The Presence of Acinetobacter baumannii DNA on the Skin of Homeless People and Its Relationship With Body Lice Infestation. Preliminary Results

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The presence of Acinetobacter baumannii was demonstrated in body lice, however, little is known about the mechanism of natural lice infection. In 2013 and 2014, cross-sectional one-day studies were therefore performed within two Marseille homeless shelters to assess the presence of A. baumannii DNA on human skin, blood and in body lice collected from the same homeless individuals. All 332 participants completed questionnaires, were examined for dermatologic signs, and provided four skin samples (hair, neck, armpits, and pelvic belt), blood samples and body lice (if any). We developed a new real-time PCR tool targeting the ompA/motB gene for the detection of A. baumannii for all collected samples. Blood culture was also performed. Body lice were found in 24/325 (7.4%) of subjects. We showed a prevalence of A. baumannii DNA skin-carriage in 33/305 (10.8%) of subjects. No difference was found in A. baumannii DNA prevalence according to body sites. A strong association between body lice infestation (OR = 3.07, p = 0.029) and A. baumannii DNA skin-carriage was noted. In lice, A. baumannii DNA was detected in 59/219 arthropods (26.9%). All blood cultures and real-time PCR on blood samples were negative for A. baumannii. Lice probably get infected with A. baumannii while biting through the colonized skin and likely transmit the bacteria in their feces. We found no evidence that lice facilitate the invasion of A. baumannii into the blood stream. Further investigations are needed to compare phenotypic and genotypic features of A. baumannii isolates from human skin and lice from the same individuals.

Keywords: body lice, homeless, Acinetobacter baumannii, skin, ompA/motB
INTRODUCTION

_Acinetobacter_ species are mostly free-living saprophytes found ubiquitously in nature and are considered part of the normal flora of the human skin (Vallenet et al., 2008). Among _Acinetobacter_ species, _A. baumannii_ is the most important member often associated with hospital-acquired infections worldwide and is responsible for opportunistic infections of the skin, bloodstream, urinary tract, and other soft tissues. Although most _A. baumannii_ infections occur in critically ill patients in the intensive care unit setting, the frequency of community-acquired _A. baumannii_ infections has been increasing gradually. Furthermore, _A. baumannii_ has been showed to rapidly develop resistance to antimicrobials (Lee et al., 2017).

_Acinetobacter_ species are hosted by several insect species (Dillon and Dillon, 2004) but the occurrence of _A. baumannii_ in hematophagous groups is poorly documented. It has been isolated from _Aedes albopictus_ in Madagascar (Minard et al., 2013), moth fly species _Clogmia albipunctata_ with colonization rates of 0–17.5% in several German hospitals (Faulde and Spiesberger, 2013) or Brazilian phlebotomine sand flies _Lutzomyia longipalpis_ (Gouveia et al., 2008). _A. baumannii_ DNA have also been detected in human body lice, suggesting that lice can possibly transmit this pathogen. It was first isolated from body lice collected from homeless people in Marseille (La-Scola et al., 2001). A 21% prevalence of _A. baumannii_ DNA was then found in a large collection of 622 body lice collected in France, Burundi, Rwanda, Peru, Algeria, Portugal, and the Netherlands (La-Scola and Raoult, 2004). It has also been isolated at high rates from body lice collected from Ethiopian subjects (Kempf et al., 2012) and finally from body lice collected from Algerian homeless people (Louni et al., 2018a). _A. baumannii_ DNA was then detected in head lice from French children (Bouvresse et al., 2011), and from infested people in Thailand (Sunantaraporn et al., 2015), Republic of Congo, Niger, and Algeria (Amanzougaghene et al., 2016; Mana et al., 2017; Louni et al., 2018b). Phenotypic and genotypic features of _A. baumannii_ isolates from body lice differ significantly from _A. baumannii_ clinical isolate from humans (Vallenet et al., 2008). Experimental infection of lice by feeding on infected rabbits demonstrated that they were able to acquire and maintain a persistent life-long infection with _A. baumannii_. Moreover, infected body lice excreted living _A. baumannii_ within their feces and did not transmit their infection to their nurse host during feeding or transovarially (Houhamdi and Raoult, 2006).

Natural lice infection may result from the ingestion of infective blood meals from patients with ongoing bacteremia, or from the penetration of colonized human skin by lice mouth parts during feeding. To challenge this hypothesis, we conducted an epidemiological study among Marseille homeless individuals addressing the presence of _A. baumannii_ DNA on human skin and blood and in body lice collected from the same individuals.

METHODS

Cohorts

In our one-shot studies, all adult homeless people residing in two Marseille emergency shelters were enrolled on a voluntary basis in winter in 2013 and 2014. The participants completed a specially designed questionnaire providing information on demographics, chronic medical conditions, substance abuse, cutaneous symptoms (pruritus, scratch lesions), and were physically examined by the medical doctor.

Samples

Body lice were removed from the clothes and body of the infested participants, transferred to sterile plastic tubes and were subsequently processed for molecular analysis (Supplementary Table 1). Four skin (hair, neck, armpits, and pelvic belt) swabs and one blood sample (collected in EDTA tube) were obtained from each participant. In the laboratory, each louse was washed with 200 µl of sterile water and then decontaminated by immersion in 70% ethanol and 0.2% eosin for 5 min as previously described (La-Scola et al., 2001). After being crushed, lice were placed in tubes containing 180 µl tampon G2 and 20 µl proteinase K (QIAGEN, Hilden, Germany) and the samples were incubated at 56°C overnight. The skin swabs were resuspended in 1 ml of HBSS (Hank’s balanced salt solution). The blood samples were incubated in the BACTEC 9240 system (Reisner and Woods, 1999) and were considered negative for _A. baumannii_ if no bacterial growth was detected after 5 days.

DNA Extraction

The automated DNA extraction was performed on 190 µl collected samples from skin swabs, blood, lice-washing liquid, crushed lice using a BioRobot® EZ1 Advanced XL instrument (QIAGEN, Hilden, Germany) and DNeasy® Blood & Tissue according to the manufacturer's instructions. The quality of all DNA extracts was assessed by real-time PCR (qPCR) targeting internal control TISS phage that was added to each extraction (Sow et al., 2017).

Real-Time PCR

The ready-to-use reaction mix Light-Cycler® 480 Probes Master (Roche Diagnostics, Meylan, France) was used for PCR assay performed in the C1000 Touch™ Thermal Cycle (Bio-Rad, USA) according to the manufacturer’s recommendations. Positive control (Plasmid DNA) and negative control template (PCR mix + sterile H2O or _A. spp._ other than _A. baumannii_) were incorporated in each experimental run. For homeless samples, results were considered positive accepted when the cycle threshold value of real-time PCR was ≤35.

Specific Identification of _Acinetobacter baumannii_

We designed a novel qPCR system by choosing _A. baumannii_-specific gene encoding for Type VI secretion system _OmpA/MotB_ (accession number CP019034.1, GenBank) because of its presence in all sequenced genomes of _A. baumannii_ available in the public domain (Hassan et al., 2016). Our detailed experimental procedures are described in supplementary material (Technical Appendix).
Statistical Analysis
Collected data were statistically treated using SPSS 23.0 software. Missing data and unidentified samples were not analyzed. Statistical differences in baseline characteristics were evaluated by Pearson’s chi-square or Fisher’s exact tests as categorical variables. A two-tailed p-value < 0.05 was considered as statistically significant. We performed a binomial logistic regression with A. baumannii DNA carriage on the skin as a dependent variable. Univariate analysis based on only variables with a prevalence ≥ 5.0% by descriptive analysis was used to examine associations between multiple factors (demographic, chronic medical condition) and cutaneous clinical presentations toward prevalence of A. baumannii DNA skin-carriage. The initial model, therefore, included variables presenting a p-value < 0.2. The step-wise regression procedure and likelihood-ratio tests were applied to determine the final model.

RESULTS
Participant Characteristics (Table 1)
At enrolment, the population of 332 homeless people (shelter A [56%] and B [44%]) was mainly men with a mean age of 41 ± 14.1 years old (range, 19–84 years). About 16% were French while the rest (84%) were migrants, originating mostly from African countries and having settled in France ~9 years before the survey was conducted. Overall, the average duration of homelessness was about 3 years and chronic homelessness, defined as an episode of homelessness ≥1 year, accounted for 42.8% of cases. A 22.3% prevalence of pruritus was recorded, in line with 17.5% individuals presenting with scratch lesions. Body lice were observed on 24 participants (7.4%); however, only 15 among them allowed our team to collect their lice.

Acinetobacter baumannii DNA on Human Skin (Table 2)
A total of 1,266 skin swabs were obtained from 332 participants and of these, 33 individuals (of 305, [10.8%]) had A. baumannii DNA on at least 1 swab. We found that 9/319 (2.8%) hair swabs, 16/319 (5.0%) neck swabs, 6/317 (1.9%) armpit swabs, and 12/311 (3.9%) pelvic belt swabs were positive for A. baumannii DNA. There was no statically significant difference in the prevalence of A. baumannii DNA according to sampling site (p = 0.2).

Factor Associated With Acinetobacter baumannii DNA Skin-Carriage: Multivariate Model (Table 1)
The prevalence of A. baumannii DNA skin-carriage was lower in migrants, compared to French individuals, but was significantly higher in those infested by lice compared to others in univariate. In the multivariate analysis, only individuals infested by lice OR = 3.07 (1.13–8.4), p = 0.029 remained associated with an increased prevalence of A. baumannii DNA skin-carriage.

Acinetobacter baumannii DNA in Lice
We collected 1,780 lice from 15 participants. The most infested individual had 560 lice on his clothes (Supplementary Table 4). Of the 219 lice analyzed, 59 (23.9%) were positive for A. baumannii DNA in the crushed lice, 40 (18.3%) were positive in the lice-washing liquid (Table 2) and 38 (17.4%) were also positive in both crushed lice and the same lice-washing liquid. Overall, among 15 individuals whose lice were tested, 9 (60%) had at least one louse positive for A. baumannii DNA, 4 (26.7%) had A. baumannii DNA in both lice and skin samples.

Acinetobacter baumannii in Blood
All bacterial qPCRs and cultures performed on the 298 homeless blood samples were negative.

DISCUSSION
Acinetobacter has gained increasing attention in recent decades due to its ability to develop resistance on a large scale to almost all major classes of antibiotics and its capacity to survive for a long period in the environment (Peleg et al., 2008). While routine clinical diagnostic laboratories often have difficulties in differentiating A. baumannii from other Acinetobacter spp. (from the same genetic group but less implicated as human pathogens), herein, we established a specific real-time PCR for A. baumannii designed from the virulence gene encoding for Type VI secretion system OmpA/MotB, which showed 100% conservation pattern among all strains of A. baumannii.

A 10.8% prevalence of A. baumannii DNA skin-carriage was detected in our cohorts and no significant difference in A. baumannii DNA prevalence was observed between body sites. In previous studies, the prevalence of bacterium has been reported to be rare on the surface of the skin, about 3% in different human populations (Seifert et al., 1997; Ackern et al., 1999). Nevertheless, A. baumannii hand-carriage rates up to 23% were reported in healthcare workers (Almasaudi, 2018). The A. baumannii strains that cause nosocomial infections are common and highly resistant to antimicrobials (Peleg et al., 2008). Conversely, A. baumannii causing community-acquired infections are rare and highly susceptible to antimicrobial treatment (Farrugia et al., 2013).

A. baumannii DNA was present in 26.9% of lice, and the positive association between the presence of body lice and A. baumannii skin-carriage was highly significant in this work. An experimental study conducted in 2004 showed that lice that ingest the blood of infected rabbits become infected with A. baumannii but do not transmit the bacterium to healthy rabbits or to their offspring; however, they excreted the living bacteria in their feces (Houhamdi and Raoult, 2006). Our preliminary results suggest that lice ingest A. baumannii while penetrating the colonized skin. Then, as soon as lice excrete viable A. baumannii through lice feces that are deposed directly on the skin, the transmission may occur when the subjects scratch their skin. Lice can move from one subject to another and, as a result, epidemic transmissions of A. baumannii strains may occur. The bacterial transmission by lice via the ingestion of A. baumannii from blood could not be documented here because no subject had A. baumannii bacteremia in our study. A. baumannii bacteremia has only been reported in two homeless in our previous surveys, making this event very unlikely (Brouqui et al., 2005). Consequently, as in studies on Bartonella quintana endocarditis,
TABLE 1 | Univariate analysis and multivariate analysis with *Acinetobacter baumannii* DNA-carriage on the skin as a dependent variable.

| Characteristics | Total N | *Acinetobacter baumannii* on the skin N (%) | No *Acinetobacter baumannii* on the skin N (%) | Univariate analysis Odds ratio (95% CI), p-value | Multivariate analysis Odds ratio (95% CI), p-value |
|-----------------|---------|--------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|
| **Total**       | 332     | 33 (10.8)                                   | 272 (89.2)                                    |                                               |                                               |
| Year of study   |         |                                            |                                               |                                               |                                               |
| 2014            | 144     | 15 (12.8)                                   | 129 (90.4)                                    | 0.72 (0.35–0.49), p = 0.38                   |                                               |
| 2013            | 188     | 15 (9.6)                                    | 173 (90.4)                                    |                                               |                                               |
| **Shelter**     |         |                                            |                                               |                                               |                                               |
| B               | 146     | 12 (12.9)                                   | 132 (90.9)                                    | 0.68 (0.33–1.40), p = 0.29                   |                                               |
| A               | 186     | 15 (9.1)                                    | 150 (90.9)                                    |                                               |                                               |
| **Genre**       |         |                                            |                                               |                                               |                                               |
| Female          | 11      | 0 (0)                                       | 5 (100)                                       |                                               |                                               |
| Male            | 321     | 33 (11)                                     | 267 (89)                                      |                                               |                                               |
| **Age**         |         |                                            |                                               |                                               |                                               |
| Mean age (SD) (years) | 41 ± 14.1 | N/A                                      |                                               |                                               |                                               |
| Age range (years) | 19–24  | N/A                                        |                                               |                                               |                                               |
| ≤40 years of age | 170 (52.1) | 15 (9)                                     | 155 (91.1)                                    |                                               |                                               |
| >40 years of age | 156 (47.9) | 16 (12)                                    | 138 (89)                                      |                                               |                                               |
| Unknown^p       | 6       |                                            |                                               |                                               |                                               |
| **Birthplace**  |         |                                            |                                               |                                               |                                               |
| France (mainland) | 52 (15.9) | 9 (20.5)                                   | 43 (79.5)                                     | 0.40 (0.17–0.93), p = 0.03                   |                                               |
| Migrants        | 275 (84.1) | 24 (9.3)                                   | 226 (90.7)                                    |                                               |                                               |
| Africa          | 214 (62.1) | 19 (8.9)                                   | 195 (91.1)                                    |                                               |                                               |
| Europe          | 36 (11.0) | 2 (5.8)                                     | 32 (94.2)                                     |                                               |                                               |
| Asia            | 16 (5.0)  | 2 (14.3)                                    | 10 (85.7)                                     |                                               |                                               |
| Other           | 9 (2.8)   | 1 (11.1)                                    | 5 (88.9)                                      |                                               |                                               |
| Unknown^p       | 5       |                                            |                                               |                                               |                                               |
| Mean duration of residence in France for migrants (SD) (years) | 8.9 (0–24.4) | 5.5 (10.9) | 3.1 (89.1) |                                               |                                               |
| Range of duration of residence in France for migrants (years) | 0–66 | N/A | N/A |                                               |                                               |
| ≤1.5 years^2    | 134 (50) | 6 (4.9)                                     | 118 (95.1)                                    |                                               |                                               |
| >1.5 years      | 134 (50) | 14 (10)                                     | 117 (90)                                      |                                               |                                               |
| Unknown^p       | 12      |                                            |                                               |                                               |                                               |
| No visits to country of origin since immigration | 191 (71.8) | 15 (8.4) | 156 (91.6) |                                               |                                               |
| Visit to country of origin since immigration | 75 (28.2) | 8 (11.8) | 60 (88) | 1.45 (0.59–3.61), p = 0.41 |                                               |
| Unknown^p       | 14      |                                            |                                               |                                               |                                               |
| Mean duration of homelessness (SD), min, max (years) | 2.8 (0–7.0) | N/A | N/A |                                               |                                               |
| Range of duration of homelessness | 1 month–48 years | N/A | N/A |                                               |                                               |
| <1 year^2       | 172 (51.8) | 14 (8.9) | 158 (91.1) |                                               |                                               |
| ≥1 year         | 142 (42.8) | 17 (12.9) | 104 (87) | 1.6 (0.8–3.42), p = 0.21 |                                               |
| Unknown^p       | 18      |                                            |                                               |                                               |                                               |
| **Alcohol consumption** |         |                                            |                                               |                                               |                                               |
| Rare or never   | 274 (85.1) | 28 (11.2) | 233 (88) |                                               |                                               |
| Frequent        | 48 (14.9)  | 10 (5)                                      | 33 (88)                                       | 0.97 (0.35–2.66), p = 0.96                   |                                               |
| Unknown^p       | 10      |                                            |                                               |                                               |                                               |

(Continued)
TABLE 1 | Continued

| Characteristics | Total N | Acinetobacter baumannii on the skin N (%) | No Acinetobacter baumannii on the skin N (%) | Univariate analysis Odds ratio (95%CI), p-value | Multivariate analysis Odds ratio (95%CI), p-value |
|-----------------|---------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|

**SMOKING CIGARETTE**

| Yes | 200 (61.9) | 20 (10.6) | 168 (89.4) | 0.89 (0.42–1.87), p = 0.75 |
|-----|------------|-----------|------------|-------------------|-------------------|

**CHRONIC DISEASES**

| Chronic respiratory diseases | 41 (12.3) | 5 (13.9) | 31 (86.1) | 1.39 (0.5–3.9), p = 0.53 |
|-------------------------------|-----------|----------|-----------|-----------------|-----------------|

**BMI**

| Mean BMI (SD) (kg/m²) | 23.6 ± 5.2 |
|-----------------------|------------|
| Range of BMI (kg/m²)  | 15.1–40.2  |

**CLINICAL PRESENTATIONS**

| Pruritus | 72 (22.3) | 7 (10.8) | 58 (89.2) | 0.96 (0.39–2.32), p = 0.93 |
|----------|-----------|----------|----------|-----------------|-----------------|

**Acinetobacter baumannii N (%)**

| Crushed lice (N = 219 samples) | 59 (26.9) |
|---------------------------------|-----------|
| Lice-washing liquid (N = 219 samples) | 40 (18.3) |

**BODY LICE**

| Hair (N = 319 samples) | 9 (2.8) |
|------------------------|--------|
| Neck (N = 319 samples) | 16 (5.0) |
| Arm pits (N = 317 samples) | 6 (1.9) |
| Pelvic belt (N = 311 samples) | 12 (3.9) |

**Human skin**

| Total (N = 1,266 samples) | 43 (3.4) |

**ETHICS STATEMENT**

This protocol was approved by the Marseille Institutional Review Board/Ethics Committee (Protocol: 2010-A01406-33). Signed informed consent was signed by all individuals.

**AUTHOR CONTRIBUTIONS**

TL, JK, PB, OM, and PG contributed to experimental design, data analysis, statistics, interpretation, and writing. JK, VH, TD, SaB, SeB, and HT-D administered questionnaires, examined patients, and collected samples. SE and ML provided technical susceptibilities of isolates and genotypic profiles to challenge our hypothesis. This would directly demonstrate if viable *A. baumannii* isolates from body-lice are identical or different from those isolated from human skin.
ACKNOWLEDGMENTS

We are grateful to our colleagues Younes Laidoudi and Hacene Medkour for their technical assistance.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcimb.2019.00086/full#supplementary-material