schoolchildren in Paris. *Comp Immunol Microbiol Infect Dis, 34*(6), 475-477. https://doi.org/10.1016/j.cimid.2011.08.007

Bowler, P. G., Davies, B. J., & Jones, S. A. (1999, May). Microbial involvement in chronic wound malodour. *J Wound Care, 8*(5), 216-218. https://doi.org/10.12968/jowc.1999.8.5.25875

Brito, D. V., Brito, C. S., Resende, D. S., Moreira do, O. J., Abdallah, V. O., & Gontijo Filho, P. P. (2010, Nov-Dec). Nosocomial infections in a Brazilian neonatal intensive care unit: a 4-year surveillance study. *Rev Soc Bras Med Trop, 43*(6), 633-637. https://doi.org/10.1590/s0037-86822010000600006

Burgess, I. F., Brunton, E. R., & Burgess, N. A. (2010, Jan). Clinical trial showing superiority of a coconut and anise spray over permethrin 0.43% lotion for head louse infestation, ISRCTN96469780. *Eur J Pediatr, 169*(1), 55-62. https://doi.org/10.1007/s00431-009-0978-0

Burgess, N. R. H., & Cowan, G. O. (1998). *A Colour Atlas of Medical Entomology*. Springer, Dordrecht. https://doi.org/https://doi.org/10.1007/978-94-011-1548-3_10

Burkhart, C. G. (2004, May). Relationship of treatment-resistant head lice to the safety and efficacy of pediculicides. *Mayo Clin Proc, 79*(5), 661-666. https://doi.org/10.4065/79.5.661

Burkhart, C. N. (2003, Jan 11). Fomite transmission with head lice: a continuing controversy. *Lancet, 361*(9352), 99-100. https://doi.org/10.1016/S0140-6736(03)12243-X

Burkhart, C. N., & Burkhart, C. G. (2007, Jun). Fomite transmission in head lice. *J Am Acad Dermatol, 56*(6), 1044-1047. https://doi.org/10.1016/j.jaad.2006.10.979

Cadima, L. R. S., Borges-Moroni, R., Mendes, J., & Moroni, F. T. (2021). Occurence of head lice in children and adolescents seen at a hospital in Uberlândia, Central Brazil. *J Trop Pathol, 50*(1), 61-72. https://doi.org/doi: 10.5216/rpt.v50i1.67885

Campbell, S., & Soman-Faulkner, K. (2021). Antiparasitic Drugs. In *StatPearls*. https://www.ncbi.nlm.nih.gov/pubmed/31334971

Candy, K., Amanzougaghene, N., Izri, A., Brun, S., Durand, R., Louni, M., Raoult, D., Fenollar, F., & Mediannikov, O. (2018, May). Molecular Survey of Head and Body Lice, Pediculus humanus, in France. *Vector Borne Zoonotic Dis, 18*(5), 243-251. https://doi.org/10.1089/vbz.2017.2206

CCAC, C. C. o. A. C. (2020). *CCAC animal data report 2019*. https://ccac.ca/Documents/Publications/AnnualReports/AR_2019-2020.pdf

CDC. (2017). *Pediculosis*. https://www.cdc.gov/dpdx/pediculosis/index.html

CDC. (2018a). *Malaria glossary of terms*. https://www.cdc.gov/malaria/glossary.html

CDC. (2018b, March 15, 2018). *Pthiriasis*. https://www.cdc.gov/dpdx/pthiriasis/index.html

CDC. (2019). *Healthcare-associated infections: Acinetobacter*. https://www.cdc.gov/hai/organisms/acinetobacter.html

Challa, V. S. R., Mahalingam, S., & Bhat, K. (2020). A case report of neonatal pediculosis: a simple challenge, yet complex. *Iranian Journal of Neonatology, 11*(3), 123-125. https://doi.org/doi: 10.22038/ijn.2020.44666.1742

Chapartegui-Gonzalez, I., Lazaro-Diez, M., Bravo, Z., Navas, J., Icardo, J. M., & Ramos-Vivas, J. (2018). Acinetobacter baumannii maintains its virulence after long-time starvation. *PLoS One, 13*(8), e0201961. https://doi.org/10.1371/journal.pone.0201961

Chosidow, O. (2000, Mar 4). Scabies and pediculosis. *Lancet, 355*(9206), 819-826. https://doi.org/10.1016/s0140-6736(99)09458-1

Chularojanamontri, L., Wongpraparut, C., Tuchinda, P., Winayanuwattikun, W., Boonyasiri, A., Kulthanan, K., & Thamlikitkul, V. (2016, Apr). Prevalence of Cutaneous Bacterial Colonization in Thai Patients with Psoriasis. *J Med Assoc Thai, 99*(4), 418-423. https://www.ncbi.nlm.nih.gov/pubmed/27396227

Chusri, S., Silpapojakul, K., McNeil, E., Singkhamanan, K., & Chongsuvivatwong, V. (2015, Feb). Impact of antibiotic exposure on occurrence of nosocomial carbapenem-resistant Acinetobacter baumannii infection: a case control study. *J Infect Chemother, 21*(2), 90-95. https://doi.org/10.1016/j.jiac.2014.10.002

Colombia Reports. (2021). https://colombiareports.com/medellin/

Conner, R. (2000). Head lice; formaldehyde storage; chewing gum in the OR; building design. *AORN Journal, 71*(3), 688. https://go.gale.com/ps/i.do?id=GALE%7CA64424019&sid=googleScholar&v=2.1&it=r&linkaccess=abs&issn=00012092&p=AONE&sw=w&userGroupName=anon%7E88e0b216

Cruickshank, R. H., Johnson, K. P., Smith, V. S., Adams, R. J., Clayton, D. H., & Page, R. D. (2001, May). Phylogenetic analysis of partial sequences of elongation factor 1alpha identifies major groups of lice (Insecta: Phthiraptera). *Mol Phylogenet Evol, 19*(2), 202-215. https://doi.org/10.1006/mpev.2001.0928

Cumming, B. F. (1915). Note on the mouthparts of a species of Polyplax (Anoplura) and the reltionship between Anouplura and Malophaga. . *Magazine of Natural History, 15*(86), 256-259. https://doi.org/DOI: 10.1080/00222931508693635

Cummings, C., Finlay, J. C., & MacDonald, N. E. (2018, Feb). Head lice infestations: A clinical update. *Paediatr Child Health, 23*(1), e18-e24. https://doi.org/10.1093/pch/pxx165

Cutler, S., Abdissa, A., Adamu, H., Tolosa, T., & Gashaw, A. (2012, Jan). Bartonella quintana in Ethiopian lice. *Comp Immunol Microbiol Infect Dis, 35*(1), 17-21. https://doi.org/10.1016/j.cimid.2011.09.007

Dal-Bo, K., Silva, R. M., & Sakae, T. M. (2012, Dec). Nosocomial infections in a neonatal intensive care unit in South Brazil. *Rev Bras Ter Intensiva, 24*(4), 381-385. https://doi.org/10.1590/s0103-507x2012000400015 (Infeccao hospitalar em uma unidade de terapia intensiva neonatal do Sul do Brasil.)

Data Africa. (2015). *Southern Nations, Nationalities, and Peoples' Region*. https://dataafrica.io/profile/snnp-eth#poverty

Diatta, G., Mediannikov, O., Sokhna, C., Bassene, H., Socolovschi, C., Ratmanov, P., Fenollar, F., & Raoult, D. (2014, Aug). Prevalence of Bartonella quintana in patients with fever and

head lice from rural areas of Sine-Saloum, Senegal. *Am J Trop Med Hyg, 91*(2), 291-293. https://doi.org/10.4269/ajtmh.13-0685

Drali, R., Boutellis, A., Raoult, D., Rolain, J. M., & Brouqui, P. (2013). Distinguishing body lice from head lice by multiplex real-time PCR analysis of the Phum_PHUM540560 gene. *PLoS One, 8*(2), e58088. https://doi.org/10.1371/journal.pone.0058088

Drali, R., Shako, J. C., Davoust, B., Diatta, G., & Raoult, D. (2015, Nov). A New Clade of African Body and Head Lice Infected by Bartonella quintana and Yersinia pestis-Democratic Republic of the Congo. *Am J Trop Med Hyg, 93*(5), 990-993. https://doi.org/10.4269/ajtmh.14-0686

Durden, L. A. (2019). *Lice (Phthiraptera). Medical and Veterinary Entomology*. https://doi.org/10.1016/b978-0-12-814043-7.00007-8

Eberle, B. M., Schnuriger, B., Putty, B., Barmparas, G., Kobayashi, L., Inaba, K., Belzberg, H., & Demetriades, D. (2010, Nov). The impact of Acinetobacter baumannii infections on outcome in trauma patients: a matched cohort study. *Crit Care Med, 38*(11), 2133-2138. https://doi.org/10.1097/CCM.0b013e3181f17af4

Edgecombe, G. D., Legg, D. A. (2014). Origins and early evolution of arthropods. *Palaeontology, 57*, 457-468. https://doi.org/doi: 10.1111/pala.12105

Eremeeva, M. E., Capps, D., Winful, E. B., Warang, S. S., Braswell, S. E., Tokarevich, N. K., Bonilla, D. L., & Durden, L. A. (2017, Jul 1). Molecular Markers of Pesticide Resistance and Pathogens in Human Head Lice (Phthiraptera: Pediculidae) From Rural Georgia, USA. *J Med Entomol, 54*(4), 1067-1072. https://doi.org/10.1093/jme/tjx039

Falagas, M. E., Matthaiou, D. K., Rafailidis, P. I., Panos, G., & Pappas, G. (2008, Sep). Worldwide prevalence of head lice. *Emerg Infect Dis, 14*(9), 1493-1494. https://doi.org/10.3201/eid1409.080368

Feldmeier, H. (2014, Oct). Treatment of pediculosis capitis: a critical appraisal of the current literature. *Am J Clin Dermatol, 15*(5), 401-412. https://doi.org/10.1007/s40257-014-0094-4

Firooziyan, S., Sadaghianifar, A., Taghilou, B., Galavani, H., Ghaffari, E., & Gholizadeh, S. (2017, Sep 1). Identification of Novel Voltage-Gated Sodium Channel Mutations in Human Head and Body Lice (Phthiraptera: Pediculidae). *J Med Entomol, 54*(5), 1337-1343. https://doi.org/10.1093/jme/tjx107

Galassi, F., Gonzalez-Audino, P., & Picollo, M. I. (2019, Sep 3). Head Lice Recognize and Prefer Head Odor Over Foot and Forearms Odors. *J Med Entomol, 56*(5), 1204-1207. https://doi.org/10.1093/jme/tjz060

Galassi, F., Ortega-Insaurralde, I., Adjemian, V., Gonzalez-Audino, P., Picollo, M. I., & Toloza, A. C. (2021, Feb). Head lice were also affected by COVID-19: a decrease on Pediculosis infestation during lockdown in Buenos Aires. *Parasitol Res, 120*(2), 443-450. https://doi.org/10.1007/s00436-020-07038-y

Gellatly, K. J., Krim, S., Palenchar, D. J., Shepherd, K., Yoon, K. S., Rhodes, C. J., Lee, S. H., & Marshall Clark, J. (2016, May). Expansion of the Knockdown Resistance Frequency Map for Human Head Lice (Phthiraptera: Pediculidae) in the United States Using Quantitative Sequencing. *J Med Entomol, 53*(3), 653-659. https://doi.org/10.1093/jme/tjw023

Giribet, G., & Edgecombe, G. D. (2019, Jun 17). The Phylogeny and Evolutionary History of Arthropods. *Curr Biol, 29*(12), R592-R602. https://doi.org/10.1016/j.cub.2019.04.057

Guo, N., Xue, W., Tang, D., Ding, J., & Zhao, B. (2016, Apr 1). Risk factors and outcomes of hospitalized patients with blood infections caused by multidrug-resistant Acinetobacter baumannii complex in a hospital of Northern China. *Am J Infect Control, 44*(4), e37-39. https://doi.org/10.1016/j.ajic.2015.11.019

Haidamak, J., Davila Dos Santos, G., Lima, B., Soares, V. M., de Menezes, R. V., Bisson, A. A., Talevi, A. S., Gomes, R. R., Vicente, V. A., Valero, M. A., & Klisiowicz, D. D. R. (2019, Sep). Scalp microbiota alterations in children with pediculosis. *Infect Genet Evol, 73*, 322-331. https://doi.org/10.1016/j.meegid.2019.05.016

Henig, O., Weber, G., Hoshen, M. B., Paul, M., German, L., Neuberger, A., Gluzman, I., Berlin, A., Shapira, C., & Balicer, R. D. (2015, Oct). Risk factors for and impact of carbapenem-resistant Acinetobacter baumannii colonization and infection: matched case-control study. *Eur J Clin Microbiol Infect Dis, 34*(10), 2063-2068. https://doi.org/10.1007/s10096-015-2452-4

Higgins, P. G., Wisplinghoff, H., Krut, O., & Seifert, H. (2007, Dec). A PCR-based method to differentiate between Acinetobacter baumannii and Acinetobacter genomic species 13TU. *Clin Microbiol Infect, 13*(12), 1199-1201. https://doi.org/10.1111/j.1469-0691.2007.01819.x

Hodgdon, H. E., Yoon, K. S., Previte, D. J., Kim, H. J., Aboelghar, G. E., Lee, S. H., & Clark, J. M. (2010, Sep). Determination of knockdown resistance allele frequencies in global human head louse populations using the serial invasive signal amplification reaction. *Pest Manag Sci, 66*(9), 1031-1040. https://doi.org/10.1002/ps.1979

Houhamdi, L., & Raoult, D. (2006, Apr). Experimental infection of human body lice with Acinetobacter baumannii. *Am J Trop Med Hyg, 74*(4), 526-531. https://www.ncbi.nlm.nih.gov/pubmed/16606978

Howard, A., O'Donoghue, M., Feeney, A., & Sleator, R. D. (2012, May 1). Acinetobacter baumannii: an emerging opportunistic pathogen. *Virulence, 3*(3), 243-250. https://doi.org/10.4161/viru.19700

Jamani, S., Rodriguez, C., Rueda, M. M., Matamoros, G., Canales, M., Bearman, G., Stevens, M., & Sanchez, A. (2019, May). Head lice infestations in rural Honduras: the need for an integrated approach to control neglected tropical diseases. *Int J Dermatol, 58*(5), 548-556. https://doi.org/10.1111/ijd.14331

Jang, T. N., Lee, S. H., Huang, C. H., Lee, C. L., & Chen, W. Y. (2009, Oct). Risk factors and impact of nosocomial Acinetobacter baumannii bloodstream infections in the adult intensive care unit: a case-control study. *J Hosp Infect, 73*(2), 143-150. https://doi.org/10.1016/j.jhin.2009.06.007

Johnson, K. P., Yoshizawa, K., & Smith, V. S. (2004, Sep 7). Multiple origins of parasitism in lice. *Proc Biol Sci, 271*(1550), 1771-1776. https://doi.org/10.1098/rspb.2004.2798

Kanafani, Z. A., Zahreddine, N., Tayyar, R., Sfeir, J., Araj, G. F., Matar, G. M., & Kanj, S. S. (2018). Multi-drug resistant Acinetobacter species: a seven-year experience from a tertiary care center in Lebanon. *Antimicrob Resist Infect Control, 7*, 9. https://doi.org/10.1186/s13756-017-0297-6

Karaiskos, I., Lagou, S., Pontikis, K., Rapti, V., & Poulakou, G. (2019). The "Old" and the "New" Antibiotics for MDR Gram-Negative Pathogens: For Whom, When, and How. *Front Public Health, 7*, 151. https://doi.org/10.3389/fpubh.2019.00151

Kempf, M., Abdissa, A., Diatta, G., Trape, J. F., Angelakis, E., Mediannikov, O., La Scola, B., & Raoult, D. (2012, Sep). Detection of Acinetobacter baumannii in human head and body lice from Ethiopia and identification of new genotypes. *Int J Infect Dis, 16*(9), e680-683. https://doi.org/10.1016/j.ijid.2012.05.1024

Kim, J. H., Previte, D. J., Yoon, K. S., Murenzi, E., Koehler, J. E., Pittendrigh, B. R., Lee, S. H., & Clark, J. M. (2017, Jun). Comparison of the proliferation and excretion of Bartonella quintana between body and head lice following oral challenge. *Insect Mol Biol, 26*(3), 266-276. https://doi.org/10.1111/imb.12292

Knoema. (2012). *World Data Atlas Thailand*. https://knoema.com/atlas/Thailand/Northern-Region?compareTo=TH-10,TH-1,TH-3,TH-4

Ko, C. J., & Elston, D. M. (2004, Jan). Pediculosis. *J Am Acad Dermatol, 50*(1), 1-12; quiz 13-14. https://doi.org/10.1016/s0190-9622(03)02729-4

Kurihara, M. N. L., Sales, R. O., Silva, K. E. D., Maciel, W. G., & Simionatto, S. (2020). Multidrug-resistant Acinetobacter baumannii outbreaks: a global problem in healthcare settings. *Rev Soc Bras Med Trop, 53*, e20200248. https://doi.org/10.1590/0037-8682-0248-2020

Larkin, K., Rodriguez, C. A., Jamani, S., Fronza, G., Roca-Acevedo, G., Sanchez, A., & Toloza, A. C. (2020, Jun 16). First evidence of the mutations associated with pyrethroid resistance in head lice (Phthiraptera: Pediculidae) from Honduras. *Parasit Vectors, 13*(1), 312. https://doi.org/10.1186/s13071-020-04183-2

Lee, S. H., Gao, J.-R., Yoon, K. S., Mumcuoglu, K. Y., Taplin, D., Edman, J. D., Takano-Lee, M., & Clark, J. M. (2003). Sodium channel mutations associated with knockdown resistance in the human head louse, Pediculus capitis (De Geer). *Pesticide Biochemistry and Physiology, 75*(3), 79-91. https://doi.org/https://doi.org/10.1016/S0048-3575(03)00018-X

Light, J. E., Allen, J. M., Long, L. M., Carter, T. E., Barrow, L., Suren, G., Raoult, D., & Reed, D. L. (2008, Dec). Geographic distributions and origins of human head lice (Pediculus humanus capitis) based on mitochondrial data. *J Parasitol, 94*(6), 1275-1281. https://doi.org/10.1645/GE-1618.1

Louni, M., Amanzougaghene, N., Mana, N., Fenollar, F., Raoult, D., Bitam, I., & Mediannikov, O. (2018, Jun 15). Detection of bacterial pathogens in clade E head lice collected from Niger's refugees in Algeria. *Parasit Vectors, 11*(1), 348. https://doi.org/10.1186/s13071-018-2930-5

Ly, T. D. A., Kerbaj, J., Edouard, S., Hoang, V. T., Louni, M., Dao, T. L., Benkouiten, S., Badiaga, S., Tissot-Dupont, H., Raoult, D., Brouqui, P., Mediannikov, O., & Gautret, P. (2019). The Presence of Acinetobacter baumannii DNA on the Skin of Homeless People and Its Relationship With Body Lice Infestation. Preliminary Results. *Front Cell Infect Microbiol, 9*, 86. https://doi.org/10.3389/fcimb.2019.00086

Mackie, F. P. (1907, Dec 14). The Part Played by Pediculus Corporis in the Transmission of Relapsing Fever. *Br Med J, 2*(2450), 1706-1709. https://doi.org/10.1136/bmj.2.2450.1706

Macrotrends. (2022). *Buenos Aires, Argentina Metro Area Population 1950-2022*. https://www.macrotrends.net/cities/20058/buenos-aires/population

Mana, N., Louni, M., Parola, P., & Bitam, I. (2017, Aug). Human head lice and pubic lice reveal the presence of several Acinetobacter species in Algiers, Algeria. *Comp Immunol Microbiol Infect Dis, 53*, 33-39. https://doi.org/10.1016/j.cimid.2017.06.003

Marinho, M. M., Milan, B. A., Borges-Moroni, R., Mendes, J., & Moroni, F. T. (2018). Epidemiological aspects of head lice in children attended to at a public hospital in

Uberlândia, Minas Gerais State, Brazil. *Rev Patol Trop, 47*(4), 1-11. https://doi.org/doi: 10.5216/rpt.v4i4.56741

Mehlhorn, H. (2012). *Arthropods as vectors of emerging diseases*. Springer.

Merino, M., Poza, M., Roca, I., Barba, M. J., Sousa, M. D., Vila, J., & Bou, G. (2014, Aug). Nosocomial outbreak of a multiresistant Acinetobacter baumannii expressing OXA-23 carbapenemase in Spain. *Microb Drug Resist, 20*(4), 259-263. https://doi.org/10.1089/mdr.2013.0127

Morfin-Otero, R., Alcantar-Curiel, M. D., Rocha, M. J., Alpuche-Aranda, C. M., Santos-Preciado, J. I., Gayosso-Vazquez, C., Araiza-Navarro, J. R., Flores-Vaca, M., Esparza-Ahumada, S., Gonzalez-Diaz, E., Perez-Gomez, H. R., & Rodriguez-Noriega, E. (2013). Acinetobacter baumannii infections in a tertiary care hospital in Mexico over the past 13 years. *Chemotherapy, 59*(1), 57-65. https://doi.org/10.1159/000351098

Mumcuoglu, K. Y. (1999, Jul-Sep). Prevention and treatment of head lice in children. *Paediatr Drugs, 1*(3), 211-218. https://doi.org/10.2165/00128072-199901030-00005

Mumcuoglu, K. Y., Gilead, L, Ingber, A. (2009). New insights in pediculosis and scabies. *Expert Review of Dermatology, 4*(3), 285-302. https://doi.org/10.1586/EDM.09.18

Mumcuoglu, K. Y., Miller, J., Zamir, C., Zentner, G., Helbin, V., & Ingber, A. (2002, Oct). The in vivo pediculicidal efficacy of a natural remedy. *Isr Med Assoc J, 4*(10), 790-793. https://www.ncbi.nlm.nih.gov/pubmed/12389342

Mumcuoglu, K. Y., Pollack, R. J., Reed, D. L., Barker, S. C., Gordon, S., Toloza, A. C., Picollo, M. I., Taylan-Ozkan, A., Chosidow, O., Habedank, B., Ibarra, J., Meinking, T. L., & Vander Stichele, R. H. (2021, Mar). International recommendations for an effective control of head louse infestations. *Int J Dermatol, 60*(3), 272-280. https://doi.org/10.1111/ijd.15096

Murray, E. S., & Torrey, S. B. (1975). Virulence of Rickettsia prowazeki for head lice. *Ann N Y Acad Sci, 266*, 25-34. https://doi.org/10.1111/j.1749-6632.1975.tb35086.x

Nelson, R. E., Schweizer, M. L., Perencevich, E. N., Nelson, S. D., Khader, K., Chiang, H. Y., Chorazy, M. L., Blevins, A., Ward, M. A., & Samore, M. H. (2016, Oct). Costs and Mortality Associated With Multidrug-Resistant Healthcare-Associated Acinetobacter Infections. *Infect Control Hosp Epidemiol, 37*(10), 1212-1218. https://doi.org/10.1017/ice.2016.145

Nutman, A., Glick, R., Temkin, E., Hoshen, M., Edgar, R., Braun, T., & Carmeli, Y. (2014, Dec). A case-control study to identify predictors of 14-day mortality following carbapenem-

resistant Acinetobacter baumannii bacteraemia. *Clin Microbiol Infect, 20*(12), O1028-1034. https://doi.org/10.1111/1469-0691.12716

Nuttall, H. F. G. (1918). The Biology of Phthirus Pubis. *Parasitology, 10*(03), 383-403. https://doi.org/doi:10.1017/s0031182000003954

Pakharukova, N., Tuittila, M., Paavilainen, S., Malmi, H., Parilova, O., Teneberg, S., Knight, S. D., & Zavialov, A. V. (2018, May 22). Structural basis for Acinetobacter baumannii biofilm formation. *Proc Natl Acad Sci U S A, 115*(21), 5558-5563. https://doi.org/10.1073/pnas.1800961115

Pappenheimer, A. M., Vermilye, H. N., & Mueller, J. H. (1917, Oct 13). ON THE ETIOLOGY OF TRENCH FEVER: (A Preliminary Communication.). *Br Med J, 2*(2963), 474-476. https://doi.org/10.1136/bmj.2.2963.474

Pearson, C. A., Stevens, M. P., Sanogo, K., & Bearman, G. M. (2012). Access and Barriers to Healthcare Vary among Three Neighboring Communities in Northern Honduras. *Int J Family Med, 2012*, 298472. https://doi.org/10.1155/2012/298472

Peleg, A. Y., Seifert, H., & Paterson, D. L. (2008, Jul). Acinetobacter baumannii: emergence of a successful pathogen. *Clin Microbiol Rev, 21*(3), 538-582. https://doi.org/10.1128/CMR.00058-07

Percival, S. L. W., David W. (2014). *Microbiology of Waterborne Diseases 2nd ed.* (S. Percival, Yates, M. V., Williams, D., Chlamers, R., Gray, N., , Ed.). Elservier Inc. https://doi.org/https://doi.org/10.1016/B978-0-12-415846-7.00002-0

Piarroux, R., Abedi, A. A., Shako, J. C., Kebela, B., Karhemere, S., Diatta, G., Davoust, B., Raoult, D., & Drancourt, M. (2013, Mar). Plague epidemics and lice, Democratic Republic of the Congo. *Emerg Infect Dis, 19*(3), 505-506. https://doi.org/10.3201/eid1903.121542

Picollo, M. I., Vassena, C. V., Casadio, A. A., Massimo, J., & Zerba, E. N. (1998, Sep). Laboratory studies of susceptibility and resistance to insecticides in Pediculus capitis (Anoplura; Pediculidae). *J Med Entomol, 35*(5), 814-817. https://doi.org/10.1093/jmedent/35.5.814

Pietri, J. E., Yax, J. A., Agany, D. D. M., Gnimpieba, E. Z., & Sheele, J. M. (2020). Body lice and bed bug co-infestation in an emergency department patient, Ohio, USA. *IDCases, 19*, e00696. https://doi.org/10.1016/j.idcr.2020.e00696

Pollack, R. J., Kiszewski, A. E., & Spielman, A. (2000, Aug). Overdiagnosis and consequent mismanagement of head louse infestations in North America. *Pediatr Infect Dis J, 19*(8), 689-693; discussion 694. https://doi.org/10.1097/00006454-200008000-00003

Portillo, A., Ruiz-Arrondo, I., & Oteo, J. A. (2018, Dec 14). Arthropods as vectors of transmissible diseases in Spain. *Med Clin (Engl Ed), 151*(11), 450-459. https://doi.org/10.1016/j.medcle.2018.10.008

Powers, J., & Badri, T. (2021). Pediculosis Corporis. In *StatPearls*. https://www.ncbi.nlm.nih.gov/pubmed/29489282

Raoult, D., Dutour, O., Houhamdi, L., Jankauskas, R., Fournier, P. E., Ardagna, Y., Drancourt, M., Signoli, M., La, V. D., Macia, Y., & Aboudharam, G. (2006, Jan 1). Evidence for louse-transmitted diseases in soldiers of Napoleon's Grand Army in Vilnius. *J Infect Dis, 193*(1), 112-120. https://doi.org/10.1086/498534

Raoult, D., & Parola, P. (2007). *Rickettsial Diseases*. CRC Press. https://books.google.ca/books?hl=en&lr=&id=QHjt676mCs0C&oi=fnd&pg=PP1&dq=rickettsial+disease+raoult&ots=sEgJpfdfcc&sig=6FfnrgEBzG_nkkHDdCVr4jmIbuk&redir_esc=y#v=onepage&q=rickettsial%20disease%20raoult&f=false

Raoult, D., & Roux, V. (1999, Oct). The body louse as a vector of reemerging human diseases. *Clin Infect Dis, 29*(4), 888-911. https://doi.org/10.1086/520454

Reed, D. L., Allen, J. M., Toups, M. A., Boyd, B. M., Ascunce, M. S. (2015). *The study of primate evolution from a lousy perspective* (S. Morand, Krasnov, B. R., Littlewood, D. T. J, Ed.). Cambridge University Press. https://doi.org/DOI: http://dx.doi.org/10.1017/CBO9781139794749

Reed, D. L., Light, J. E., Allen, J. M., & Kirchman, J. J. (2007, Mar 7). Pair of lice lost or parasites regained: the evolutionary history of anthropoid primate lice. *BMC Biol, 5*, 7. https://doi.org/10.1186/1741-7007-5-7

Reed, D. L., Smith, V. S., Hammond, S. L., Rogers, A. R., & Clayton, D. H. (2004, Nov). Genetic analysis of lice supports direct contact between modern and archaic humans. *PLoS Biol, 2*(11), e340. https://doi.org/10.1371/journal.pbio.0020340

Rocha Lde, A., Vilela, C. A., Cezario, R. C., Almeida, A. B., & Gontijo Filho, P. (2008, Feb). Ventilator-associated pneumonia in an adult clinical-surgical intensive care unit of a Brazilian university hospital: incidence, risk factors, etiology, and antibiotic resistance. *Braz J Infect Dis, 12*(1), 80-85. https://doi.org/10.1590/s1413-86702008000100017

Rogers, A. R., Iltis, D., & Wooding, S. (2004). Genetic variation at the MC1R locus and the time since loss of body hair. *Current Anthropology, 45*, 105-108. https://doi.org/https://doi.org/10.1086/381006

Romanelli, R. M., Anchieta, L. M., Mourao, M. V., Campos, F. A., Loyola, F. C., Mourao, P. H., Armond, G. A., Clemente, W. T., & Bouzada, M. C. (2013, Mar-Apr). Risk factors and

lethality of laboratory-confirmed bloodstream infection caused by non-skin contaminant pathogens in neonates. *J Pediatr (Rio J), 89*(2), 189-196. https://doi.org/10.1016/j.jped.2013.03.002

Sangare, A. K., Boutellis, A., Drali, R., Socolovschi, C., Barker, S. C., Diatta, G., Rogier, C., Olive, M. M., Doumbo, O. K., & Raoult, D. (2014, Aug). Detection of Bartonella quintana in African body and head lice. *Am J Trop Med Hyg, 91*(2), 294-301. https://doi.org/10.4269/ajtmh.13-0707

Saraswat, N., Shankar, P., Chopra, A., Mitra, B., & Kumar, S. (2020, Jan-Feb). Risk Factors Associated with Head Lice Infestation in Rural Pediatric Patients. *Indian Dermatol Online J, 11*(1), 25-28. https://doi.org/10.4103/idoj.IDOJ_48_19

Sasaki, T., Poudel, S. K., Isawa, H., Hayashi, T., Seki, N., Tomita, T., Sawabe, K., & Kobayashi, M. (2006, Jan). First molecular evidence of Bartonella quintana in Pediculus humanus capitis (Phthiraptera: Pediculidae), collected from Nepalese children. *J Med Entomol, 43*(1), 110-112. https://doi.org/10.1093/jmedent/43.1.110

Schultz, M. G., Morens, D. M. (2009, 2009). Charles-Jules-Henri Nicolle. *Emerg Infect Dis, 15*(9), 1519-1522. https://doi.org/doi: 10.3201/eid1509.090891

Seifert, H., Dijkshoorn, L., Gerner-Smidt, P., Pelzer, N., Tjernberg, I., & Vaneechoutte, M. (1997, Nov). Distribution of Acinetobacter species on human skin: comparison of phenotypic and genotypic identification methods. *J Clin Microbiol, 35*(11), 2819-2825. https://doi.org/10.1128/jcm.35.11.2819-2825.1997

Shami, A., Al-Mijalli, S., Pongchaikul, P., Al-Barrag, A., & AbduRahim, S. (2019, Aug 16). The prevalence of the culturable human skin aerobic bacteria in Riyadh, Saudi Arabia. *BMC Microbiol, 19*(1), 189. https://doi.org/10.1186/s12866-019-1569-5

Shi, J., Sun, T., Cui, Y., Wang, C., Wang, F., Zhou, Y., Miao, H., Shan, Y., & Zhang, Y. (2020, Aug 12). Multidrug resistant and extensively drug resistant Acinetobacter baumannii hospital infection associated with high mortality: a retrospective study in the pediatric intensive care unit. *BMC Infect Dis, 20*(1), 597. https://doi.org/10.1186/s12879-020-05321-y

Sunantaraporn, S., Sanprasert, V., Pengsakul, T., Phumee, A., Boonserm, R., Tawatsin, A., Thavara, U., & Siriyasatien, P. (2015, Feb 26). Molecular survey of the head louse Pediculus humanus capitis in Thailand and its potential role for transmitting Acinetobacter spp. *Parasit Vectors, 8*, 127. https://doi.org/10.1186/s13071-015-0742-4

Taplin, D., & Zaias, N. (1963, Dec 7). The Human Skin as a Source of Mima-Herellea Infections. *JAMA, 186*, 952-955. https://doi.org/10.1001/jama.1963.63710100030023a

Taravati, S. (2019). *How to distinguish between head, body, and crab lice?* Integrated Pest Management for Structural Pests in Southern California. https://urbanipmsocal.com/ipm/articles/how-to-distinguish-between-head-body-and-crab-lice/

Toloza, A. C., Lucia, A., Zerba, E., Masuh, H., & Picollo, M. I. (2010, Jan). Eucalyptus essential oil toxicity against permethrin-resistant Pediculus humanus capitis (Phthiraptera: Pediculidae). *Parasitol Res, 106*(2), 409-414. https://doi.org/10.1007/s00436-009-1676-6

Toloza, A. C., Zygadlo, J., Biurrun, F., Rotman, A., & Picollo, M. I. (2010). Bioactivity of Argentinean essential oils against permethrin-resistant head lice, Pediculus humanus capitis. *J Insect Sci, 10*, 185. https://doi.org/10.1673/031.010.14145

United States Census. (2021). https://www.census.gov/

Vincent, J. L., Rello, J., Marshall, J., Silva, E., Anzueto, A., Martin, C. D., Moreno, R., Lipman, J., Gomersall, C., Sakr, Y., Reinhart, K., & Investigators, E. I. G. o. (2009, Dec 2). International study of the prevalence and outcomes of infection in intensive care units. *JAMA, 302*(21), 2323-2329. https://doi.org/10.1001/jama.2009.1754

von Dolinger de Brito, D., Oliveira, E. J., Abdallah, V. O., da Costa Darini, A. L., & Filho, P. P. (2005, Aug). An outbreak of Acinetobacter baumannii septicemia in a neonatal intensive care unit of a university hospital in Brazil. *Braz J Infect Dis, 9*(4), 301-309. https://doi.org/10.1590/s1413-86702005000400006

WHO. (2017). *Global vector control response 2017–2030: A strategic approach to tackle vector-borne diseases*. https://www.who.int/publications/i/item/WHO-HTM-GVCR-2017.01

WHO. (2019). *Antibacterial agents in preclinical development: an open access database* https://www.who.int/publications/i/item/WHO-EMP-IAU-2019.12

World Population Review. (2021a). *Algiers Population 2021*. https://worldpopulationreview.com/world-cities/algiers-population

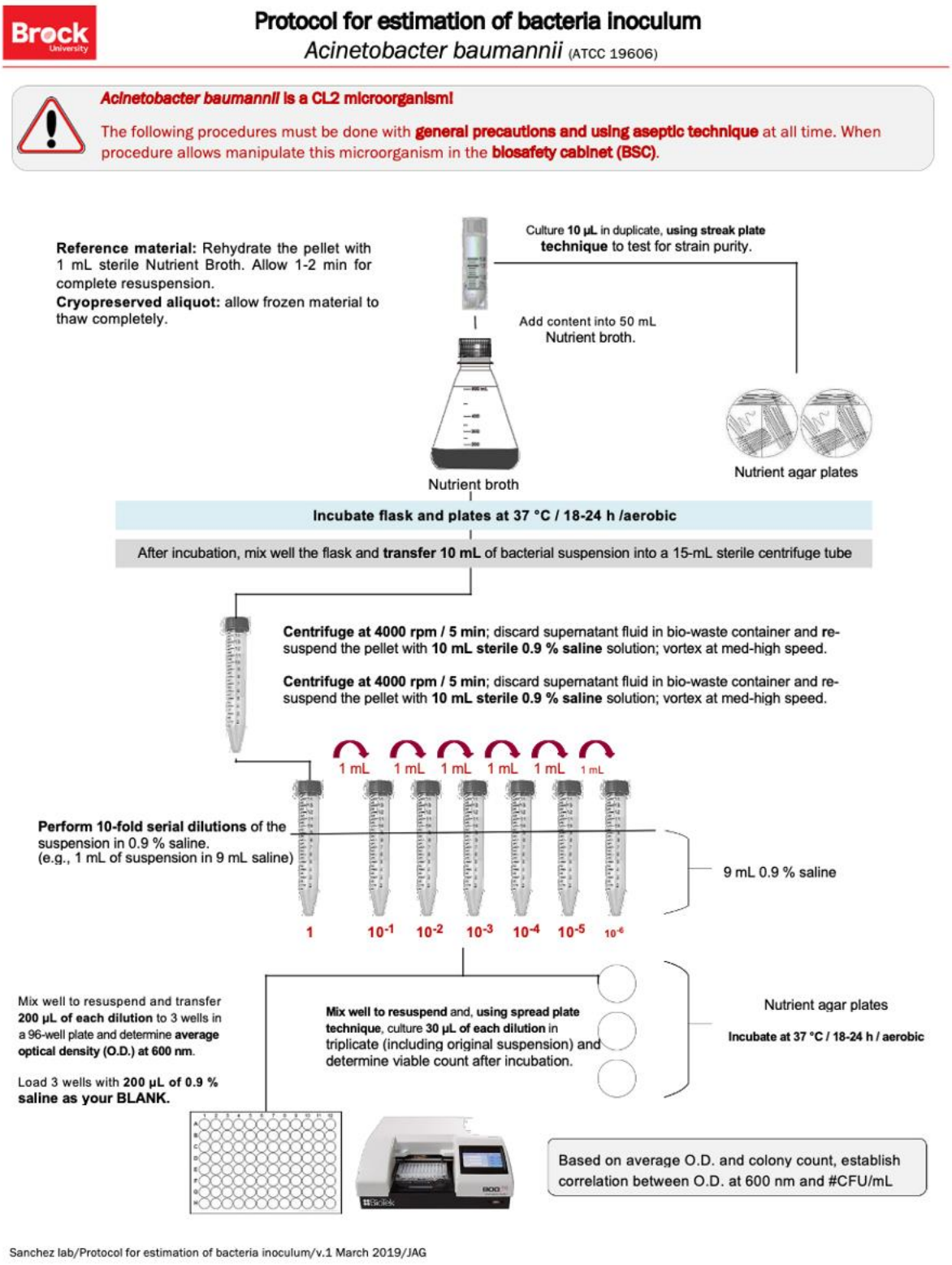
World Population Review. (2021b). Paris, France. https://worldpopulationreview.com/world-cities/paris-population

Yasar Durmus, S., Tanir, G., Oz, F. N., Kaman, A., & Aydin Teke, T. (2020, Jun 2). A Rare Cause of Healthcare-associated Infection in a Pediatric Infectious Diseases Unit. *Turkiye Parazitol Derg, 44*(2), 122-123. https://doi.org/10.4274/tpd.galenos.2020.6766

Yoon, K. S., Gao, J. R., Lee, S. H., Clark, J. M., Brown, L., & Taplin, D. (2003, Aug). Permethrin-resistant human head lice, Pediculus capitis, and their treatment. *Arch Dermatol, 139*(8), 994-1000. https://doi.org/10.1001/archderm.139.8.994

Zhang, Z. Q. (2013). Animal biodiversity: An outline of higher-level classification and survey of taxonomic richness (Addenda 2013). *Zootaxa, 3703*, 1-82. https://doi.org/10.11646/zootaxa.3703.1.1

APPENDIX:


Protocol for estimation of bacteria inoculum
Acinetobacter baumannii (ATCC 19606)
Acinetobacter baumannii is a CL2 microorganism!
The following procedures must be done with general precautions and using aseptic technique at all time. When procedure allows manipulate this microorganism in the biosafety cabinet (BSC).
Reference material: Rehydrate the pellet with 1 mL sterile Nutrient Broth. Allow 1-2 min for complete resuspension.
Cryopreserved aliquot: allow frozen material to thaw completely.
Culture 10 µL in duplicate, using streak plate technique to test for strain purity.
Add content into 50 mL Nutrient broth.
Nutrient broth
Nutrient agar plates
Incubate flask and plates at 37 °C / 18-24 h /aerobic
After incubation, mix well the flask and transfer 10 mL of bacterial suspension into a 15-mL sterile centrifuge tube
Centrifuge at 4000 rpm / 5 min; discard supernatant fluid in bio-waste container and re-suspend the pellet with 10 mL sterile 0.9 % saline solution; vortex at med-high speed.
Centrifuge at 4000 rpm / 5 min; discard supernatant fluid in bio-waste container and re-suspend the pellet with 10 mL sterile 0.9 % saline solution; vortex at med-high speed.
1 mL 1 mL 1 mL 1 mL 1 mL 1 mL
Perform 10-fold serial dilutions of the suspension in 0.9 % saline.
(e.g., 1 mL of suspension in 9 mL saline)
9 mL 0.9 % saline
1 10-1 10-2 10-3 10-4 10-5 10-6
Mix well to resuspend and transfer 200 µL of each dilution to 3 wells in a 96-well plate and determine average optical density (O.D.) at 600 nm.
Load 3 wells with 200 µL of 0.9 % saline as your BLANK.
Mix well to resuspend and, using spread plate technique, culture 30 µL of each dilution in triplicate (including original suspension) and determine viable count after incubation.
Nutrient agar plates
Incubate at 37 °C / 18-24 h / aerobic
Based on average O.D. and colony count, establish correlation between O.D. at 600 nm and #CFU/mL
Sanchez lab/Protocol for estimation of bacteria inoculum/v.1 March 2019/JAG

Protocol for cryopreservation

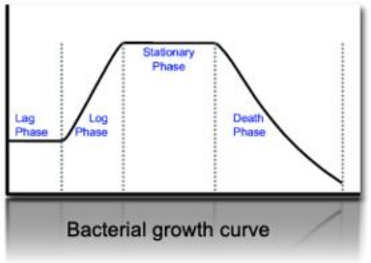
Acinetobacter baumannii (ATCC 19606)

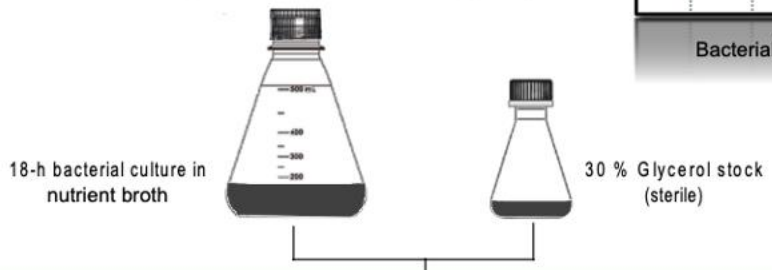
***Acinetobacter baumannii* is a CL2 microorganism!**

The following procedures must be done with **general precautions and using aseptic technique** at all time. When procedure allows manipulate this microorganism in the **biosafety cabinet**.

To obtain good viability in cryopreserved aliquots, bacteria must be still in the LOG phase of growing (~18 h).

Sterile glycerol is added to prevent intracellular ice formation which will destroy the bacterial cells (final concentration is 15 % glycerol).





- Transfer **500 µL of 30 % sterile glycerol** stock to several 1.8-mL cryovials.
- Mix well the culture flask and transfer **500 µL of bacterial suspension** into each cryovial with glycerol; mix well by pipetting up and down on several times. Discard the tip.
- Close the vials tightly and **label them properly** (Bacterium name & reference code; Passage; Date; Technician initials)

- Place all vials at **-80 °C using a freezing container** (special device to control freezing of cells in a -1 °C/min cooling rate). Leave them freezing **overnight**.

- Take vials out of the freezing container and place them into a properly labeled **cryobox; store at -80 °C.**

Sanchez lab/Protocol for cryopreservation/v.1 March 2019/JAG

Colony and cell morphology

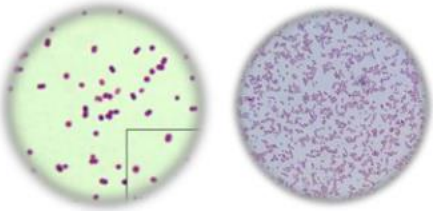
Acinetobacter baumannii (ATCC 19606)

Colony Morphology:

In Nutrient Agar and Trypticase Soy Agar, *Acinetobacter baumannii* form smooth, sometimes mucoid, pale yellow to greyish white colonies, about 1–2 mm in diameter.

Cell Morphology:

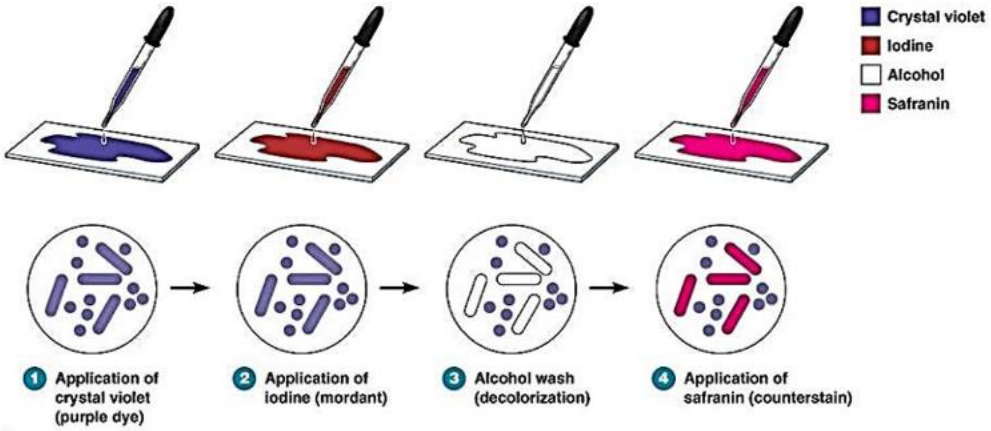
Acinetobacter baumannii are Gram-negative, strictly aerobic, catalase-positive, oxidase-negative bacteria which, **in log phase**, have the appearance of **short, Gram-negative rods**, but **in stationary phase** the organism has a **coccoid appearance**. They are difficult to destain, which can lead to initial misidentification as Gram -positive or Gram-variable [1].



Gram staining technique

Summary:

Heat-fixed smear | 1 min Crystal violet | wash | 1 min Iodine | wash | ~5 sec Alcohol-base decolorizer | 10 sec Safranin .



http://www.medicinehack.com/2012/02/gram-staining-procedure-mechanism.html

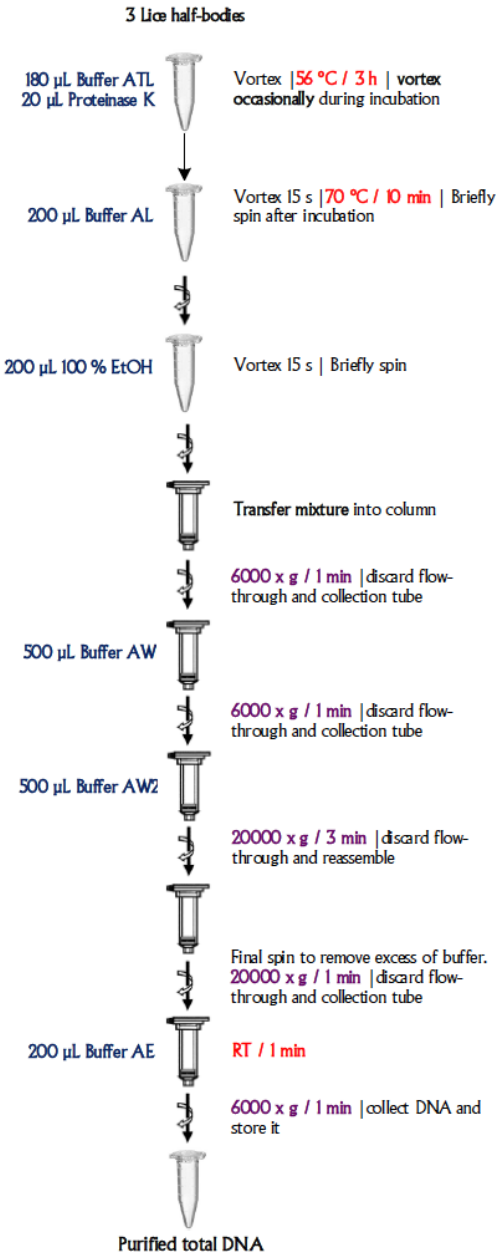
Reference:

Percival S.L., Williams D.W. Chapter Two – *Acinetobacter*. In: Percival S., Yates M.V., Williams D., Chalmers R., Gray N., editors. Microbiology of Waterborne Diseases. 2nd ed. Amsterdam: Elsevier Inc.; 2014. pp. 35–48.

DNA extraction and purification
Lice and pathogens

ⓘ Procedure based on "QIAmp DNA mini kit" (QIAGEN, cat# 51304)

1. **Cut 3 lice in half (longitudinally)** and place them into a 1.5-mL tube.
2. Add **180 µL Buffer ATL and 20 µL Proteinase K.** Vortex to mix.
3. Incubate at **56 °C for 3 h. Vortex occasionally.**
4. Add **200 µL Buffer AL** and vortex thoroughly for 15 s to mix.
5. Incubate at **70 °C for 10 min.**
6. Briefly spin the samples to remove drops from the lid.
7. Add **200 µL Ethanol (100 %)** and vortex thoroughly for 15 s to mix.
8. Briefly spin the samples to remove drops from the lid.
9. Pipet the mixture onto the **QIAmp Mini spin column** (in a 2-mL collection tube).
10. Centrifuge at **6000 x g (8000 rpm) for 1 min** and discard the flow-through and collection tube.
11. Place the QIAmp Mini spin column in a new 2-mL collection tube and add **500 µL Buffer AW1.**
12. Centrifuge at **6000 x g (8000 rpm) for 1 min** and discard the flow-through and collection tube.
13. Place the QIAmp Mini spin column in a new 2-mL collection tube and add **500 µL Buffer AW2.**
14. Centrifuge at **20000 x g (14000 rpm) for 3 min.** Discard the flow-through and **reassemble spin column and collection tube.**
15. Centrifuge at **20000 x g (14000 rpm) for 1 min** to eliminate the chance of possible Buffer AW2 carryover. Discard the flow-through and collection tube.
16. Place the QIAmp Mini spin column in a new 1.5-mL microcentrifuge tube and add **200 µL Buffer AE.**
17. Incubate at **room temperature for 1 min.**
18. Centrifuge at **6000 x g (8000 rpm) for 1 min** to elute DNA.
19. **Store purified genomic DNA at – 20 °C** or continue with PCR protocol.



A. Primer sets

1. Louini et al._2018_Detection of bacterial pathogens in clade E head lice_Algeria.

   Product size = 348 bp

   a) Forward: IDT | Phc-IF | GAGCGACTGTAATTACTAATC

   b) Reverse: IDT | Phc-IR | CAACAAAATTATCCGGGTCC

2. Raoult et al._2006_Evidence for louse-transmitted diseases in soldiers of Napoleon's Grand Army in Vilnius.

   Product size = 216 bp

   a) Forward: IDT | Phc-2F | TTGTTGTGCTTTTGACTTCTTG

   b) Reverse: IDT | Phc-2R | CCCTGATTTGAAGTATTAAAGAAACTC

B. Master mix

-> T-500 | Taq DNA Polymerase | Froggabio

C. PCR Mix (25 µL)

1. 10X PCR Buffer (Mg2+ plus) ——— 2.5 µL
2. dNTPs (10 mM each) ——————— 0.5 µL
3. FWD (10 µM) ——————————— 1.5 µL
4. REV (10 µM) ——————————— 1.5 µL
5. Taq DNA Polymerase (5 U/µL) —- 0.25 µL
6. NFW ——————————————— 17.75 µL
7. DNA template ——————————— 1 µL

D. PCR conditions

Primer set #1

-> 95 °C / 3 min (1X) | 95 °C / 60 sec — 55 °C / 30 sec — 72 °C / 60 sec (40X) | 72 °C / 7 min (1X)

Primer set #2

-> 95 °C / 3 min (1X) | 95 °C / 30 sec — 58 °C / 30 sec — 72 °C / 90 sec (44X) | 72 °C / 7 min (1X)

E. Electrophoresis conditions

-> Add 6X loading buffer to DNA samples before loading the gel

-> 1.5 % Agarose gel + EtBr

-> 120 V

-> 45 min

A. Primer sets

1. Higgins et al._2007_A PCR-based method to differentiate between *A. baumannii*. Two products = 490 and 294 bp
   a) Forward: IDT | Abau-IF1 | CACGCCGTAAGAGTGCATTA
   b) Forward: IDT | Abau-IF2 | GTTCCTGATCCGAAATTCTCG
   c) Reverse: IDT | Abau-IR | AACGGAGCTTGTCAGGGTTA

2. JAG. Product size = 366 bp
   a) Forward: IDT | Abau-2F | ACATCGCATTGGGGATTGGT
   b) Reverse: IDT | Abau-2R | GACGCGCTTGTAGACCCATA

B. Master mix

-> FBTAQM | 2X Taq FroggaMix | Froggabio

C. PCR Mix (25 µL)

Primer set #1		Primer set #2	
1. Master Mix	12.5 µL	1. Master Mix	12.5 µL
2. FWD1 (10 µM)	1.5 µL	2. FWD (10 µM)	1.5 µL
3. FWD2 (10 µM)	1.5 µL	3. REV (10 µM)	1.5 µL
4. REV (10 µM)	1.5 µL	4. NFW	8.5 µL
5. NFW	7 µL	5. DNA template	1 µL
6. DNA template	1 µL		

D. PCR conditions

-> 94 °C / 3 min (1X) | 94 °C / 60 sec — 60 °C / 30 sec — 72 °C / 60 sec (25X) | 72 °C / 10 min (1X)

E. Electrophoresis conditions

-> 1.5 % Agarose gel + EtBr
-> 120 V
-> 45 min