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Use of MALDI-TOF MS for the Identification of Chad Mosquitoes and the Origin of Their Blood Meal

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Abstract. Matrix-assisted desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) is a clinical microbiology tool for the systematic identification of microorganisms. It has recently been presented as an innovative tool for the rapid and accurate identification of mosquitoes and their blood meal. To evaluate the capacity of this tool to identify mosquitoes collected in a tropical environment and preserved with silica gel, we analyzed 188 mosquitoes of different species collected in Chad, which were preserved with silica gel for 2 months. The MALDI-TOF MS analysis correctly identified 96% of the mosquitoes and 37.5% of their blood meals. Using MALDI-TOF MS and molecular biology, eight mosquito species were identified, including *Anopheles gambiae* s.l., *Anopheles rufipes*, *Culex quinquefasciatus*, *Culex neavei*, *Culex pipiens*, *Culex perexiguus*, *Culex rima*, and *Culex watti*. Blood meal identification revealed that mosquitoes fed mainly on humans, birds, and cows. Matrix-assisted desorption/ionization time-of-flight mass spectrometry appears to be a promising, fast, and reliable tool to identify mosquitoes and the origin of their blood meal for samples stored with silica gel.

INTRODUCTION

Mosquitoes are the primary arthropod vectors of infectious diseases, posing serious economic and public health problems because of their role in the transmission of numerous human and veterinary pathogens.[1] To human, they are capable of transmitting not only parasitic diseases such as malaria and lymphatic filariasis but also serious arboviruses including yellow fever, dengue fever, chikungunya, Zika virus, and West Nile virus (WNV) infections.[2] Malaria, caused by several species of *Plasmodium* parasites (*Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae*, *Plasmodium ovale wallikeri*, *P. ovale curtisi*, *Plasmodium knowlesi*, and *Plasmodium simium*), is transmitted to humans by female *Anopheles* spp. mosquitoes.[3,4] According to the World Health Organization, approximately five million additional cases of malaria in 2016 compared with 2015 and 445,000 deaths were reported.[5]

Despite the availability of antimalarial treatments, vector control measures are needed to control the mosquito vectors.[6] Long-lasting insecticide-treated bed net, indoor residual spraying, larviciding, and community education to promote vector avoidance are commonly used approaches.[7] The implementation of vector control and surveillance strategies against mosquitoes requires entomological surveys including correct identification not only of the vectors but also of their blood meal for a better understanding of their biting behavior (endophilic or exophilic and anthropophilic or zoophilic).[8,9]

Mosquito identification is most often performed using morphological criteria using identification keys and/or molecular methods.[10] These methods, however, have limitations, which may be the absence of identification keys or specific documentation, expertise in entomology, and inability to differentiate species from the same complex for the morphological method. On the other hand, molecular approaches are time consuming, expensive, and limited by the completeness of online sequence databases.[10] Similarly, the origin of the blood meal is identified by several methods such as precipitin, enzyme immunoassay, and molecular tests.[11,12] However, these methods also have drawbacks, such as the difficulty of obtaining specific antisera against a wide variety of host species, the effect of blood meal digestion and DNA extraction protocol, the high cost, handling time, and the need for bulky equipment.[13,14]

Matrix-assisted desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) is a clinical microbiology tool used for the systematic identification of bacteria,[15,16] archaea,[17] fungus, and parasites.[18] Recently, MALDI-TOF MS has been presented as an alternative tool for the rapid identification of many arthropods including mosquitoes[19–22] and the origin of their blood meal.[23,24] This involved different entomological studies using fresh samples from the laboratory and samples collected from the fields which were either frozen, preserved, preserved in alcohol, or crushed on Whatman paper.[23–26]

The aim of this study was to evaluate the ability of MALDI-TOF MS to identify mosquitoes and the origin of their blood meal using samples collected in the field in a tropical setting, preserved with silica gel, and sent to a place which has a MALDI-TOF MS device available.

MATERIALS AND METHODS

Mosquito collection. All mosquitoes were collected as part of an entomological survey made by the French Army Centre for Epidemiology and Public Health in Chad in October 2017. Mosquitoes were collected using BG sentinel traps (Biogents AG, Weißenburgstraße, Regensburg, Germany)[27] and CDC light traps (John W. Hock Company, Gainesville, États-Unis),[28] which were inspected every day between 6:00 AM and 7:00 AM and between 6:00 PM and 7:00 PM. The mosquitoes were morphologically identified every day to the genus and at the species level for *Anopheles* females, using morphological criteria.[29]

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A convenient sample of mosquitoes was selected for the present study. The mosquitoes were stored individually (for *Anopheles* spp.) or by a pool of 3–10 specimens (for *Culex* spp.) in a 1.5-mL Eppendorf tube with silica gel (Carl Roth GmbH, Karlsruhe, Germany) before being sent to Marseille, France, on November 3, 2017, for subsequent analysis.

Preparation of samples for MALDI-TOF MS analysis. *Matrix-assisted desorption/ionization time-of-flight mass spectrometry identification of mosquito samples.* Two months after being maintained at room temperature, the legs of each mosquito were removed and placed individually in 1.5 mL Eppendorf tubes with glass powder (Sigma, Lyon, France), 15 µL of 70% (v/v) formic acid (Sigma), and 15 µL of 50% (v/v) acetonitrile (Fluka, Buchs, Switzerland). The samples were ground using a tissue lyzer (Qiagen, Hilden, Germany) over three cycles of 30 ms^{-1} for 60 seconds.[30] The samples were then centrifuged at 10.000 rpg for 1 minute, and 1 µL of supernatant of each homogenate was deposited on a MALDI-TOF MS target plate in quadruplicate (Bruker Daltonics, Wissembourg, France) and covered with 1 µL of α-cyano-4-hydroxycynnamic acid (CHCA) matrix solution composed of saturated α-cyano-4-hydroxycynnamic acid (Sigma), 50% acetonitrile (v/v), 2.5% trifluoroacetic acid (v/v) (Aldrich, Dorset, UK), and high-performance liquid chromatography (HPLC) grade water.[26,30] After drying for several minutes at room temperature, the target was introduced into the MALDI-TOF Microflex LT mass spectrometer (Bruker Daltonics, Breman, Germany) for analysis.

Matrix-assisted desorption/ionization time-of-flight mass spectrometry identification of blood meal sources. The engorged abdomen of each female mosquito was individually ground in an Eppendorf tube containing 50 µL of HPLC grade water. After centrifugation, 10 µL of the supernatant was used for 10 µL for the MALDI-TOF analysis, as previously described.[23] Ten microliter of the abdomen supernatant was mixed with 20 µL of 70% (v/v) formic acid and 20 µL of 50% (v/v) acetonitrile (Fluka) and then centrifuged at 10. 000 rpm for 20 seconds. One microliter of supernatant from each sample was placed on the MALDI-TOF target plate in quadruple (Bruker Daltonics) and recovered with 1 µL of CHCA matrix solution composed of saturated α-cyano-4-hydroxycynnamic acid (Sigma), 50% acetonitrile (v/v), 2.5% trifluoroacetic acid (v/v) (Aldrich), and HPLC grade water. After drying for several minutes at room temperature, the target was introduced into the MALDI-TOF Microflex LT mass spectrometer (Bruker Daltonics) for analysis.

Spectral analysis. Protein mass profiles were acquired using a Microflex LT MALDI-TOF mass spectrometer, with detection in the linear positive ion mode at a laser frequency of 50 Hz in a mass range of 2–20 kDa. The acceleration voltage was 20 kV and the extraction time was 200 ns. Each spectrum corresponds to the ions obtained from the 240 laser shots performed in six regions in the same location and acquired automatically using the AutoXecute Flex Control software v.2.4 (Bruker Daltonics). Spectrum profiles obtained from mosquito legs and engorged abdomens were visualized with FlexAnalysis software v.3.3, and low-quality spectra were excluded from the study based on their intensity, reproducibility, and noise. They were then exported to ClinProTools version v.2.2 (Bruker Daltonics) and MALDI-Biotyper v.3.0 (Bruker Daltonics) for data processing.

Blind tests for the identification of mosquitoes and blood meals. To determine the mosquito species and the origin of blood meals, MALDI-TOF MS spectra from the legs and abdominal protein extracts of blood-engorged females were queried against the homemade MS reference spectra database (Table 1) using the MALDI-Biotyper software v3.0. tool (Bruker Daltonics). The level of significance was determined using the log score values (LSVs) provided by the MALDI-Biotyper software v.3.3. corresponding to a matched degree of signal intensities of mass spectra of the query and the reference spectra. Log score values ranged from zero to three. The samples were correctly considered and significantly identified when the spectrum queried had an LSV ≥ 1.8.[23] After molecular identification, the reference spectra of *Culex perexiguus* (n = 1), *Culex watti* (n = 1), and *Culex rima* (n = 1) were added in the homemade MS reference spectra database and a second blind test was made against the new database.

Molecular identification. Mosquito samples with high-quality spectra and LSV ≥ 1.8 but showing discrepancies between morphological identification and MALDI-TOF MS, and those with high-quality spectra and LSV < 1.8 were all identified at the species level by molecular tools. Quality of spectra was evaluated based on overall intensity of peaks, absence of noise, and reproducibility among each species, and visualized on both FlexAnalysis and ClinProTools software. Besides, some randomly selected well-identified samples (LSV ≥ 1.8 with concordance between morphological identification and MALDI-TOF MS) were also identified at the species level by molecular tools. We used the same workflow for the molecular identification of blood meals.

DNA extractions from individual mosquito heads and thorax samples or 10 µL supernatant of engorged abdomen of females were performed using the EZ1 DNA Tissue kit (Qiagen) according to the manufacturer's recommendations. To determine the origin of the blood meal, we used primers that specifically amplified the vertebrate cytochrome c oxidase I gene (v*COI*) (v*COI*_long forward: 5′-AAGAATCAGAATARGTTG-3′; v*COI*_long reverse: 5′-AACCACAAAGACATTGGCAC-3′).[31] As for mosquitoes, a region of the cytochrome c oxidase I gene (m*COI*) was amplified using the following primers: (LCO1490 (before): 5′-GGTCAAC AAATCATAAGATATTGG-3′; HC02198 (reverse): 5′-TAAACTTCAGGGTGACCAAAAAATCA-3′,[32] and the internal transcribed spacer 2 (ITS2) was amplified using the following primers: forward: 5′-ATCACTCGGCTCATGGATCG-3′; reverse: 5′-ATGCTTAAATTTAGGGGGTAGTC-3′.[33] Positive polymerase chain reaction (PCR) products were then purified and sequenced using the same primers with the BigDye version 1-1 Cycle Sequencing Ready Reaction Mix (Applied Biosystems, Foster City, CA) and an ABI 3100 automated sequencer (Applied Biosystems). The sequences were assembled and analyzed using the ChromasPro software (version 1.34) (Technelysium Pty. Ltd., Tewantin, Australia) and the National Center for Biotechnology Information, Basic Local Alignment Search Tool (NCBI BLAST) website (http://blast.ncbi.nlm.nih.gov).

RESULTS

Mosquitoes collection and morphological identification. A total of 188 mosquitoes were selected randomly to have a varied number of species and sex, but not as representative of the entire collection during the entomological survey, which will be reported elsewhere. According to the morphological identification, the selected mosquitoes belonged to two genera: *Culex* spp. represented 112/188 (59.6%) of which 13 were males and 99 were females, and *Anopheles* spp. represented

TABLE 1

List of the arthropod species present in our homemade MALDI-TOF MS database

Mosquitoes	Imago: *Aedes aegypti*, *Aedes albopictus*, *Aedes alternans*, *Aedes australis*, *Aedes caspius*, *Aedes cinereus*, *Aedes dufouri*, *Aedes flavifrons*, *Aedes fowleri*, *Aedes multiplex*, *Aedes notoscriptus*, *Aedes polynesiensis*, *Aedes procax*, *Aedes vexans*, *Aedes vigilax*, *Aedes vittiger*, *Anopheles annulipes*, *Anopheles arabiensis*, *Anopheles claviger*, *Anopheles coluzzi*, *Anopheles coustani*, *Anopheles funestus*, *Anopheles gambiae* Giles, *Anopheles hyrcanus*, *Anopheles maculipennis*, *Anopheles pharoensis*, *Anopheles rufipes*, *Anopheles wellcomei*, *Anopheles ziemani*, *Coquillettidia richiardii*, *Coquillettidia xanthogaster*, *Culex annulirostris*, *Culex australicus*, *Culex insignis*, *Culex modestus*, *Culex molestus*, *Culex neavei*, *Culex orbostiensis*, *Culex pipiens*, *Culex quinquefasciatus*, *Culex perexiguus*, *Culex rima*, *Cx. sitiens*, *Culex watti*, *Culiseta longiareolata*, *Lutzia tigripes*, *Mansonia uniformis*, *Ochlerothatus rusticus*, *O. excrucians*, *Orthopodomyia reunionensis*, and *Verralina funerea*
	Larvae: *Ae. albopictus*, *Ae. aegypti*, *An. coluzzi*, *An. gambiae*, *Cx. molestus*, *Cx. pipiens*, and *Culiseta* sp.
Lice	*Pediculus humanus*, *Damalinia bovis*, *Damalinia caprae*, *Damalinia ovis*, *Haematopinus eurysternus*, *Linognatus vituli*, and *L. africanus*
Fleas	*Archaeopsylla erinacei*, *Ctenocephalides felis*, *Culex canis*, and *Xenopsylla chopis*
	Pulex irritans, *Stenoponia tripectinata*, *Nosopsyllus fasciatus*, and *Cx. canis*
Ticks	Legs: *Amblyomma variegatum*, *Dermacentor marginatus*, *D. marginatus*–infected with *R. slovaca*, *D. reticulatus*, *Haemaphysalis concinna*, *Haemaphysalis punctata*, *Hyalomma m. rufipes*, *Ixodes hexagonus*, *Ixodes ricinus*, *Rhipicephalus bursa*, *Rhipicephalus sanguineus*, *Rh. sanguineus*–infected with *Rickettsia conorii*, *Rh. sanguineus*–infected with *R. massiliae*, and *Rh. sulcatus*
	Amblyomma gemma, *Amblyomma cohaerens*, *Am. variegatum*, *Argas persicus*, *Haemaphysalis leachi*, *Hae. punctata*, *Haemaphysalis spinulosa*, *Hyalomma detritum*, *Hy. m. rufipes*, *Hyalomma truncatum*, *I. ricinus*, *Ornithodoros sonrai*, *Rhipicephalus annulatus*, *Rhipicephalus bergeoni*, *Rh. bursa*, *Rhipicephalus decoloratus*, *Rhipicephalus e. evertsi*, *Rhipicephalus microplus*, *Rhipicephalus praetextatus*, *Rhipicephalus pulchellus*, and *Rh. sanguineus*
	Hemolymph: *Am. variegatum*–infected with *Rickettsia africae*, *D. marginatus*, *Hy. m. rufipes*, *Rh. bursa*, and *Rh. sanguineus*
Bed bugs	*Cimex lectularius* and *Cimex hemipterus*
Triatominae	*Eratyrus mucronatus*, *Panstrongylus geniculatus*, *Rhodnius prolixus*, *Rhodnius pictipes*, *Rhodnius robustus*, and *Triatoma infestans*
Sand flies	*Phlebotomus papatasi*, *Phlebotomus longicuspis*, *Phlebotomus perfiliewi*, *Phlebotomus perniciosus*, *Phlebotomus sergenti*, *Sergentomyia minuta*
Mite	*Leptotrombidium chiangraiensis*, *Leptotrombidium imphalum*, and *Leptotrombidium deliense*
Blattidae	*Supella longipalpa*, *Periplaneta americana*, *Blatta orientalis*, *Blatella germanica*, and *Blaptica dubia*
Flies	*Melophagus ovinus* and *Hippobosca equina*
Abdomen of mosquitoes engorged	*An. gambiae* Giles fed on *Homo sapiens*, *Equus caballus*, *Ovis aries*, rabbit, Balb/*C. mouse*, *Rattus norvegicus*, *Canis familiaris*, *Bos taurus*, *Capra hircus*, *Gallus gallus*, *Equus asinus*, *Tapirus indicus*, *Tapirus terrestris*, *Carollia perspicillata*, *Thraupis episcopus*, *Erythrocebus patas*, and *Callithrix pygmaea* blood
	Ae. albopictus fed on *H. sapiens* blood

76/188 (40.4%) of which 44 were males and 32 were females (Table 2). A total of 62.5% (20/32) of *Anopheles* females were morphologically identified as belonging to *Anopheles gambiae* s.l. and 37.5% (12/32) to *Anopheles rufipes* species.

Matrix-assisted desorption/ionization time-of-flight mass spectrometry and molecular identification of mosquitoes. Among the 188 mosquitoes that were preserved with silica gel, 169/188 (89.9%) had at least four legs and were selected for MALDI-TOF MS analysis (Table 2). Of these 169 mosquitoes, 104 (61.5%) provided good-quality MS spectra and were included for further MS analysis.

The spectra obtained from the legs of these 104 mosquitoes were then queried against the in-lab MS arthropod database. A total of 93.3% (97/104) were identified with LSVs ranging from 1.84 to 2.427 (average: 2.119). The other including six *Culex* spp. (females only) and one *Anopheles* spp. male had LSV less than 1.8 (Table 3).

Regarding the molecular identification, the seven mosquitoes that had LSVs less than 1.8 and a high-quality spectra, and 26 mosquitoes that had LSVs greater than 1.8 and discrepancies with morphological identification (or obtained from specimen with identification to the genus only) were subjected to standard PCR and sequencing. Among the mosquitoes identified with LSVs greater than 1.8, 22/26 (84.6%) were definitively confirmed by molecular biology with unambiguous similarities with the *COI* gene of the corresponding species (Table 3). For 4/26 (15.4%) morphologically identified as male *Anopheles* spp. and as *An. rufipes* by MS, they showed 99.7% identity with *Anopheles* sp. M36YA (GenBank accession number: KU187107.1), and 98.8% identity with *An. rhodesiensis* and *An. rufipes*, (GenBank accession numbers: KU187106.1 and KJ522838.1), with the *COI* gene. Samples that could not be identified using the *COI* gene (not enough divergence between *An. rhodesiensis* and *An. rufipes*) were sequenced using the ITS2 gene and revealed 100% identity with *An. rufipes* reference sequences (GenBank accession number: KJ522822.1). Therefore, MALDI-TOF identification of selected mosquitoes has been confirmed by molecular biology.

Partial COI gene sequences were obtained from the seven mosquitoes that had LSVs less than 1.8: one had 99% identity with *An. rufipes* (GenBank accession number: KJ522838.1), three were identified as *Cx. perexiguus* (100% identity; GenBank accession number: KU380423.1), two as *Cx. watti* (99.2%; GenBank accession number: KU187063.1), and one as *Cx. rima* (99.6%; GenBank accession number: KU187034.1). Our homemade MS reference spectra database did not contain any of the *Cx. rima*, *Cx. perexiguus*, and *Cx. watti* spectrum. The spectra of the new species not present in our in-lab database before this study (*Cx. rima*, *Cx. watti*, and *Cx. perexiguus*) have been added.

After the molecular identification and upgrade of the database with the reference spectra of *Cx. rima*, *Cx. perexiguus*, and *Cx. watti* from Chad, the second blind test against this updated database identified another *Cx. perexiguus* that had not been identified during the first blind test with LSV greater than 1.8. After the second blind test analysis, the percentage of MS correct identification reached 96.4%.

Identification of blood meals. A total 59 abdomens of engorged female mosquitoes (58 *Culex quinquefasciatus* and

TABLE 2

The number of mosquitoes collected in Chad preserved on silica gel by sex and gender, with the legs, those with the good matrix-assisted desorption/ionization time-of-flight mass spectrometry spectra, and the percentage of identification after the first blind test

Mosquitos species or genus	Number collected	Number with legs	Number of spectra of good quality	Percentage of identification to the species level
Anopheles sp. ♂	44	38 (62.3%)	18 (47%)	94.4% (17/18)
Anopheles gambiae ♀	22	18 (81.8%)	11 (61.1%)	100% (11/11)
Anopheles rufipes ♀	10	5 (50%)	1 (20%)	100% (1/1)
Culex sp. ♂	13	11 (84.6%)	7 (63.6%)	100% (7/7)
Culex sp. ♀	99	97 (97.9%)	67 (69%)	91% (61/67)
Total	188	169 (89.9%)	104 (61.5%)	93.3% (97/104)

one *An. gambiae* s.l.) were used for MALDI-TOF MS analysis. Of these, 24/59 (40.7%) samples had good quality MS spectra. A total of 9/24 (37.5%) were identified as human blood with LSVs between 1.901 and 2.308. The remaining 15 samples were not realibily identified (LSVs between 1.222 and 1.681, Because of low LSVs, these identifications were considered unreliable[23] (Table 4). The abdomens of engorged mosquitoes with MS spectra of good quality were subjected to sequencing using the *COI* gene vertebrate to determine the origin of the blood meal (Table 4). Of these, nine that had already been identified as human blood by MALDI-TOF MS with LSVs greater than 1.8 were confirmed by molecular biology with identities ranging from 99.7% to 100% (GenBank accession numbers: MF621085.1, MG970575.2, MG272704.1, MH161386.1, and MF696131.1). Among the 15 samples with low LSVs, sequencing showed that eight had identities ranging from 99.8% to 100% with human blood reference sequences (GenBank accession numbers: MF621085.1, MG970575.2, MG272704.1, MH161386.1, and MF696131.1), two had identities ranging from 95.3% to 96.4% with the European roller (*Coracias garrulous*) blood reference sequences (GenBank accession number: GQ481616.1), and one had 98.2% identity with domestic goat (*Capra hircus*) blood reference sequence (GenBank accession number: KX845672.1). No sequence was obtained for three samples.

DISCUSSION

Matrix-assisted desorption/ionization time-of-flight mass spectrometry, a widely used tool for the identification biomolecules, is based on the acidic extraction and ionization of

TABLE 3

Molecular identification of mosquitoes collected in Chad in October 2017 randomly selected for confirmation of MALDI-TOF MS identification

Number of samples	Morphological identification	Log score value	MALDI-TOF MS identification	Molecular identification (COI gene)	Molecular identification (internal transcribed spacer 2 gene)
632	*Anopheles* sp. ♂	1.79	/	*Anopheles rhodesiensis/Anopheles rufipes**	*An. rufipes*
595	*Anopheles* sp. ♂	1.84	*An. rufipes*	*An. rufipes*	*An. rufipes*
657	*Anopheles* sp. ♂	1.84	*An. rufipes*	*An. rufipes*	–
693	*Anopheles* sp. ♂	1.854	*An. rufipes*	*An. rhodesiensis/An. rufipes**	*An. rufipes*
599	*Anopheles* sp. ♂	1.869	*An. rufipes*	*An. rufipes*	–
698	*Anopheles* sp. ♂	1.879	*An. rufipes*	*An. rhodesiensis/An. rufipes**	*An. rufipes*
621	*Anopheles* sp. ♂	1.89	*An. rufipes*	*An. rufipes*	–
677	*Anopheles* sp. ♂	1.91	*An. rufipes*	*An. rufipes*	–
587	*Anopheles* sp. ♂	1.977	*An. rufipes*	*An. rufipes*	–
585	*Anopheles* sp. ♂	2.005	*An. rufipes*	*An. rhodesiensis/An. rufipes**	–
583	*Anopheles* sp. ♂	2.023	*An. rufipes*	*An. rufipes*	–
563	*Anopheles* sp. ♂	2.065	*An. rufipes*	*An. rufipes*	–
487	*Anopheles* sp. ♂	2.11	*An. rufipes*	*An. rufipes*	–
491	*Anopheles gambiae* ♀	2.126	*An. gambiae*	*An. gambiae*	–
593	*An. rufipes* ♀	2.128	*An. rufipes*	*An. rufipes*	–
297	*An. gambiae* ♀	2.23	*An. gambiae*	*An. gambiae*	–
572	*An. gambiae* ♀	2.261	*An. gambiae*	*An. gambiae*	–
533	*Culex* sp. ♀	1.037	/	*Culex watti*	–
694	*Culex* sp. ♀	1.452	/	*Culex rima*	–
618	*Culex* sp. ♀	1.513	/	*Culex perexiguus*	–
374	*Culex* sp. ♀	1.635	/	*Cx. perexiguus*	–
556	*Culex* sp. ♀	1.679	/	*Cx. perexiguus*	–
720	*Culex* sp. ♀	1.73	/	*Cx. watti*	–
624	*Culex* sp. ♀	1.956	*Culex pipiens*	*Cx. pipiens*	–
521	*Culex* sp. ♂	2.131	*Culex quinquefasciatus*	*Cx. quinquefasciatus*	–
522	*Culex* sp. ♂	2.139	*Cx. quinquefasciatus*	*Cx. quinquefasciatus*	–
740	*Culex* sp. ♀	2.145	*Cx. pipiens*	*Cx. pipiens*	–
705	*Culex* sp. ♀	2.161	*Cx. pipiens*	*Cx. pipiens*	–
715	*Culex* sp. ♀	2.161	*Cx. pipiens*	*Cx. pipiens*	–
568	*Culex* sp. ♀	2.253	*Cx. quinquefasciatus*	*Cx. quinquefasciatus*	–
682	*Culex* sp. ♀	2.267	*Cx. quinquefasciatus*	*Cx. quinquefasciatus*	–
523	*Culex* sp. ♂	2.314	*Cx. quinquefasciatus*	*Cx. quinquefasciatus*	–
520	*Culex* sp. ♀	2.337	*Cx. pipiens*	*Cx. pipiens*	–
592	*Culex* sp. ♀	2.427	*Cx. quinquefasciatus*	*Cx. quinquefasciatus*	–

MALDI-TOF MS = Matrix-assisted desorption/ionization time-of-flight mass spectrometry.
* Non-discriminative results (identical cover and identity values).

TABLE 4
MALDI-TOF MS and molecular identification of blood meal origin from abdomens of engorged females

Sample numbers	Log score value	MALDI-TOF MS identification	Molecular identification	% Identity	GenBank accession number
762	1.222	/	No sequence	/	–
605	1.267	/	*Coracias garrulus*	95.27	GQ481616.1
506	1.271	/	*Coracias garrulus*	96.40	GQ481616.1
681	1.290	/	*Capra hircus*	98.17	KX845672.1
623	1.309	/	*Homo sapiens*	100	MF621085.1
671	1.334	/	*H. sapiens*	99.68	MH378688.1
646	1.339	/	*H. sapiens*	100	MH378688.1
691	1.343	/	No sequence	/	–
670	1.377	/	No sequence	/	–
714	1.421	/	*H. sapiens*	99.07	MF621085.1
550	1.438	/	No sequence	/	–
673	1.453	/	*H. sapiens*	99.68	MG936624.1
761–3	1.457	/	*H. sapiens*	99.69	MF621085.1
505	1.509	/	*H. sapiens*	99.37	MF621085.1
716	1.681	/	*H. sapiens*	99.69	MH378688.1
695	1.901	Crushed abdomen of *Aedes albopictus* containing human blood	*H. sapiens*	100	MH378688.1
663	1.929	Crushed abdomen of *Ae. albopictus* containing human blood	*H. sapiens*	99.84	MH161386.1
717	1.953	Crushed abdomen of *Ae. albopictus* containing human blood	*H. sapiens*	99.68	MH378688.1
761-7	2.019	Crushed abdomen of *Ae. albopictus* containing human blood	*H. sapiens*	99.84	MF696131.1
644	2.078	Crushed abdomen of *Ae. albopictus* containing human blood	*H. sapiens*	100	MG272704.1
680	2.102	Crushed abdomen of *Ae. albopictus* containing human blood	*H. sapiens*	99.37	MH378688.1
727	2.147	Crushed abdomen of *Ae. albopictus* containing human blood	*H. sapiens*	100	MF621085.1
627	2.148	Crushed abdomen of *Ae. albopictus* containing human blood	*H. sapiens*	100	MH378688.1
537	2.308	Crushed abdomen of *Ae. albopictus* containing human blood	*H. sapiens*	100	MH378688.1

MALDI-TOF MS = Matrix-assisted desorption/ionization time-of-flight mass spectrometry. Results below the threshold of reliable identification (< 1.8) are not reported.

the proteins of an organism of interest. The extract is deposited on a steel target, covered with a MALDI matrix, and then dried at room temperature until co-crystallization. The crystallized target is then introduced into the apparatus, where the crystal is irradiated with laser pulses, performing desorption and "soft" ionization.[10] These desorbed and ionized molecules are accelerated in an electric field and separated by a flight tube in the linear or reflectron mode according to their mass–load ratio until they reach a detector.[34] Thus, the mass/charge values and intensities, that is, the so-called mass fingerprint of a generated sample, are then compared with a database containing species reference mass fingerprints for species identification.[35] Matrix-assisted desorption/ionization time-of-flight mass spectrometry has revolutionized clinical microbiology by its use in the systematic identification of bacteria,[15,16,36] archaea,[17] parasites, and fungi.[18] Recently, it has been introduced in medical entomology as a tool for the rapid and accurate identification of arthropods, detection of the origin of their blood meal, and detection of associated microorganisms.[23,24,26,37–41] In entomology, adjustments such as arthropod-based body selection and sample crushing protocol are required for proper MALDI-TOF MS identification of arthropods or detection of associated pathogens.[10,26,38,39]

The method of conservation may also be a limiting factor of this method. Generally, arthropods are collected in the field, far from the laboratories. Therefore, they are usually stored dry with silica gel, in 70% ethanol at +4°C or −20°C for transport to laboratories.[25] It is easier and less expensive to transport samples preserved with silica gel or in 70% ethanol than samples preserved at −20° C, and these methods are widely used in African countries.[25] However, it was reported that storage for a long time in 70% alcohol may impact MALDI-TOF MS profiles, resulting in lower intensity and lower overall quality than those of fresh or frozen samples.[25,30,42] No MALDI-TOF MS study has yet been conducted on arthropods stored with silica gel despite the advantages of this method such as limited cost and simplicity.

In this study, 96% of the mosquito legs with good spectra were correctly identified with LSV ≥ 1.8 by the MALDI-TOF MS. This confirms the reliability of this method for mosquito identification, as long as the spectra are of good quality. This allowed us to confirm the morphological identification of the female *Anopheles* spp. up to the species level and of the *Culex* spp. up to the genus level. It helped us to identify not only male *Anopheles* spp. but also minority species such as *Cx. rima*, *Cx. perexiguus*, and *Cx. watti* which were not included in our database before this work. The results of this study can be considered robust and reliable as they have been confirmed by molecular biology.

The two species of *Anopheles* identified in this study, that is, *An. gambiae* s.l. and *An. rufipes*, have already been reported in Chad.[43] *Anopheles gambiae* s.l. were the most abundant mosquitoes included in our study and considered the main vector of malaria in Chad.[43] *Anopheles rufipes* is a mosquito that rests frequently in human habitation but feeds on domestic animals and accidentally on humans.[29] In this study, we have also identified several *Culex* species such as *Cx. quinquefasciatus*, *Culex pipiens*, *Cx. perexiguus*, *Cx. watti*, and *Cx. rima*. Many of these species have been implicated or

suspected in the transmission of parasitic or viral pathogens. The *Cx. pipiens* species consists of two morphologically identical subspecies, that is, *Cx. pipiens pipiens* and *Cx. pipiens molestus*, with distinct trophic preferences: *Cx. pipiens pipiens* feeds on birds, whereas *Cx. pipiens molestus* prefers mammals.[44,45] *Culex pipiens* has been identified as the most important vector species of WNV in the United States because of their vectorial competence and summer abundance.[46] Presently, only one study has reported the presence of *Cx. pipiens* in Chad.[47] *Culex quinquefasciatus*, known vector of lymphatic filariasis and arboviruses including WNV, has been reported in some African countries.[48–50] *Culex perexiguus*, considered a potential vector for WNV transmission in birds and horses, has recently been reported in Mali.[24] *Culex watti* has been reported in Madagascar and is believed to have played a role in the WNV transmission.[51] As for *Cx. rima*, its presence has already been reported in some African countries,[52–54] but its vector role remains unknown to this day. We found that the fragment of the *COI* gene amplified in this study could not distinguish *An. rufipes* and *An. rhodesiensis*. This limitation of molecular biology had already been reported.[55] Therefore, MALDI-TOF MS could be an alternative tool to meet this challenge because this tool has been proven relevant to discriminate cryptic mosquito species.[30]

Only 40.7% of abdomens engorged had good quality MS spectra and 37.5% were correctly identified as human blood. *Niare* et al.[56] had shown that identification of the origin of the blood meal was relevant up to 24 hours because of blood digestion altering the resulting spectrum. We support the idea that this low percentage of good spectra could be explained by the fact that the Sella score to describe the digestive state of blood, from engorged mosquitoes from zero (non-nourished mosquitoes) to seven (females without visible blood and fully developed eggs in their abdomen), was poorly appreciated visually.[14]

CONCLUSION

The present study demonstrated that MALDI-TOF MS appears to be a promising tool for identifying mosquitoes stored in silica gel and moderately the origin of their blood meal. Although the number of samples used in our study is relatively small, the results obtained are robust and reliable. Thus, it opens the way for future studies with a large number of samples to confirm these preliminary results.

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REFERENCES

1. Fernandes JN, Moise IK, Maranto GL, Beier JC, 2018. Revamping mosquito-borne disease control to tackle future threats. *Trends Parasitol 34:* 359–368.
2. Moise IK, Riegel C, Muturi EJ, 2018. Environmental and social-demographic predictors of the southern house mosquito *Culex quinquefasciatus* in New Orleans, Louisiana. *Parasit Vectors 11:* 249.
3. Brasil P et al., 2017. Outbreak of human malaria caused by *Plasmodium simium* in the Atlantic Forest in Rio de Janeiro: a molecular epidemiological investigation. *Lancet Glob Health 5:* e1038–e1046.
4. WHO, 2016. *World Malaria Report 2016*. Geneva, Switzerland: World Health Organization.
5. WHO, 2017. *World Malaria Report 2017*. Geneva, Switzerland: World Health Organization.
6. Pluess B, Tanser FC, Lengeler C, Sharp BL, 2010. Indoor residual spraying for preventing malaria. *Cochrane Database Syst Rev 4:* CD006657.
7. Medzihradsky OF et al., 2018. Study protocol for a cluster randomised controlled factorial design trial to assess the effectiveness and feasibility of reactive focal mass drug administration and vector control to reduce malaria transmission in the low endemic setting of Namibia. *BMJ Open 8:* e019294.
8. Bass C, Williamson MS, Wilding CS, Donnelly MJ, Field LM, 2007. Identification of the main malaria vectors in the *Anopheles gambiae* species complex using a TaqMan real-time PCR assay. *Malar J 6:* 155.
9. Muturi EJ, Mwangangi JM, Beier JC, Blackshear M, Wauna J, Sang R, Mukabana WR, 2013. Ecology and behavior of *Anopheles arabiensis* in relation to agricultural practices in central Kenya. *J Am Mosq Control Assoc 29:* 222–230.
10. Yssouf A, Almeras L, Raoult D, Parola P, 2016. Emerging tools for identification of arthropod vectors. *Future Microbiol 11:* 549–566.
11. Fyodorova MV, Savage HM, Lopatina JV, Bulgakova TA, Ivanitsky AV, Platonova OV, Platonov AE, 2006. Evaluation of potential west Nile virus vectors in Volgograd region, Russia, 2003 (Diptera: Culicidae): species composition, bloodmeal host utilization, and virus infection rates of mosquitoes. *J Med Entomol 43:* 552–563.
12. Ngo KA, Kramer LD, 2003. Identification of mosquito bloodmeals using polymerase chain reaction (PCR) with order-specific primers. *J Med Entomol 40:* 215–222.
13. Kent RJ, 2009. Molecular methods for arthropod bloodmeal identification and applications to ecological and vector-borne disease studies. *Mol Ecol Resour 9:* 4–18.
14. Martinez-de la Puente J, Ruiz S, Soriguer R, Figuerola J, 2013. Effect of blood meal digestion and DNA extraction protocol on the success of blood meal source determination in the malaria vector *Anopheles atroparvus*. *Malar J 12:* 109.
15. Bizzini A, Greub G, 2010. Matrix-assisted laser desorption ionization time-of-flight mass spectrometry, a revolution in clinical microbial identification. *Clin Microbiol Infect 16:* 1614–1619.
16. Seng P, Drancourt M, Gouriet F, La Scola B, Fournier PE, Rolain JM, Raoult D, 2009. Ongoing revolution in bacteriology: routine identification of bacteria by matrix-assisted laser desorption ionization time-of-flight mass spectrometry. *Clin Infect Dis 49:* 543–551.
17. Dridi B, Raoult D, Drancourt M, 2012. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry identification of Archaea: towards the universal identification of living organisms. *APMIS 120:* 85–91.

18. Murugaiyan J, Roesler U, 2017. MALDI-TOF MS profiling-advances in species identification of pests, parasites, and vectors. *Front Cell Infect Microbiol 7:* 184.
19. Halada P, Hlavackova K, Dvorak V, Volf P, 2018. Identification of immature stages of phlebotomine sand flies using MALDI-TOF MS and mapping of mass spectra during sand fly life cycle. *Insect Biochem Mol Biol 93:* 47–56.
20. Hoppenheit A, Murugaiyan J, Bauer B, Steuber S, Clausen PH, Roesler U, 2013. Identification of tsetse (Glossina spp.) using matrix-assisted laser desorption/ionisation time of flight mass spectrometry. *PLoS Negl Trop Dis 7:* e2305.
21. Raharimalala FN, Andrianinarivomanana TM, Rakotondrasoa A, Collard JM, Boyer S, 2017. Usefulness and accuracy of MALDI-TOF mass spectrometry as a supplementary tool to identify mosquito vector species and to invest in development of international database. *Med Vet Entomol 31:* 289–298.
22. Rothen J, Githaka N, Kanduma EG, Olds C, Pflüger V, Mwaura S, Bishop RP, Daubenberger C, 2016. Matrix-assisted laser desorption/ionization time of flight mass spectrometry for comprehensive indexing of East African ixodid tick species. *Parasit Vectors 9:* 151.
23. Niare S, Tandina F, Davoust B, Doumbo O, Raoult D, Parola P, Almeras L, 2017. Accurate identification of *Anopheles gambiae* Giles trophic preferences by MALDI-TOF MS. *Infect Genet Evol 63:* 410–419.
24. Tandina F, Niaré S, Laroche M, Koné AK, Diarra AZ, Ongoiba A, Berenger JM, Doumbo OK, Raoult D, Parola P, 2018. Using MALDI-TOF MS to identify mosquitoes collected in Mali and their blood meals. *Parasitology 145:* 1170–1182.
25. Diarra AZ, Almeras L, Laroche M, Berenger JM, Koné AK, Bocoum Z, Dabo A, Doumbo O, Raoult D, Parola P, 2017. Molecular and MALDI-TOF identification of ticks and tick-associated bacteria in Mali. *PLoS Negl Trop Dis 11:* e0005762.
26. Yssouf A, 2013. Matrix-assisted laser desorption ionization—time of flight mass spectrometry: an emerging tool for the rapid identification of mosquito vectors. *PLoS One 8:* e72380.
27. Vogels CB, Möhlmann TW, Melsen D, Favia G, Wennergren U, Koenraadt CJ, 2016. Latitudinal diversity of *Culex pipiens* biotypes and hybrids in farm, peri-urban, and wetland habitats in Europe. *PLoS One 11:* e0166959.
28. Biteye B, Fall AG, Ciss M, Seck MT, Apolloni A, Fall M, Tran A, Gimonneau G, 2018. Ecological distribution and population dynamics of Rift Valley fever virus mosquito vectors (Diptera, Culicidae) in Senegal. *Parasit Vectors 11:* 27.
29. Diagne N, Fontenille D, Konate L, Faye O, Lamizana MT, Legros F, Molez JF, Trape JF, 1994. Anopheles of Senegal. An annotated and illustrated list. *Bull Soc Pathol Exot 87:* 267–277.
30. Nebbak A, Willcox AC, Bitam I, Raoult D, Parola P, Almeras L, 2016. Standardization of sample homogenization for mosquito identification using an innovative proteomic tool based on protein profiling. *Proteomics 16:* 3148–3160.
31. Townzen JS, Brower AV, Judd DD, 2008. Identification of mosquito bloodmeals using mitochondrial cytochrome oxidase subunit I and cytochrome b gene sequences. *Med Vet Entomol 22:* 386–393.
32. Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R, 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Mol Mar Biol Biotechnol 3:* 294–299.
33. Weeraratne TC, Surendran SN, Reimer LJ, Wondji CS, Perera MDB, Walton C, Parakrama Karunaratne SHP, 2017. Molecular characterization of *Anopheline* (Diptera: Culicidae) mosquitoes from eight geographical locations of Sri Lanka. *Malar J 16:* 234.
34. Carbonnelle E, Mesquita C, Bille E, Day N, Dauphin B, Beretti JL, Ferroni A, Gutmann L, Nassif X, 2011. MALDI-TOF mass spectrometry tools for bacterial identification in clinical microbiology laboratory. *Clin Biochem 44:* 104–109.
35. Welker M, Moore ER, 2011. Applications of whole-cell matrix-assisted laser-desorption/ionization time-of-flight mass spectrometry in systematic microbiology. *Syst Appl Microbiol 34:* 2–11.
36. Gregory D, Chaudet H, Lagier JC, Raoult D, 2018. How mass spectrometric approaches applied to bacterial identification have revolutionized the study of human gut microbiota. *Expert Rev Proteomics 15:* 217–229.
37. El HB, Laroche M, Almeras L, Bérenger JM, Raoult D, Parola P, 2018. Detection of *Bartonella* spp. in fleas by MALDI-TOF MS. *PLoS Negl Trop Dis 12:* e0006189.
38. Laroche M, Almeras L, Pecchi E, Bechah Y, Raoult D, Viola A, Parola P, 2017. MALDI-TOF MS as an innovative tool for detection of plasmodium parasites in *Anopheles* mosquitoes. *Malar J 16:* 5.
39. Tahir D, Almeras L, Varloud M, Raoult D, Davoust B, Parola P, 2017. Assessment of MALDI-TOF mass spectrometry for filariae detection in *Aedes aegypti* mosquitoes. *PLoS Negl Trop Dis 11:* e0006093.
40. Yssouf A, Flaudrops C, Drali R, Kernif T, Socolovschi C, Berenger JM, Raoult D, Parola P, 2013. Matrix-assisted laser desorption ionization-time of flight mass spectrometry for rapid identification of tick vectors. *J Clin Microbiol 51:* 522–528.
41. Yssouf A, Socolovschi C, Leulmi H, Kernif T, Bitam I, Audoly G, Almeras L, Raoult D, Parola P, 2014. Identification of flea species using MALDI-TOF/MS. *Comp Immunol Microbiol Infect Dis 37:* 153–157.
42. Mathis A et al., 2015. Identification of phlebotomine sand flies using one MALDI-TOF MS reference database and two mass spectrometer systems. *Parasit Vectors 8:* 266.
43. Kerah-Hinzoumbe C, Péka M, Antonio-Nkondjio C, Donan-Gouni I, Awono-Ambene P, Samè-Ekobo A, Simard F, 2009. Malaria vectors and transmission dynamics in Goulmoun, a rural city in south-western Chad. *BMC Infect Dis 9:* 71.
44. Byrne K, Nichols RA, 1999. *Culex pipiens* in London underground tunnels: differentiation between surface and subterranean populations. *Heredity (Edinb.) 82:* 7–15.
45. Vinogradova EB, Shaikevich EV, 2005. Differentiation between the urban mosquito *Culex pipiens pipiens F. molestus* and *Culex torrentium* (Diptera: Culicidae) by the molecular genetic methods. *Parazitologiia 39:* 574–576.
46. Kilpatrick AM, Kramer LD, Campbell SR, Alleyne EO, Dobson AP, Daszak P, 2005. West Nile virus risk assessment and the bridge vector paradigm. *Emerg Infect Dis 11:* 425–429.
47. Hamon J, Burnett GF, Adam JP, Rickenbach A, Grjebine A, 1967. *Culex pipiens* fatigans Wiedemann, *Wuchereria bancrofti* Cobbold and the economic development of tropical Africa. *Bull World Health Organ 37:* 217–237.
48. Chavasse DC, Lines JD, Ichimori K, 1996. The relationship between mosquito density and mosquito coil sales in Dar es Salaam. *Trans R Soc Trop Med Hyg 90:* 493.
49. Fofana D, Koné AB, Koné N, Konan YL, Doannio JM, N'goran KE, 2012. *Culex quinquefasciatus* sensitivity to insecticides in relation to the urbanization level and sewage water in Yopougon, a township of Abidjan (Cote-d'Ivoire). *Bull Soc Pathol Exot 105:* 230–236.
50. Yadouleton A, Badirou K, Agbanrin R, Jöst H, Attolou R, Srinivasan R, Padonou G, Akogbéto M, 2015. Insecticide resistance status in *Culex quinquefasciatus* in Benin. *Parasit Vectors 8:* 17.
51. Rodhain F, Clerc Y, Albignac R, Ricklin B, Ranaivosata J, Coulanges P, 1982. Arboviruses and lemurs in Madagascar: a preliminary note. *Trans R Soc Trop Med Hyg 76:* 227–231.
52. Rickenbach A, Eouzan JP, Ferrara L, Bailly-Choumara H, 1976. *Données Nouvelles sur la Présence, la Fréquence et la Répartition des Toxorhynchitinae et Culicinae (Diptera, Culicidae) au Cameroun2. Genres Eretmapodites et* Culex. Cah. O.R.S.T.O.M., sér. Ent. méd. et Parasitol., vol. XN, no 2, 1976: 93–10.
53. Obame-Nkoghe J et al., 2017. Exploring the diversity of blood-sucking *Diptera* in caves of Central Africa. *Sci Rep 7:* 250.
54. Peters W, 1956. *The Mosquitos of Liberia (Diptera: Culicidae), A General Survey*. *Bull Entomol Res 47:* 525–551.
55. Erlank E, Koekemoer LL, Coetzee M, 2018. The importance of morphological identification of African anopheline mosquitoes (Diptera: Culicidae) for malaria control programmes. *Malar J 17:* 43.
56. Niare S, Berenger JM, Dieme C, Doumbo O, Raoult D, Parola P, Almeras L, 2016. Identification of blood meal sources in the main African malaria mosquito vector by MALDI-TOF MS. *Malar J 15:* 87.