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Detection of *Acinetobacter baumannii* in human head and body lice from Ethiopia and identification of new genotypes

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SUMMARY

Background: *Acinetobacter baumannii* has previously been detected and genotyped in human body lice. The objectives of this study were to determine the presence of this bacterium in head and body lice collected from healthy individuals in Ethiopia by molecular methods and to characterize the genotype.
Methods: Human lice from locations at different altitudes in Ethiopia were screened for the presence of *Acinetobacter sp* by targeting the *rpo*B gene. *Acinetobacter baumannii* was detected and genotyped using *rec*A PCR amplification.
Results: A total of 115 head and 109 body lice were collected from 134 healthy individuals. *Acinetobacter sp* were found in 54 head (47%) and 77 body (71%) lice. The *rec*A gene was sequenced for 60 of the *Acinetobacter sp* and 67% were positive for *A. baumannii*; genotype 1 was retrieved the most frequently.
Conclusion: Our study is the first to show the presence of *A. baumannii* in human body lice, and also in head lice, in Ethiopia.

1. Introduction

Human body lice and head lice are closely related obligate parasites that feed exclusively on human blood.[1] In nature, the body louse has been demonstrated to be the vector of three human pathogens: *Rickettsia prowazekii*, the agent of epidemic typhus; *Bartonella quintana*, the agent of trench fever; and *Borrelia recurrentis*, the agent of louse-borne recurrent fever. Our laboratory has reported the isolation of *Acinetobacter baumannii* from body lice collected from homeless people in Marseille (France), as well as from diverse countries such as Burundi, Rwanda, Peru, Algeria, Portugal, and the Netherlands, indicating that epidemic *A. baumannii* infections among human body lice could be a source of human infection.[2,3]

A. baumannii, which is widespread in nature (water, soil, living organisms, vegetables, and the skin of patients and healthy subjects),[4,5] is an important pathogen of critically ill patients that can cause a range of infections, including ventilator-associated pneumonia, bloodstream infections, wound infections, and nosocomial meningitis.[6–8] An increase in the frequency of community-acquired infections with *A. baumannii* has been reported during the last decade, mainly from countries located in inter-tropical areas or during international armed conflicts or natural disasters, which raises the question of a potential environmental reservoir.[9–12] Our laboratory found that 21% of body lice collected worldwide were naturally infected with *A. baumannii*.[3] However, it is still unknown how all these body lice acquire their *A. baumannii* infections.

This infection could occur after the ingestion of an infective blood meal from patients with ongoing bacteremia,[2,3] or possibly by passage through the human skin while feeding. In an experimental model of a human body louse infection, it has been shown that *A. baumannii* is able to maintain a persistent life-long infection with a generalized septicemia.[13] In 2006, the genome of *A. baumannii* SDF strain isolated from a human body louse was sequenced and analyzed.[14] Results showed that this remarkably susceptible strain harbored several hundred insertion sequence elements that have played a crucial role in its genome reduction (gene disruptions and simple DNA loss). Moreover, it was shown to have low catabolic capacities compared to the human multidrug-resistant *A. baumannii* AYE strain,[14–16] suggesting specific adaptation of this bacterium to the louse environment.

The objective of this study was to determine the presence of *A. baumannii* in head and body lice collected from individuals in Ethiopia by molecular methods and to characterize the genotype.

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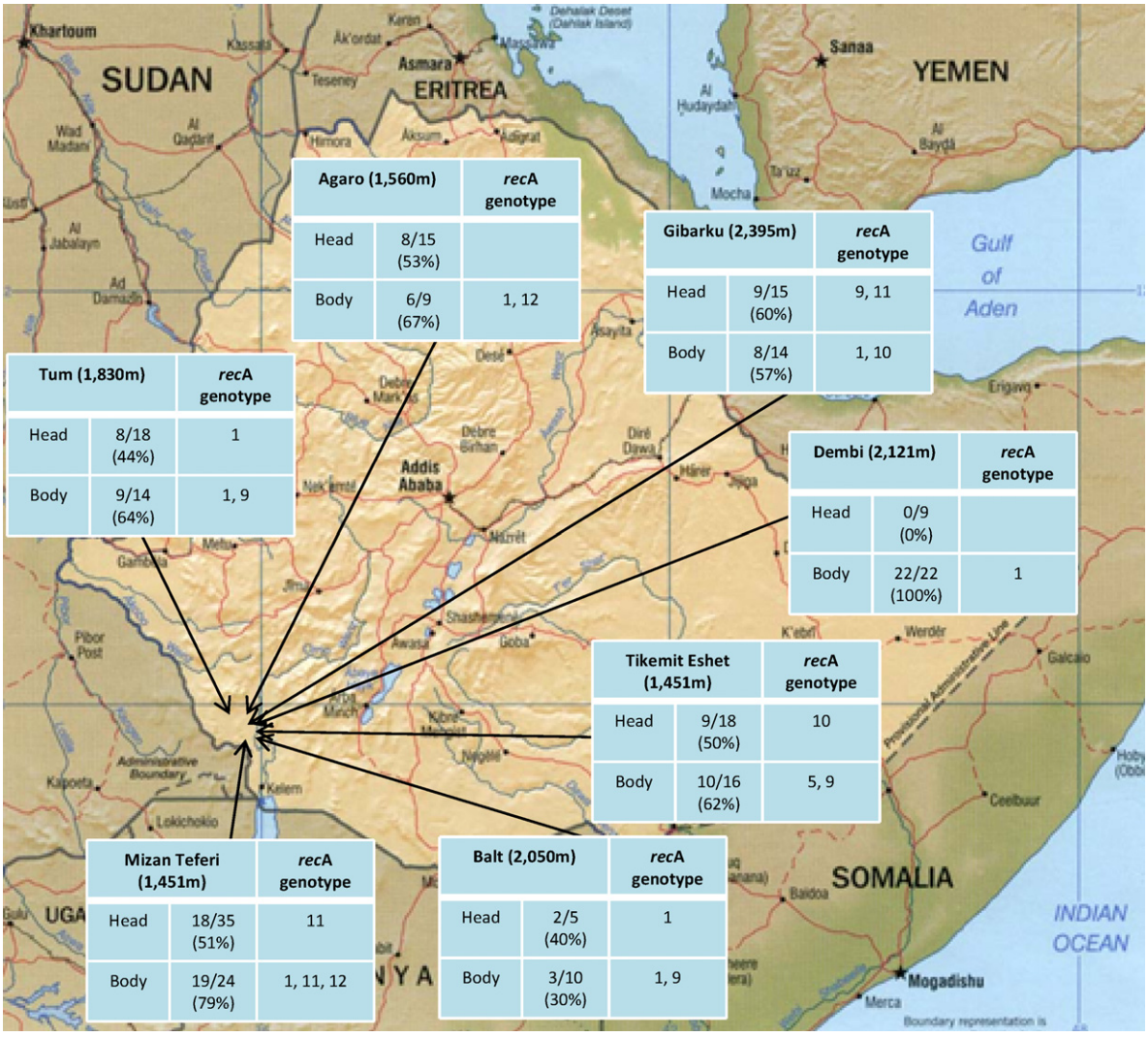

Figure 1. Distribution of *Acinetobacter sp*-positive head and body lice at seven locations in Ethiopia (expressed as the number of positive lice and percentage positivity) and the distribution of *A. baumannii recA* genotypes.

2. Materials and methods

Head and body lice were collected from persons with no clinical signs, from seven locations at different altitudes in Ethiopia (Figure 1). Lice were then transferred to Marseille in sterile conditions at room temperature. Before DNA isolation, each louse was rinsed twice in sterile water for 15 min and cut in half lengthwise. The total genomic DNA of each half louse was then extracted using the QIAamp Tissue Kit (Qiagen, Hilden, Germany) following the manufacturer's recommendations. The presence of *Acinetobacter sp* was determined by screening the samples with a real-time PCR assay targeting the *rpo*B gene (Table 1). Negative and positive controls were added in each assay. Some of the samples positive for *Acinetobacter sp* were subjected to *rec*A gene amplification and sequencing using specific primers, as previously described.[3,17] Genotypes were determined by comparison of *recA* sequences with the genotypes previously deposited in the GenBank database.[3] EpiInfo version 6.0 software was used for data comparisons (Centers for Disease Control and Prevention, Atlanta, GA, USA). A *p*-value of <0.05 was considered significant.

3. Results

A total of 115 head and 109 body lice were collected from 134 healthy individuals (109 women and 25 men) and tested. All subjects had black head lice, except for two who each had a single gray louse among the population of black head lice. All body lice were gray–transparent. *Acinetobacter sp* were found in 54 head (47%) and 77 body (71%) lice. The distribution of *Acinetobacter sp*-positive lice is shown in Figure 1. Globally, no difference was found in the presence of *Acinetobacter sp* between men and women ($p = 0.34$). Moreover, no difference was found in the presence of *Acinetobacter sp* in head ($p = 0.55$) or body ($p = 0.38$) lice by altitude. However, the bacteria were more frequently isolated from body lice than from head lice ($p < 0.01$).

More than 50% of the head lice were infected with *Acinetobacter sp* in all the regions investigated, except in Balt (40%), Tum (44%), and Dembi (0%; no head louse contained the bacterium). Surprisingly, in Dembi, all the body lice (22/22) collected were infected with *Acinetobacter sp*. A high proportion of body lice were also infected with *Acinetobacter sp* in Mizan Teferi (79%), followed by Agaro (67%), Tum (64%), and Tikemit Eshet (62%). Balt was the region in which *Acinetobacter sp* was the least commonly detected for both head lice (40%) and body lice (30%). Among the individuals with positive lice, 12 had *Acinetobacter sp* in both body and head lice. These persons were located in Tikemit Eshet, Gibarku, and Tum; 10 were women and two were men.

Sixty lice positive for the *rpo*B gene underwent sequencing of the *rec*A gene. Forty (67%) were positive for *A. baumannii*

Table 1
Oligonucleotide primers and Taqman[a] fluorescent probe sequences of the *rpo*B and *rec*A genes used for PCR and sequencing of *Acinetobacter baumannii*

Gene	Oligonucleotide	Species	Sequence	Amplicon size (bp)
*rpo*B	AcropBF	*Acinetobacter sp*	5′-TACTCATATACCGAAAAGAAACGG-3′	238
	AcropBR		5′-GGYTTACCAAGRCTATACTCAAC-3′	
	AcropP		6′ FAM-CGCGAAGATATCGGTCTSCAAGC-TAMRA	
*rec*A	rA1F	*A. baumannii*	5′-CCTGAATCTTCTGGTAAAAC-3′	424
	rA2R		5′-GTTTCTGGGCTGCCAAACATTAC-3′	

[a] Applied Biosystems, Courtaboeuf, France.

Table 2
Detection of *Acinetobacter baumannii* in human lice from Ethiopia using *rec*A PCR amplification and sequencing

Location	*rec*A sequenced (head/body)	Detected *A. baumannii* (head/body)	*rec*A type (number)
Balt	5 (2/3)	3 (1/2)	1 (2), 9 (1)
Tikemit Eshet	8 (3/5)	4 (1/3)	5 (2), 9 (1), 10 (1)
Gibarku	8 (5/3)	7 (4/3)	1 (1), 9 (3), 10 (2), 11 (1)
Tum	6 (2/4)	3 (1/2)	1 (3)
Dembi	7 (0/7)	7 (0/7)	1 (7)
Mizan Teferi	21 (10/11)	12 (6/6)	1 (1), 11 (10), 12 (1)
Agaro	5 (1/4)	4 (0/4)	1 (1), 12 (3)
Total	60 (23/37)	40 (13/27)	1 (15), 5 (2), 9 (5), 10 (3), 11 (11), 12 (4)

(Table 2). Amplifying the *rec*A gene allowed unambiguous determination of the sequence of a 336-bp fragment. Four new genotypes were identified: (1) genotype 9, which is similar to genotype 1 but with a single nucleotide polymorphism (SNP) at position 251; (2) genotype 10, similar to genotype 5 with a SNP at position 293; (3) genotype 11, similar to genotype 5 with a SNP at position 50; and (4) genotype 12, similar to genotype 5 but with a SNP at position 146. The sequences have been deposited in the GenBank database with the following accession numbers: 9, **JF895617**; 10, **JF895618**; 11, **JF895619**; 12, **JF895620**.

The translated protein sequences were all identical, except for genotype 12 in which an aspartic acid is replaced by a glutamic acid at position 56. The most common genotype isolated was genotype 1 (15/40; 38%). The second most frequent genotype found was *A. baumannii* genotype 11, a new genotype that was mostly isolated from the head and body lice from Mizan Teferi (10 out of 12 lice). With regard to the *rec*A sequences of the non-baumannii strains (20/60), *Acinetobacter radioresistens* and *Acinetobacter rhizosphaerae* were the most commonly identified species using the BLAST method. It is interesting to note that in the regions of Gibarku and Tikemit Eshet, the *A. baumannii* genotypes observed in head lice differed from those observed in body lice.

4. Discussion

Our study is the first showing the presence of *A. baumannii* in human body lice, and also in head lice, in Ethiopia. For a long time *A. baumannii* was found only in body lice,[2] and it was in Marseille that the bacterium was first identified in the body lice of homeless persons.[3] The percentage of the total body lice infected with *Acinetobacter sp* in Ethiopia was 71%, and among 37 of the positive lice tested, 27 were found to be *A. baumannii*, showing a global infection of the lice due to *A. baumannii* of 52%. This percentage of body lice infection was found to be much higher than that in European countries: in Portugal, the Netherlands, and France, 10%, 18%, and 32%, respectively, of body lice were found to be infected by *A. baumannii*. Concerning Sub-Saharan African countries, it was shown that 58% of body lice in Rwanda were infected with *A. baumannii*, whereas in Burundi, the rate of infection was found to be only of 3%.[3] These results are particularly interesting as no other common skin commensal agent has been isolated from body lice.[3]

In a previous study from our laboratory, we amplified and sequenced a partial *cyt*B gene from head and body lice collected in the same seven regions of Ethiopia. Results showed that all the black head lice belonged to genotype C, while all the gray lice belonged to genotype A (unpublished data). These results confirmed those of other studies, which have shown genotype C head lice to be prevalent in Ethiopia.[18–20] In our study, all subjects except two had only black head lice, and all body lice were gray–transparent. The bacteria were more frequently isolated from body lice than from head lice ($p < 0.001$). Moreover, 12 patients with *Acinetobacter sp*-positive lice had *Acinetobacter sp* in both head and body lice. As a result, it appears that *A. baumannii* lice infection is not specific to a specific louse genotype.

The *rec*A gene of *A. baumannii* present in head and body lice was sequenced and the results showed a certain diversity of genotypes. Genotype 1 was ubiquitous in Ethiopia and was isolated from both head and body lice. This genotype is also the most common in other countries around the world, and notably in France.[3] Genotypes 3 and 4 have been observed in other parts of the world, but they were absent in Ethiopia. On the other hand, we found genotype 5, and this is the first time that this genotype has been described in human lice. Finally, four new genotypes were discovered here, but each one exhibiting only one SNP. In 2008, the complete genome sequences of *A. baumannii* SDF strain (isolated from a louse) and AYE strain (isolated during an outbreak in France in 2003)[14–16] became available, allowing an in-depth analysis of the gene contents and highlighting the differences that might reflect adaptation to a specific environment. An in-depth analysis of the gene contents revealed that although the two organisms share a large fraction of the genes, large differences exist.[15]

A. baumannii is known as a redoubtable pathogen of nosocomial infections, but also of community-acquired infections.[6–12] *A. baumannii* was the most commonly isolated organism (32.5% of cases) in one assessment of combat victims from Iraq and Afghanistan with open tibial fractures.[21] The bacterium was also isolated from wound infections after the earthquake in Sichuan Province, China,[22] and after the 1999 Marmara earthquake.[23] Our study showed the detection of *A. baumannii* in human lice, suggesting a potential environmental reservoir. In an experimental model of infection of body lice with *A. baumannii*, it was shown that the infected lice excreted viable bacteria in feces from the first day post-infection.[13] In addition, *A. baumannii* has been reported to have the ability to survive for a long time on dry surfaces.[24] Thus, the infected feces could transmit the Acinetobacter infection to the host (humans) either by contamination of the bite wound site,

when scratched into the skin, or via an aerosol. However, it is not clear if the *A. baumannii* strains present in lice are the same as those that are responsible for human infections.

In conclusion, we identified the presence of *A. baumannii* in head and body lice in seven regions of Ethiopia at different altitudes. Further epidemiological studies of head and body lice collected from more individuals and from different regions should be carried out to see if *A. baumannii* is present only at high altitude or if this bacterium can be retrieved, for example, in humid regions.

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Ethical approval: This study was approved by the Comité d'éthique de l'IFR 48 (statement number #11-010).

Conflict of interest: No conflict of interest to declare.

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