HUMAN MICROBIOME AT YOUR FINGERTIPS: STATE-OF-THE-ART AND FORENSIC OPPORTUNITIES

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Abstract

The human skin, as the largest organ, hosts a wide range of rich and diverse microbial communities. These are a mixture of resident (or core) and transient species that are dependent on multiple variables including person-specific, and environmental. Together they contribute to an individual's unique microbial profile. The skin microbiome composition also varies across different body sites. Although the role of microbes within the human body is being progressively elucidated, particularly within the context of health and medicine, their exploitation within forensic science is still being researched. By mining the published literature between 2010 and 2026, this mini-review maps the application of "omics" techniques detecting microbial populations on skin and fingermarks, with the purpose of understanding the limitations, opportunities and what further forensic research should encompass to exploit the untapped potential of detecting, identifying and visualising the fingermark microbiome, in support to criminal investigations. Identified key gaps in knowledge derive, amongst others, from technical limitations and interpretative uncertainty with respect to genomic techniques, different severity of the challenges in disrupting the cell walls across bacterial populations when using molecular techniques such as mass spectrometry, and challenges in maintaining a contamination-free environment. These issues are compounding factors when considering the evolving nature of the microbiome and the present impossibility to directly correlate the skin and/or fingertip microbiomes to the fingermark microbiomes. This mini-review therefore also provides suggestions to overcome these challenges and mitigate/fill these gaps in knowledge.

Keywords: forensic, microbiome, mass spectrometry, skin, fingermark, identification, investigation

1. Introduction

It is well-established that criminal offenders exhibit increasing forensic awareness, including the use of gloves or protective clothing – to mitigate the deposition of physical evidence. Nevertheless, the recovery of fingermarks in non-premeditated crimes continue to yield significant forensic opportunities. Here, the term fingermark will be used according to the UK standards, to define the generation of an impression via contact of the fingertip with a surface, versus a fingerprint, deliberately taken at police stations upon arrest, at airports or for experimental research.

Crime scene investigators and accredited forensic laboratories utilise a range of optical (including spectroscopic) physical and chemical methods, often in a sequential manner to enhance the marks and maximise biometric information [1] . Spectroscopic, chemical and physical methods all target specific classes of compounds, such as amino acids and sebaceous lipids, which are selected and used on the assumption that they are present and sufficiently abundant in a given crime scene mark. However, enhancement is not always successful. This circumstance, and the importance of fingermarks as biometric evidence, underscore the necessity for experimental proof-of-concept research initiatives, such as the recently funded OPSCA STAR project, aimed at the development of alternative and complementary methods yielding human identification as well as intelligence profiling the suspect from a crime scene mark. Amongst these, the characterization of the uniqueness of both core and transient fingermark microbiomes [2] and their detection and visualisation, would provide considerable intelligence, particularly in major crimes including strangulation, domestic and gender-based violence, homicide, and sexual assault. In this light, this mini-review is intended to paint a picture of the state-of-the-art knowledge around the skin, the fingertip and the fingermark microbiomes to better direct research efforts aiming at the exploitation in skin-to skin/skin-to surface contact violent crimes.

Here we use the all-encompassing “skin microbiome” to indicate the wider context of the human body. We then discuss the “fingermark microbiome”, in a closer relation to the human “fingertip microbiome”, as, whilst not the same, it is recognised that the fingertip biogeography, which is unique within (intra) and between (inter) individuals, will have some role in dictating the community composition and distribution of the deposited

fingermark microbiome [2,3]. The latter is therefore defined as the original (and accidental) transfer the fingertip microbiome onto a deposition surface.

However, to effectively integrate fingermark-derived microbial intelligence into forensic workflows, efficient, sensitive and reproducible methods need to be developed and adapted from similar research conducted on bacterial cultures (by and large, in a clinical diagnostic setting). These methods need to be incorporated into a systematic research approach to elucidate both intra- and inter-personal variations within the human fingermark microbiome. Research has also highlighted that a fundamental understanding is required for the intra-personal latent fingerprint microbiome in response to time-since-deposition [2–4]. Therefore, given the inherent complexity of microbial sub-communities and their varying temporal persistence, a multi-modal analytical approach is essential.

There is broad recognition among forensic researchers, practitioners, and stakeholders of the need for coordinated, collaborative approaches to this research and its experimental design, rather than siloed efforts [5,6]. In this light, combinations of complementary techniques that investigate microbial community structure and composition (metagenomics), functional activity (transcriptomics), metabolites (metabolomics), proteins (proteomics), and lipids (lipidomics) would enable a more comprehensive analysis of microbial and molecular traces in fingermarks, potentially providing insight into biologically relevant activities associated with a crime (e.g. contact, environment, personal information on the individuals relevant to the scene). Multi-modality would therefore enhance the understanding required to truly extend the forensic toolkit to include skin microbial forensics, which, in the long-term, could be used to investigate major crimes and inform court proceedings.

Critical and systematic reviews, along with primary research articles, have recognised the roles of the human microbiome in health and medicine [6]. However, its application in forensic science is also under investigation. It was realised as early as 2017 that human microbiomes could be forensically exploited to derive the origin of unknown samples by distinguishing microbial strains that were characteristic of individuals [7–9] and with potential for personal identification [10]. In this context, although the hand microbiome has been explored, only a few studies have investigated the specific

microecology of the fingertip targeting inherent communities [11] or model strains [12,13] and even less primary research [2,4] is available on fingermark microbiomes.

This mini-review assesses published research on the skin microbiome within a forensic context, with the aim of synthesising existing evidence and discussing and recommending complementary analytical approaches for studying as well as extrapolating the fingermark microbiome.

2. Methods

Search strategy- Scopus, Pubmed and Google Scholar databases were used to search for and to identify relevant peer-reviewed journal articles based on specific keywords, their synonyms, and key phrases (Table 1). Key words were identified in the titles, abstracts, publication keyword lists and main text.

Table 1. Literature search Keywords

The first round of searching was conducted within the 2010 – 2026 period, to reflect the near 20-year period in which the role of microorganisms in forensic science has received attention and has been researched using molecular techniques. Inclusion criteria encompassed primary peer-reviewed articles and select peer-reviewed critical reviews that focused on the skin microbiome within a forensic context. Additional primary research articles were identified from references and/or “cited by” lists of the publications selected from the first round of the inclusion criteria. Specific book chapters, project reports and operational manuals were used to add a wider context where relevant. Grey literature and publications not available in English, non-peer reviewed books and book chapters were excluded from the assessment in this mini-review.

3. Skin Microbiome Pressures and potential forensic exploitation

The human microbiome – a complex and dynamic consortium of microorganisms – is determined by a combination of interacting factors from the host and with the surrounding environment [14,15]. As illustrated in Table 2, the complexity and composition of the human skin microbial communities are determined by endogenous host physiological factors (e.g. age, biological sex, ethnicity, personal hygiene, health status, medication and its mode of delivery), lifestyle (e.g., diet, alcohol consumption, physical activity, smoking habits, etc. and exogenous variables including environmental (geographical location climate, environmental pollution, UV exposure, migrations) [15–23], contact, personal hygiene and cohabitation [24–27], making an individual's skin microflora just as unique as the gut microbiome (the latter targeted for personalised medicine).

Table 2: Potential selection pressures acting on the human skin microbiome, including intrinsic factors, behaviours, and environmental exposures. For each pressure, the table reports the study context and main microbiome effects. Summations are presented and referenced from example primary research articles.

It is likely that some of the selection pressures in Table 2, such as, but not limited to, handedness, occupation, hand hygiene, and lifestyle choices like smoking, will have the highest impact on the fingermark microbiome.

Given the ubiquitous presence of microorganisms on the human body, particularly gut, oral cavity and skin [55], it is reasonable to speculate on the possibility to detect distinct microbiota on human skin. As the largest organ on the human body, the skin harbours a significant number of microbial populations distributed across anatomically and physiologically distinct microenvironments, with bacterial densities reaching up to 1 x 10^7 per cm^2 [56]. Specifically, the skin microbiome is recognised as unique in its structural diversity and individual specificity. It was realized as early as 2017 that human microbiomes could potentially be forensically exploited to derive the origin of unknown samples by identifying microbial strains that were characteristic of individuals [7–9] and ultimately for personal identification [10]. Brüssow [57] suggested that this uniqueness

could be exploited to even distinguish monozygotic twins. Overall, the skin microbiome can vary between specific areas on an individual's body, between different individuals [19,58] as well as being an intra-individual evolving landscape. Whilst it has been shown that once transferred on the surface this uniqueness disappears within days [2–4], this has not been proved at a metabolomics level; therefore, this heterogeneity could still be of importance to the potential profiling of fingermark in crime scene investigations, especially if the crime scene is accessed immediately. *Fingermark heterogeneity* – An early example study of intra- and inter-individual heterogeneity was shown by Fierer et al. [59] who researched the palm microbiome of 51 young adults with comparisons between dominant and nondominant hands, as analysed further by biological sex and washing. Although not focused on the fingermark, the researchers posited that, occupation, and individual habits contributed to minor asymmetries in the microbiome of the left and right side of the body. Taxa such as *Enterobacteriales, Lactobacillaceae and Peptostreptococcaceae* differentiated between dominant and nondominant hands of the same donor (intra-individual). Distinctions between different individuals (inter-individual) were exemplified by shifts in the numerical abundances of specific taxa between men (*Proprionibacterium, Corynebacterium*) and women (*Enterobacteriales, Moraxellaceae, Lactobacillaceae*). Beyond intra-and inter-individual comparisons, and with more relevance to forensic contexts, additional research assessed longevity, reporting that some of the distinguishing taxa disappear or decrease in abundance within days of the skin/fingermark microbiomes being deposited onto surfaces [3]. More recently, De Alcaraz-Fossoul et al. [2,4] focused on time-since-deposition and reported general shifts in microbial communities of latent fingerprints or fingermarks. Numerically abundant taxa were reportedly stable for longer during 21-day experiments with some clades such as *Deinococcus-Thermus* identified as time indicators, while others indicated time-since-deposition relative to unwashed (e.g. *Mycosphaerellaceae*) versus washed (e.g. *Ruminococcaceae*) hands. Table 3 presents the few empirical studies conducted between 2017 and 2026 with emphasis on the fingermark microbiome. Overall, the recorded outcomes highlight the need for well-designed research questions and comprehensive experiments to decide the minimum post-deposition sampling intervals and % taxa similarities between the donor and transferred fingermark microbiome profiles.

Table 3: Number of donors (n), sampling site, 16S region analysed, methods used, identification accuracy, and limitations for available primary research studies on the fingermark microbiome.

Also, Assenmacher et al. [60], De Alcaraz-Fossoul et al. [61] and Macneil and De Alcaraz-Fossoul [62] highlighted the importance, challenges and necessity for protocol optimisation for fingermark storage conditions, and the stage of swabbing fingerprints/fingermarks for subsequent microbial DNA extraction and community composition profiling.

4. Potential for Forensic Application

Typically, using "human skin microbiome" + "forensic" as combined keywords to mine the literature, mostly returns critical/systematic reviews and primary research articles on the human microbiome *post-mortem*. Recognition of the role of the human microbiome to determine the *post-mortem* interval has consequently led to the new terms of necrobiome [63,64], epinecrobiome [65], and thanatomicrobiome [66,67], as well as the recommendation and establishment of a Human *Post-Mortem* Microbiome Project [67]. Recognition for the potential application of the human microbiome *antemortem*, as illustrated in the Human Microbiome Project, has been captured further in critical/systematic reviews [21,68,69].

Due to individual-specific diversity, it is recognised that the human skin microbiome offers considerable potential applications in forensic identification. Also, microbial trace evidence may persist on surfaces even after DNA has degraded, offering a complementary or alternative route for identification in cases where traditional genetic material is absent or compromised [70].

Despite pronounced interpersonal variability, a core microbiome remains detectable and is largely dependent on the anatomical site [71,72]. Landmark initiatives like the Human Microbiome Project (HMP) have underscored the impact of body site on community composition [73,74]. Sebaceous regions host relatively stable, specialised

communities. Moist regions, such as the axilla, are dominated by *Corynebacterium* and *Staphylococcus* species, whereas dry areas typically harbour a more diverse and mixed populations belonging to the *β-Proteobacteria* and *Flavobacteria* taxa [73,74]. Notably, these dry regions display higher microbial diversity and significant temporal variability [75]. These findings support the potential for body-site specific microbial signatures in forensic applications, potentially allowing to infer both the donor's identity and the anatomical source of the sampled microbiome.

Although inter-site diversity has been well documented, few studies have examined intra-individual asymmetry – such as microbial variation between contralateral body sites (e.g., left versus right limbs). Fierer et al. [59] were amongst the few researchers to assess this, observing that dominant and non-dominant hands harboured significantly different bacterial communities. They also noted that skin surfaces in proximity tended to exhibit similar microbiota. These findings suggest that the dominance observed in handedness may also extend to the arms.

The potential to provide forensic biological markers was proposed and illustrated in several studies where individual microbiota was recovered from commonly touched surfaces [11,76]. Lax et al., [77] registered similar individualisation through the microbiome retrieved from bathroom doorknobs, front doorknobs, and kitchen light switches, etc., despite the donors being family members. Additionally, Flores et al., [78] and Meadow et al., [79] successfully used skin microbiomes to associate individuals with personal objects and public surfaces. However, these studies have not included the time variable (time since deposition), which is very important for the identification of microbial communities.

Inter-individual variation in skin microbiomes has also been explored for demographic classification. For example, Hospodsky et al., [80], Ying et al., [81] and Perez et al., [31] reported microbial differences at genus and phylum levels across ethnic groups. However, these findings are difficult to disentangle from behavioural and environmental confounders, such as hygiene practices or geography, which may exert greater influence than genetic ancestry. Similarly, sex-based microbial differences have shown some discriminatory potential. Tridico et al., [82] identified *Lactobacillus* as a predominant genus in females on human hair, while Phan et al., [36] reported a 77% accuracy in male

identification using *Alloiococcus* on human skin. Richardson et al. [83] further achieved 80% accuracy in female sex determination based on *Lactobacillus* abundance.

While the establishment of geographical provenance has been considered by targeting the oral cavity (e.g., Ogbanga et al., [22,23]), the selection of unique skin and fingermark microbiomes due to short-term [84] and long-term exposure to the environmental activities around an individual are yet to be investigated. Likely environment-based selection pressures include: (i) Agronomy – pesticides will select for unique skin microbial communities); (ii) Husbandry – antibiotic treatment of herds to manage diseases may enrich for antibiotic-tolerant and antibiotic-resistant strains on farmers and farm employees; (iii) Industrial activities – predominance of specific contaminant-degrading microbes; (iv) Geographical regions with endemic diseases – enrichment of skin microbes due to the medicines and ointments, used to prevent infection and/or treat symptoms may be indicative of origin of the individual.

A critical review by Edmonds-Wilson [85] captured studies of the human hand microbiome within different contexts, identifying the taxonomic relative abundances at family-level. The uniqueness of the human skin requires further research especially for areas that are already forensically important, such as fingermarks. Therefore, studies of the oral/buccal cavity as published by Lovisolo et al., [86] which will complement swabbing for human DNA analysis, could be extended to the fingertip or fingermark microbiome; the resulting capability would be critical to complement conventional physical fingerprinting.

5. Role of "Omics" in the Elucidation of Human Microbiomes

Whilst there is a real potential to harness the human skin microbiome uniqueness, there presently exist both objective and analytical challenges. For example, although based on the palms of only four donors and their respective single occupancy households, Wilkins et al., [87] conducted a 10-day study and reported changes in the skin-derived microbiomes depending on whether samples were collected in the morning or evening. Furthermore, potential similarities in the core/resident skin microbiome composition and structure of cohabiting people such as couples, families, students in shared

educational residences, and care homes for the elderly, might reduce the resolution potential to unequivocally ascribe one microbial profile to one donor over another [3,21,26]. Thus, differentiating and identifying distinct microbiomes of those co-habiting, as in cases of domestic violence, may be significantly more challenging than distinguishing microbiomes of non-cohabiting individuals.

Methodologically, there is a considerably high risk of contamination from molecular workflows while investigating the human skin microbiome and to a lesser extent, from culture-based approaches, even if using selective media. Also, it is well known that culture-dependent methods fail to capture the significant portion of skin microbes which are not culturable [88]. Therefore, research into an approach that is molecular signature-driven could highlight the viability of an alternative and culture-independent method for microbial profiling. This approach is typically delivered by "omics" science.

5.1 Human identification through genomics approaches - A substantial body of work investigating the forensic utility of skin microbiomes relies on 16S ribosomal RNA (rRNA) gene sequencing for bacterial profiling (**Figure 1**), while fungal communities are typically characterized using Internal Transcribed Spacer (ITS) or 18S rRNA sequencing [89].

In addition to these targeted approaches, shotgun metagenomic sequencing represents a broader, untargeted strategy that can be applied to skin microbiome analyses, offering the potential for more comprehensive taxonomic characterization.

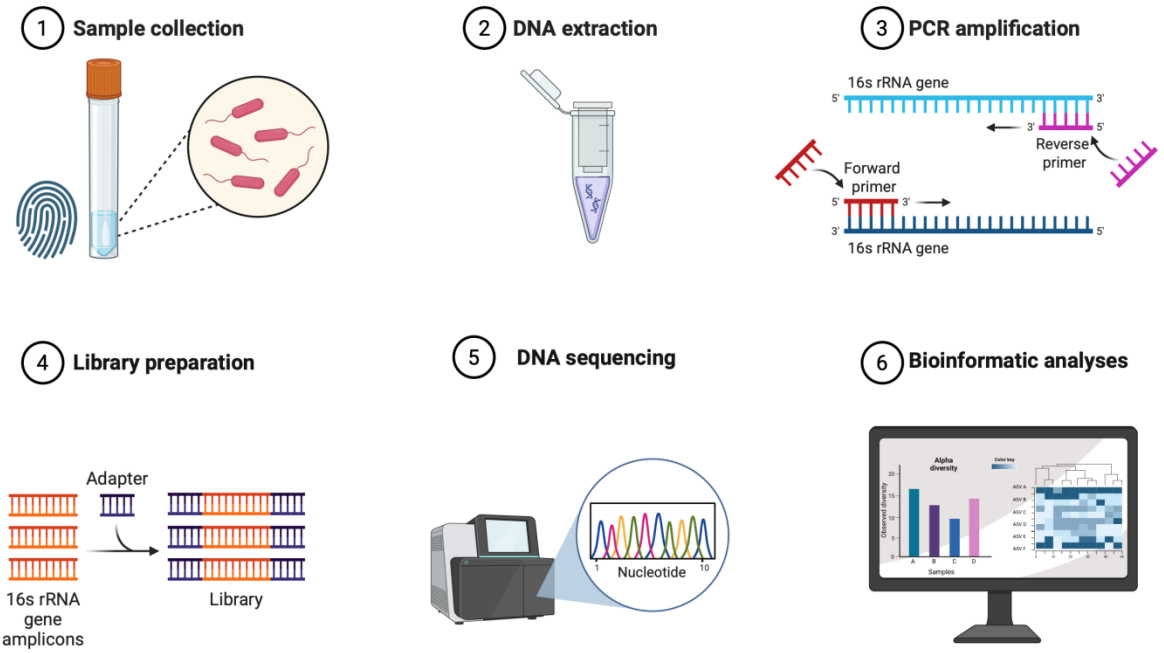


Figure 1. Metabarcoding of the 16S rRNA bacterial gene involves sampling using swabs, DNA extraction, amplification of the target gene, library preparation, NGS sequencing and bioinformatic analyses to identify taxa and relative abundances in each sample. Figure created with Biorender.com.

Their high-throughput pyrosequencing-based approach could further confirm with high confidence which individuals touched which objects, through quantitative comparison of the bacterial communities on objects and skin to link the object to the individual. Table 2 and Table 3 include examples of more recent studies showing the potential to individualize skin [36,50] and fingermark microbiomes [2], albeit with some caveats such as the taxonomic levels at which distinctions were achievable.

An early study on fingermark microbiomes concluded that there is too much variability to achieve individualisation [90]. However, this report only employed eight donors; as such, its conclusions are non-statistically significant. More recent studies have indicated the potential for 16S rRNA gene sequencing-based methodologies to identify an individual from fingermarks [91]. Next-generation sequencing (NGS) methods have shown the potential to provide significantly more data for forensic analysis and allow phylogenetic analysis of an individual's microbiome transferred during touch [92].

Despite the small sample size and a single timepoint (30 days), a pilot study demonstrated this potential through NGS analysis of fingermarks to determine specific taxa which could link an object to an individual [19]. Studies using machine-based learning of gut microbiota with random forest analysis have additionally demonstrated the potential to identify geolocation, illness and lifestyle details [7,10]. The application of computational methods to mine the dataset is also reported in Table 2, however a discussion of these methods is outside the scope of this review.

Finally, metatranscriptomics is an emerging approach that studies gene expression and is currently being explored in forensic science primarily for body fluid identification, while also holding a potential as a future tool for microbial-based individual identification [93].

5.2. Human identification through microbial Mass Spectrometry - a forensic opportunity? **-** Advances in technology including NGS, metaproteomics, lipidomics and metabolomics. Using mass spectrometry techniques such as Liquid Chromatography (LC)-MS/MS and Matrix Assisted Laser Desorption Ionisation Mass Spectrometry (MALDI-MS), have demonstrated enhanced microbial identification capabilities and impact in clinical settings, with two leading examples of implementation in hospital settings provided by the MALDI-TOF-MS based systems BioTyper® (Bruker Daltonics), and the VITEK® MS (bioMérieux), identifying bacteria through their peptides and proteins in the *m/z* range 2,000-20,000, following the work of Fenselau et al., [94]. As shown by Kostrzewa and Nagy [95], this technique overcomes the challenges of bacterial morphology assessment and the time consuming and expensive biochemical tests (which often provide genus level determination only), by providing molecular spectra signatures of the given bacterial populations present. Combinations of MALDI- and 16S rRNA gene-based techniques could afford additional detailed characterisation, functional capacity determination, quantification, and tracking of specific genera or species of core human fingermark microbiomes. Potential benefits include tracking bacterial strains, with and without known published genomes, linked directly to specific scenarios such as endemic illnesses, geographical regions, industrial activities, etc.

Indeed, MALDI-TOF-MS based systems allow the detection of bacterial signatures at a genus/species/strain level [96], and rapidly identify the microbial pathogen. Both MALDI-

MS and the traditional methods require culture of the organisms unless they are originating from high biomass samples.

Using a culture method approach, Bouslimani et al published a groundbreaking study in which both 16S rRNA gene sequencing-based methodologies and mass spectrometry techniques, including LC MS/MS and MALDI-MS, to produce a 3D molecular cartography of the skin surface through swabbing samples from 400 sites on the human body [97]; an example from their work is illustrated in **Figure 2** showing the topographical map of representative distributions of bacteria from the genera *Staphylococcus, Propionibacterium, and Corynebacterium*, for the female

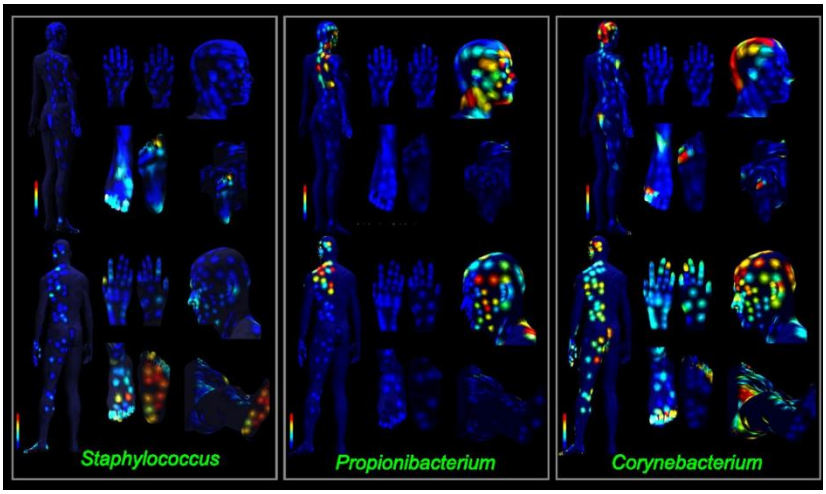


Figure 2. Topographical map of representative distributions of bacteria from the genera Staphylococcus, Propionibacterium, and Corynebacterium, for the female and male volunteers (colour scale: red is the highest percentage of each genus that was detected, and blue is the lowest percentage; other colors are in between). *With permission to publish from Bouslimani et al., Proc Natl Acad Sci USA. 2015 112(17):E2120-9.*

and male donors This study and the fact that these microorganisms are readily transferred by touch, may further support the potential for personal identification, and the provision of intelligence for (i) narrowing down the pool of suspects and (ii) reconstructing the crime dynamics in a forensic context, through fingermark microbiome detection/visualisation. However, presently, only a few studies (and no longitudinal

research) are reported exploiting the microbiome in fingermarks for human identification using mass spectrometry-based metabolomics. A signature mass spectrometry-based peer reviewed article was published by the Dorrestein group revealing how a combined Liquid Chromatography tandem mass spectrometry (LC/MSMS)-bioinformatic approach enabled intelligence on personal lifestyle via microbial signatures recovered from touched objects (e.g., mobile phones, keys etc.) by matching with those from skin swabs of palms and fingers of the donors participating in the study [98]. In essence, this exemplary paper demonstrates the mass-spectrometry based possibility to exploit the human skin microbiome in a forensic context, preceding and aligning with the findings by Procopio et al., [19] who applied genetic techniques instead. These papers are very important as, despite the advent of DNA-based methods, fingermarks remain a primary form of biometric evidence and human identification. Although liquid and gas chromatography coupled with mass spectrometry are the golden standard for many forensic applications such as drug and explosives analyses, high-throughput molecular 'omics' supported by MALDI-MS based techniques are being explored and used more in forensic science research and development [99,100]. As such they hold the potential to be harnessed in the detection and visualisation of the microbiome in fingermarks.

5.2.1 - Potential role of MALDI-MS within the recovery of microbial signatures in fingermarks - In a pioneering adaptation of the MALDI-MS imaging technology (MALDI-MS Imaging (MSI), it has been possible to reconstruct molecular images of fingermarks through the detection and mapping of biomolecules such as lipids, peptides and proteins, present on the ridges [101]. As images are generated from molecules, MALDI MSI provides both biometric information by reconstructing molecular images of the same fingermarks based on the chemicals visualised on the ridges, as well as contextual information. The technology, named molecular fingerprinting, has been recognised by the Home Office UK and Defence Science and Technology Laboratory (Dstl) in the Fingermark Visualisation Manual. It was recommended as Category C in [101] to Category B (due to its higher Technology Readiness Levels (TRL)) when the Manual was edited by the Home Office and the Dstl in 2022 [1]. The analytical power of the molecular fingerprinting technology is illustrated by the fact that it has been used in major crime investigations since 2014, with one example published by Bradshaw et al., [102].

Specific classes of molecules have been identified as potential indicators of the physiological state of an individual i.e. sex and age [103,104] or pathological state [105,106]. When mapping molecules and biological matrices of forensic interest, including drugs/medications and metabolites, biological fluids, and condom lubricants in fingermarks [107,108], a link can also be established between the biometric information (*minutiae* and ridge flow) and the circumstances of the crime or actions prior to/during a crime. Therefore MALDI-MS and MALDI-MSI can be reasonably proposed as suitable candidate techniques, not only in clinical microbiology but also in microbial forensics, to reconstruct the fingermark ridge pattern via microbial molecular signatures. This is particularly helpful in strangulation or assault cases, provided that the microflora residing within the perpetrator's mark present different biological signatures (reflecting differences in microbial populations) with respect to the victim's skin where the mark is located. Presently, visualising a mark on skin is problematic as the techniques currently used by CSI/crime laboratories would target molecular classes present in both the victim's skin and in the perpetrator's mark.

Additional to the biometric information, the type of microbial populations detected may provide invaluable intelligence on the suspect or on their activities prior to or during committing the crime, aiding in the reconstruction of the crime dynamics, as demonstrated using LC MS/MS by Bouslimani et al., [98]. However, in contrast to LC MS/MS, MALDI MS: (i) does not require extraction of the mark and hence destruction of the sample, (ii) generally requires much lower sample amounts, estimated in a study 1/300th of what would be needed for LC MS/MS analysis [97]; (iii) in terms of data acquisition, in profiling mode, is a much faster technique; (iv) preserves the integrity of the ridge detail, thus in principle providing both biometric and chemical intelligence in one analysis.

5.3. Detection of protein and peptides microbial signatures via Mass Spectrometry - As discussed earlier, a few successful metaproteomic methods have been published for the bacterial identification in clinical research. Proteins detected are generally those present on the outer microbial surface and to a minor extent, from intracellular or cytoplasmic origin [109–111]. The use of LC-MS/MS [112] with data independent

acquisition (DIA) to analyse microbial protein digests [113] has led to >70,000 gut microbiota proteins being identified in a single metaproteomic study [114]. Zhang et al have recently and comprehensively reviewed the most efficient metaproteomics approaches for microbial protein identification by LC MS/MS in clinical microbiome analysis [115]. In hospital settings, isolated microorganisms are cultured and strains/colonies are directly smeared on a target plate for intact protein analysis by MALDI-MS, allowing pathogen ID in minutes and thus permitting rapid interventions. Whilst encouraging, major challenges need to be overcome; low-abundance proteins, dynamic range, and sample heterogeneity may lead to low ion yield (low sensitivity). Bacterial cell walls exacerbate these issues. For Gram-positive bacteria in human gut microbiome, it has been reported that mechanical disruption, combined with a strong lysis buffer, is essential for breaking the cell wall and releasing proteins thus increasing sensitivity [116]. However, a bigger challenge is anticipated when attempting to detect and visualise microbial protein signatures in fingermarks by MALDI-MS/MALDI MS/MS and MALDI MS Imaging (MALDI MSI). The protein extraction methods mentioned cannot be employed as the integrity of the evidence must be preserved to provide the biometric information. Therefore, alternative protocols must be developed to circumvent the issue of the low protein abundance and hard-to-disrupt bacterial cell walls.

5.4. Detection of lipid and metabolites microbial signatures via Mass Spectrometry - There is considerable literature reporting on the detection and visualisation of bacterial lipids and metabolites through MALDI MS and MALDI MSI (**Figure 3**) [117] , with instances of Secondary Ion Mass Spectrometry Imaging (SIMS MSI) [118] and ambient mass spectrometry such as nano-Desorption Electrospray Ionisation (DESI MSI) [119,120], rapid evaporative ionisation-mass spectrometry (REIMS) [121] and the MasSpec pen [122]. However, majority of the publications report such analyses on microbial cultures [123–128] or biofilms [129–131]. Amongst these, Nartey et al., published a culture-free lipidomics-based method involving, amongst other, the use of

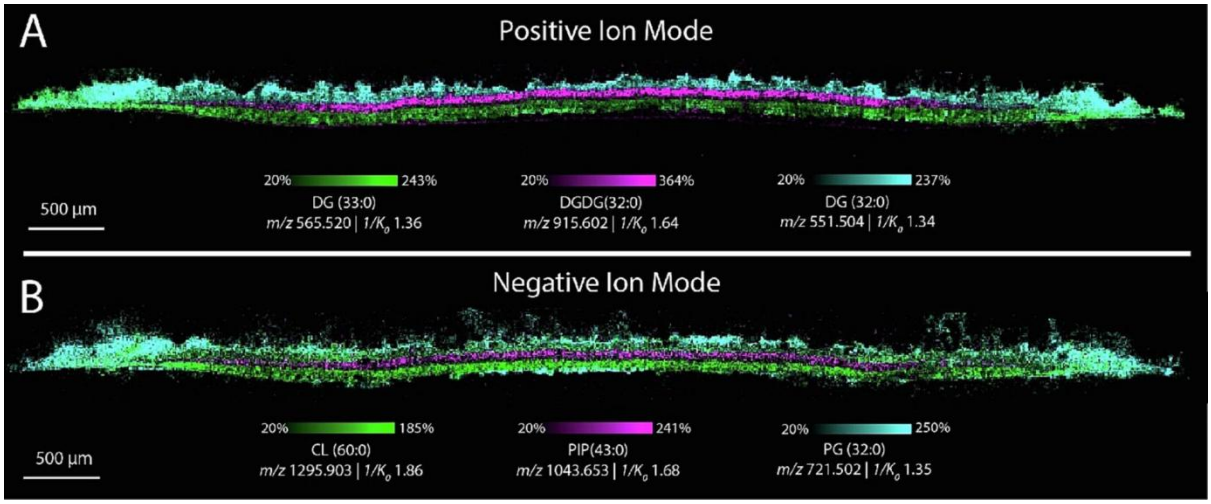


Figure 3. MALDI MSI of *S. aureus* biofilm cross-sections. Lipid distribution maps were obtained in both positive (A) and negative ion mode (B) showing Diacylglycerol (DG, 32:0) and Diacylglycerol (33:0) (A) and Cardiolipin (CL), Phosphatidylinositol-phosphate (PIP), and Phosphatidylglycerol (PG) visualised in different layers of the biofilm (B). *Reprinted from Journal of Mass Spectrometry and Advances in the Clinical Lab 2019, 26, 36–46, Copyright (2022), with permission from Elsevier*.

lysozyme to break the cell wall. This addition enabled the detection of Gram-negative bacteria in high sensitivity and, with lower sensitivity, Gram-positive bacteria too [132]. In another recent study, a metabolomic workflow, encompassing the use of REIMS and DESI, found 90% of 359 taxon specific markers from 233 bacterial species directly in clinical samples, namely gastrointestinal tissues and faecal samples imaging some of these markers too [133]. However, there is, to date, no report on detection, identification or visualisation of bacterial species in fingermarks. Lauzon et al., [134] detected a number of potentially microbial lipids from the valleys of the latent fingermarks investigated using Ag-assisted Laser Desorption Ionisation Mass Spectrometry Imaging (Ag-LDI MSI); they suggested that the valleys are less prone to friction compared to ridges and this may create a better environment for bacterial proliferation. These considerations would support the use of desorption techniques such as LDI and MALDI to detect (and image) microbial signatures in fingermarks.

6. CONCLUSIONS

Challenges, opportunities and recommendations- Our mini-review has highlighted knowledge and technological gaps hindering comprehensive fingermark microbiome detection and mapping, which could provide a valuable complementary layer of crime scene analysis.

Microbiome-related gaps/limitations - Knowledge gaps partly reflect the dynamic nature of skin and fingermark microbiomes; human microbial release rates have been estimated at between 1 x 10^6 [135,136] and 3.7 x 10^7 microbes per hour [137] using bioaerosol sampling in controlled chambers, fluorescent biological aerosol particle monitoring [135,136] and 16S rt-PCR standard curves to quantify total genome numbers [137]. Microbiomes also vary between individuals [10,138,139] but reliable identification requires high-resolution taxonomic data. Phylum-level discrimination is limited, with greater accuracy achievable at genus, species, or strain level [73,138,140]. Thus, despite its promise, genomic approaches face technical limitations and interpretative uncertainty. Larger donor cohorts are therefore needed to assess the feasibility of human identification through skin microbiomes and microbial profiling of fingermarks to provide contextual, case, crime scene, and suspect intelligence.

A further limitation of microbial forensic research is its reliance on heterogeneous populations. Although microbial distinctiveness has been demonstrated among demographically diverse individuals [31,80], te persistence of this individuality within a demographically homogenous group remains unclear; this is critical for forensic applications, where the discriminatory power of microbial profiling must remain robust across varied or demographically matched populations.

Contamination-related limitations: contamination in research (for example from researchers themselves) can compromise culture-based and molecular techniques, making aseptic sampling, whenever practicable, and appropriate controls, essential. Ideally, precautions include designated laboratories, where feasible, full PPE, ethylene oxide-sterilised swabs, microbiome- and human-DNA-free storage vials, and

environmental swabs as context controls for both human and microbial environmental DNA (eDNA). Additional good practice includes analysing collectors' PPE swabs and using blank swabs and storage vials as negative controls. Although these measures are feasible in laboratories, instrumentation such as mass spectrometers cannot currently be fully decontaminated. In addition, aseptic conditions cannot be maintained at crime scenes. Accordingly, collecting negative samples from relevant surfaces or crime-scene areas is essential to characterise background "contamination".

In the long-term, following method validation, fingermark microbiome experts will need to train analysts and crime scene officers in sample collection, analysis and storage protocols. Experts and investigators will also require training to present complex microbiome data, such that they are accessible by members of the general public and the judicial system.

Essential knowledge requirements- Generally, the factors shaping a unique and forensically acceptable microbial signature remain poorly understood. As also highlighted in recent perspectives [141], we posit that, forensic applicability requires microbiomes to be sufficiently consistent, predictive or deterministic rather than stochastic, over time and between individuals.

Research should establish the persistence of core versus transient microbiomes through larger, more diverse donor cohorts (e.g., biological sex, age, racial background, and cultural practices) and extended longitudinal sampling (e.g., 2–6 consecutive months) [51]. As with touch DNA, it is anticipated that human skin microbiome deposits and longevity are surface-dependent, including on inanimate surfaces, as demonstrated for mobile phones [91] and biological surfaces, such as skin-to-skin contact. Studies should therefore investigate surface chemistry and physical properties, alongside sampling time. For example, although based on the palms of only four donors and their respective single occupancy households, Wilkins et al. [142] conducted a 10-day study and reported morning–evening differences in skin microbiomes.

In alignment with Sreekutti et al., [143], we propose combining advanced complementary microbial (e.g., metagenomics-based) and molecular visualisation technologies (e.g., mass spectrometry-based) to investigate microbiome consistency,

longevity, viability, and detectability on household items (e.g., knives and hammers) and against recipient skin microbiomes. This has the potential to increase the opportunity and feasibility to realise the untapped potential of consistently visualise the human skin microbiome, particularly for cases of domestic violence, enabling microbial characterisation of fingermarks to identify and evidence physical (finger-to-skin) contact—an historically unresolved challenge—and provide contextual intelligence for profiling persons of interest.

Potential role and challenges of omics-techniques - Disrupting bacterial cell walls in intact fingermarks remains challenging for recovering microbial lipids/metabolites/proteins. Although mass spectrometry has been widely applied to both fingermark chemistry and microbial identification separately, their integration for fingermark microbial profiling remains underexplored. For MALDI-MS-based analyses, matrix systems should be developed to preserve the fingermark evidence, especially for imaging purposes; these could potentially incorporate low percentages of chloroform and DMSO to disrupt the phospholipids-based cell walls. This high-risk high-reward research could support homicides and domestic violence investigations, where fingermarks on skin are currently unrecoverable.

However, skin-to-skin transfer complicates forensic interpretation, particularly among cohabiting individuals, including non-genetically related couples and genetically related families [3,77]. Nonetheless, Lax et al 2014 [77] identified household members' contributions to microbial "home surface sink" including bedroom and kitchen floors, door handles, kitchen counters, and light switch. In terms of transfer and longevity, Neckovic et al. [89] reported that microbial transfer can occur through both direct and indirect contact, with microorganisms recoverable from mobile phones and clothing over extended periods. Similarly, Fierer et al. [11] had formerly demonstrated that microbial signatures can persist on computer keys and computer mice for up to two weeks at room temperature.

However, persistence alone does not establish forensic specificity or probative value, particularly where environmental contamination and microbial convergence remain insufficiently controlled [89,144]. This presents a research opportunity to longitudinally compare donor-specific STRs ("touch DNA") and microbial fingerprints ("touch

microbiome") from the same samples, as demonstrated by Procopio et al., [19]. Indeed, the consistent STR profile of a given donor could be compared longitudinally to their microbiome profile for assessing microbiome variation associated with physiological and psychological factors, including acute illnesses, emotional status, anxiety.

As observed by Schmedes, Woerner and Budowle [14], the degree of, and potential for microbiome overlap between site-specific communities and consistent colonisation patterns across individuals, mandate robust testing. Future empirical investigation should also assess the impact of lateral asymmetry on microbial load and diversity, i.e., can fingertip related/fingermark microbial profiles infer the handedness of a suspected perpetrator? to what extent will microbial sub-populations unique to the fingermarks of the same individual complicate identification?

As in other ecosystems studied in forensic science, the inherent bio- and physico-chemical characteristics of fingermarks (pH, salinity, moisture and lipid content, resilience against environmental degradation, whether members are mostly free-living or within micro-biofilms), as well as the presence of environmental contaminants or degraders (UV, bactericides, grazers, competitors), will determine how long their integrity is maintained after deposition [145].

Future admissibility in Court- Moving microbiome analyses from the laboratory into the courtroom will ultimately depend on their ability to satisfy evidentiary gatekeeping standards - the *Daubert* standard in most U.S. courts (and the older *Frye* "general acceptance" test in a minority of jurisdictions), and in England and Wales the reliability criteria of Criminal Practice Direction 7 and the expert-evidence duties of Criminal Procedure Rules Part 19 [145,146]. Common to these regimes is a demand for empirical testing, peer review, known error rates, maintained standards and controls, and general acceptance within the field. Measured against them, forensic microbiomics remains uneven: the sequencing chemistries and bioinformatic pipelines are mature, but casework-relevant applications such as individual attribution, geolocation, trace transfer, and post-mortem interval estimation still lack the inter-laboratory validation, standardised protocols, reference databases, and, in some instances, quantified error rates that admissibility requires [147]. Despite existing recommendations for the introduction and adoption of guidelines for microbiome-based analyses in specific

contexts, such as post-mortem microbiology [148], these frameworks have yet to translate into the cross-laboratory reproducibility that courtroom use demands.

The contrast with STR profiling is instructive, as STR evidence rests on a mature probabilistic architecture (validated allele-frequency databases and random-match-probability or likelihood-ratio frameworks that express the strength of a match in transparent, reproducible terms), whereas microbial classifiers are typically reported as predictive accuracy (commonly 85–95%) rather than calibrated likelihood ratios, with no consensus reference database to weight a "match." Because the human STR genotype is stable and lifelong while the microbiome is dynamic and context-dependent, microbial signatures cannot presently be treated as the probative equivalent of an STR profile. For now, microbiome evidence is therefore best positioned as corroborative or investigative intelligence rather than stand-alone identification, and expert testimony should carefully distinguish analytically validated findings from investigational inference.

Declaration of generative AI and AI-assisted technologies in the manuscript preparation process - During the submission of this paper, the corresponding author, Simona Francese, used ChatGTP to generate graphical abstract for purely concept illustration purpose, employing the following instruction: "generate an image of microbial populations in a fingerprint". The corresponding author reviewed and edited the output as needed and take full responsibility for the content of the published article.

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