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Microbial genomic scars: a novel paradigm for reconstructing events and behaviors in forensic investigations

Shujie Dou[1,2], Guanju Ma[1], Shujin Li[1]

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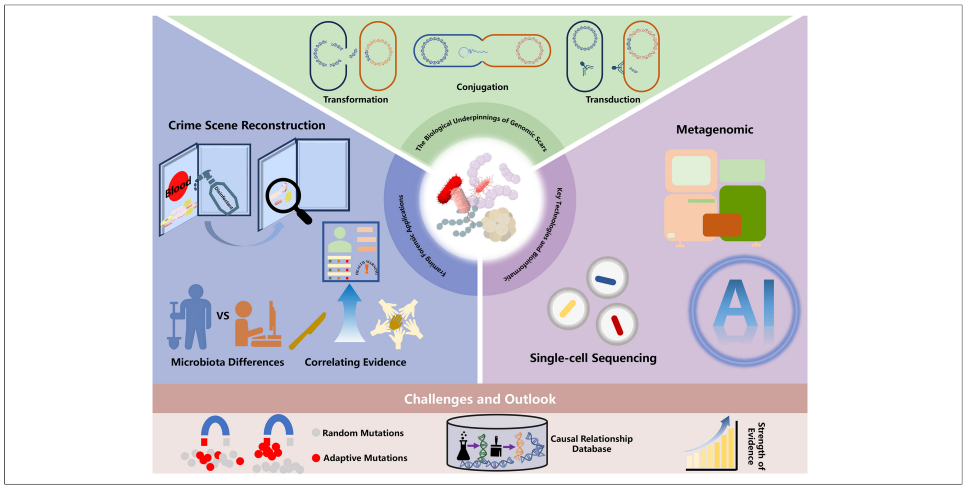

Abstract

Traditional forensic microbiology treats microbial communities as static "taxonomic labels", relying on species composition for associative analysis. However, this paradigm struggles to reconstruct the dynamic events underlying criminal activities. This review proposes a new paradigm: recasting microbes as dynamic "environmental sensors" and "event recorders." Under stressors such as disinfectants or antibiotics, microbes heritably alter their genomes via horizontal gene transfer, phage induction, and adaptive mutations, leaving specific "genomic scars". These scars can potentially document critical events, such as crime scene sanitization or specific occupational exposures. Decoding these scars through ultra-deep metagenomics, single-cell genomics, and artificial intelligence (AI) offers a new avenue for reconstructing behaviors, individual profiling, and evidence linkage. The article systematically elaborates the biological basis, forensic applications, and technical challenges of this paradigm, propelling microbial evidence from associative to behavioral inference.

[1]Hebei Key Laboratory of Forensic Medicine, Hebei Collaborative Innovation Center of Forensic Medical Molecular Identification, College of Forensic Medicine, Hebei Medical University, Shijiazhuang 050017, Hebei, China.
[2]Postdoctoral Research Station in Biology, Hebei Medical University, Shijiazhuang 050017, Hebei, China.

Correspondence to: Prof. Shujin Li, Hebei Key Laboratory of Forensic Medicine, Hebei Collaborative Innovation Center of Forensic Medical Molecular Identification, College of Forensic Medicine, Hebei Medical University, Shijiazhuang 050017, Hebei, China. E-mail: shujinli@hebmu.edu.cn

INTRODUCTION

Over the past decade, forensic microbiology has evolved from a nascent concept into a powerful tool for connecting crime scenes, suspects, and victims[1,2]. Enabled by high-throughput sequencing, researchers can now delineate the microbial "fingerprints" on physical evidence or individuals with unprecedented resolution. By comparing the taxonomic composition and community structure between samples, significant strides have been made in human identification, geographic sourcing, and post-mortem interval estimation[2-8]. The central tenet of this research has been to treat microbial communities primarily as stable, static taxonomic labels. This paradigm presumes that a microbial census, captured at a specific moment, faithfully reflects its origin and thus serves as the core basis for associative evidence.

However, this foundational view of microbes as static taxonomic identifiers fails to fully harness their informational potential. This conventional approach has inherent limitations in reconstructing the specifics of an event. First, while it acknowledges the dynamic responsiveness of organisms to environmental change[9-11], its focus on community-level shifts often provides limited insight into the causal pressures driving these changes. For instance, analyses of taxonomic succession are valuable for estimating the post-mortem interval, but they may not distinguish between different types of stressors that could lead to similar community structures. Consequently, a static species list provides an incomplete record of critical events, such as scene sanitization or body disposal, which impose intense selective pressures sufficient to reshape the microbial genomic landscape within hours to days[12-14]. Second, taxonomic-level comparisons can be confounded by ecological convergence, leading to ambiguity and diminishing the specificity and strength of the evidence[15,16]. Therefore, while this approach is effective at addressing "who was here?", it is often less equipped to resolve the more granular forensic question: "what happened here?".

Here, we propose a paradigm shift to transcend these limitations: recasting microbes from passive "taxonomic labels" to active "dynamic environmental sensors" and "event recorders." The core idea of this paradigm is that the microbial genome is not immutable but is a molecular ledger that responds to environmental stress in real time, leaving heritable alterations[17]. When confronted with specific stimuli - such as the chemical assault of a disinfectant, the selective pressure of antibiotics, or contamination with heavy metals - microbes undergo adaptive evolution at the genomic level through mechanisms such as horizontal gene transfer (HGT), phage activity, or the rapid accumulation of point mutations[18-24]. These genomic alterations, which we term "genomic scars", are driven by environmental pressures and fixed by natural selection. In principle, these scars can document the specific events a microbial community has endured, offering a dimension of information far richer and more profound than a simple species roster.

In this Review, we will systematically elucidate this emerging field. We first delve into the key biological mechanisms that forge these "genomic scars", including the roles of mobile genetic elements (MGEs), phage dynamics, and the accumulation of micro-scale genomic variations. Building on this foundation, we will construct a framework of potential forensic applications, demonstrating how decoding these scars can be used to reconstruct criminal actions, infer an individual's lifestyle and provenance, and forge novel evidentiary links. Furthermore, we will highlight the key analytical technologies required to achieve these goals and, finally, discuss the challenges and future directions for the field. We contend that this paradigm shift - from taxonomic labels to event recorders - will open a new dimension for forensic science, marking a pivotal step for microbial evidence to evolve from being purely associative to becoming truly behavioral.

THE BIOLOGICAL UNDERPINNINGS OF GENOMIC SCARS

The concept of microbes as dynamic event recorders is scientifically grounded in a suite of rapid genomic adaptation mechanisms that microorganisms have evolved to survive drastic environmental shifts[25-27]. These mechanisms enable microbial populations to transduce external physical or chemical stimuli into stable,

heritable genomic alterations - what we define as "genomic scars" - on timescales ranging from hours to a few generations. However, the persistence of these scars is not indefinite, as their maintenance can impose a "fitness cost" on the organism once the stressor is removed. Therefore, understanding this temporal dynamic, or the "half-life" of a specific scar, is essential for accurately reconstructing the timeline of a forensic event and inferring how recently it occurred. These scars are not merely a testament to microbial survival strategies; they provide a layer of high-resolution information crucial for forensic reconstruction.

HGT

HGT serves as a primary conduit for genetic information exchange within the microbial world, enabling the rapid dissemination of genetic material - particularly adaptive functional genes - across species[28,29]. This process is primarily mediated by MGEs, such as plasmids, transposons, and integrons[28-30]. When a microbial community confronts an acute and intense selective pressure - such as the disinfection of a crime scene with biocides [e.g., quaternary ammonium compounds (QACs), bleach], or chronic exposure to specific antibiotics (e.g., in healthcare workers) or heavy metals (e.g., in industrial workers) - MGEs harboring the corresponding resistance genes become critical determinants of survival[30,31]. The few cells that possess these MGEs are thereby selected for, enabling them to survive and proliferate rapidly, which in turn leads to a dramatic increase in the frequency of these resistance genes throughout the entire community[30]. Therefore, the significant enrichment of plasmids encoding specific disinfectant efflux pumps or antibiotic-degrading enzymes, as identified through metagenomic analysis of physical evidence, could provide strong indicators of prior treatment with corresponding chemical agents, potentially enabling the reconstruction of critical activities such as scene cleanup[32-34].

Phage dynamics and the CRISPR-Cas system

Phages, as ubiquitous viruses, are engaged in a perpetual co-evolutionary arms race with their bacterial hosts[35]. This dynamic interplay offers a unique lens for documenting environmental perturbations. Prophages are commonly integrated into bacterial genomes in a dormant, or lysogenic, state. However, a diverse array of internal and external stimuli, such as DNA damage, oxidative stress, nutrient availability, host immune responses, quorum sensing, diet, secondary metabolites, antibiotics, and lifestyle changes, can trigger prophage induction and prompt their switch into the lytic cycle[36]. Consequently, a burst of free virions in the environment or the detection of a high frequency of phage excision sites (att sites) within bacterial genomes can serve as potential indicators of a preceding, specific environmental stimulus. Furthermore, the bacterial clustered regularly interspaced short palindromic repeats and CRISPR-associated proteins (CRISPR-Cas) adaptive immune system provides an even more sophisticated mechanism, acting as a "molecular recorder". It chronologically captures and archives DNA fragments (known as spacers) from invading phages or plasmids. Upon each new invasion event, a novel spacer sequence is integrated at the leader end of the CRISPR array[37,38]. This implies that the sequential arrangement of spacers within the array constitutes a "molecular fossil record" of the invasion history experienced by that cell lineage.

Adaptive point mutations and indels

Beyond the acquisition or loss of gene cassettes, the adaptive evolution of microbial genomes also manifests at a finer scale: through the rapid accumulation and positive selection of single nucleotide polymorphisms (SNPs) and short insertions/deletions (indels)[39,40]. Under sustained, sub-lethal selective pressures - for instance, long-term exposure to sub-minimum inhibitory concentrations (sub-MIC) of antibiotics or industrial pollutants - microbial populations experience intense directional selection[41]. In this process, stochastic mutations that confer even minor fitness advantages, such as SNPs that reduce drug-target affinity or indels that alter regulatory elements to modulate gene expression, are rapidly selected for, leading to a significant increase in their allele frequencies within the population[42]. This phenomenon of population-level genetic convergence at specific loci constitutes an "evolutionary snapshot" that chronicles the population's

response to a particular chronic stress[43]. In a forensic context, the detection of an identical, non-random profile of adaptive SNPs and indels within the same dominant bacterial strain across different evidence samples provides strong evidence that these samples share a common origin, reflecting their exposure to the same unique selective pressure[44,45]. This provides a novel dimension of evidence for linkage, independent of species composition and HGT.

FRAMING FORENSIC APPLICATIONS

A paradigm shift that views microbial genomes as dynamic information carriers has opened up broad prospects for forensic applications, ranging from behavioral reconstruction to physical evidence association. These deeply embedded "genomic scars", serving as direct responses to specific environmental stresses and events, can provide higher-dimensional and more targeted evidence compared to traditional species composition analysis. Building upon the robust foundations of existing research in forensic genetics, microbial ecology, evolutionary biology, and clinical microbiology, this chapter systematically elaborates on how to translate these "genomic scars" into actionable forensic practices, constructing three core application scenarios.

Reconstructing crime scene actions

Post-crime scene contamination, particularly through the application of chemical agents for cleanup, represents a common strategy to obstruct forensic investigations. Conventional physicochemical analytical techniques exhibit limited efficacy in detecting such deliberate alterations. In contrast, microbial genomics offers a novel paradigm for forensic breakthrough. The deployment of potent disinfectants imposes a severe chemical bottleneck on indigenous microbial communities, wherein the genomic signatures of surviving microorganisms can bear distinctive imprints of this anthropogenic disturbance. Numerous environmental microbiology studies have confirmed that exposure to disinfectants such as QACs, chlorine-based agents, and phenolics triggers rapid selection and enrichment of tolerant microbial populations within hours to days[46-49]. For example, a global phenomenon observed during the COVID-19 pandemic involved the discharge of excessive amounts of chlorine-based disinfectants (e.g., sodium hypochlorite) into sewer systems. Research revealed that although the microbial community structure gradually recovered after disinfection ceased, the composition of antibiotic resistance genes (ARGs) underwent persistent and irreversible changes. The core mechanism lies in chlorine stress, which strongly selected for and enriched chlorine-tolerant bacteria carrying specific biocide resistance genes (BRGs), such as the *chtR* subtype. These surviving bacteria then efficiently facilitated the co-selection and stabilization of biocide and antibiotic resistance via plasmids and integrative and conjugative elements[47]. A separate study on QACs revealed a similar pattern. It found that QAC resistance genes prevalent in environmental samples were significantly correlated with multiple ARGs. At environmental concentrations, QACs enhanced bacterial resistance to multiple antibiotics and significantly promoted the conjugation transfer of the RP4 plasmid. This promotion, reaching up to approximately 15-fold, is achieved by increasing bacterial membrane permeability and stimulating reactive oxygen species production[46]. Beyond the direct effects of disinfectants, their unintended by-products also play a critical role. Disinfection by-products (DBPs), such as trichloromethane (TCM) and dichloroacetonitrile (DCAN), are commonly detected in various water environments. A recent study demonstrated that exposure to low concentrations of TCM (25 μg/L) and DCAN (10 μg/L) significantly stimulated the conjugative transfer of the RP4 plasmid in *Escherichia coli*, resulting in maximum transfer fold changes of approximately 5.5 and 6.0, respectively. Mechanistic investigations revealed that DBPs promote ARG dissemination through intracellular reactive oxygen species generation, SOS response activation, increased membrane permeability, and upregulation of genes and proteins related to pilus generation, adenosine triphosphate (ATP) synthesis, and plasmid transfer[50]. Together, these cases demonstrate that the application of disinfectants such as chlorine-based agents or QACs at a scene constitutes far more than a simple "clean-up". Instead, it acts as a powerful anthropogenic intervention into the indigenous microbial

community, triggering a cascade of stress-selection-enrichment-transfer events that leave a traceable genomic signature. This provides a novel and powerful avenue for forensic microbial traceability.

This biological phenomenon provides a novel molecular basis for the forensic reconstruction of cleaning activities. By performing differential sampling of suspected cleaning areas (e.g., wiped floors) and adjacent undisturbed control areas (e.g., corners or beneath heavy furniture) at a crime scene, followed by deep metagenomic sequencing, researchers can conduct a quantitative comparative analysis of the resistome. If samples from a suspected cleaning site not only reveal the co-localization of *qac* genes with ARGs (e.g., *dfrA*/*sul1*) on a class 1 integron, forming a co-selected genetic cassette, and a marked increase in their overall abundance, but also genomic analysis confirms their location on plasmids prone to HGT, this would constitute a significant molecular signature suggesting a "cleaning event"[51]. Such evidence could offer a higher degree of specificity than taxonomic shifts alone, which can be confounded by a variety of non-specific factors. More importantly, the precision of this approach has the potential to discriminate between the chemical classes of the cleaning agents used. For instance, the enrichment of *qac* genes could serve as a potential indicator for the use of QAC disinfectants, while an abnormal proliferation of certain BRGs might be associated with exposure to chlorine-based cleaning agents. It is crucial, however, to interpret such signatures with caution, as they could also arise from background environmental contamination or prior selective pressures rather than a single, specific event. This molecular profile, when corroborated by the analysis of cleaning products seized from a suspect, could theoretically form a powerful evidence chain from the act to the physical object, aiming to provide objective and precise evidence for courtroom reconstruction.

It is crucial to acknowledge that natural abiotic stressors, such as extreme temperatures or ultraviolet (UV) radiation common at many crime scenes, can also induce specific genomic adaptations in microbes. Therefore, a key future challenge will be to differentiate these naturally induced signatures from those arising from anthropogenic activities to avoid confounding forensic interpretations.

Inferring individual occupation and lifestyle

An individual's distinctive profession, lifestyle, and geographical trajectory continually sculpt the genomes of their commensal microbiota. These adaptive genomic features collectively constitute a dynamically updated "microbial passport", which holds significant promise as a powerful supplementary tool for forensic inference of personal background.

Occupational exposure distinctly shapes an individual's antibiotic resistome. Recent studies demonstrate that intensive care unit (ICU) healthcare workers harbor a gut microbiota with a significantly higher abundance and diversity of ARGs compared to the general population[52]. Furthermore, the hand microbiota of nursing staff is characterized not only by an enrichment of multi-drug resistance ARGs but also by a greater load of potential pathogens, underscoring the profound impact of hospital environmental exposure[53]. The detection of an exceptionally complex resistome from a suspect's skin swab metagenome, after excluding recent infection or hospitalization, could be indicative of a healthcare or related occupational background. Occupational exposure has been empirically demonstrated to shape the individual microbiome across various industries. In slaughterhouse settings, nasal carriage of Methicillin-Resistant *Staphylococcus aureus* (MRSA) among workers (3.2%) showed significant association with specific work zones, with the highest risk observed in the lairage and scalding/dehairing areas. Notably, 73% of the isolates were identified as livestock-associated ST398 lineage (LA-MRSA). The decreasing gradient of MRSA concentration in environmental samples along the slaughter line further corroborates the dynamic interplay between occupational exposure and microbial colonization[54]. Additionally, metagenomic analyses revealed distinct oral and gut microbial structures between occupational groups (e.g., students *vs.* manual laborers). These differences remained stable after controlling for confounders such as sex, smoking, and alcohol consumption, and in this specific

study, machine learning models were reported to achieve 100% classification accuracy on the tested cohorts, indicating that specific microbial taxa and functional pathways (e.g., genes related to the "Phagosome" pathway) hold promise as biomarkers for occupational inference[55].

The integration of the CRISPR-Cas system with phage biogeography holds promise for providing high-resolution spatiotemporal markers for both individual geographic origin tracing and behavioral trajectory inference[6,56]. CRISPR typing serves as a complementary tool for genomic source tracking and has been successfully applied in foodborne disease outbreak investigations. For instance, comparing the CRISPR arrays of *Salmonella* isolates can effectively distinguish outbreak-related strains from unrelated ones, thereby aiding in determining the infection source[57]. This principle can be extended to forensic science, offering a novel conceptual framework for determining an individual's geographic origin and reconstructing their activity trajectories. Therefore, if shared, recently acquired spacer sequences that are rare in public databases are identified in the same strain isolated from crime scene evidence and a suspect, this provides strong evidence for their recent co-exposure to the same micro-environment containing a specific phage, thereby supporting the establishment of a spatiotemporal association between the suspect and the crime scene. By comparing the "new" and "old" spacers in the CRISPR array, the temporal sequence of the divergence between the two samples can even be inferred, providing key information for reconstructing the event timeline.

Linking physical evidence

The rapid transfer of microorganisms makes them a useful tool for linking physical evidence. A 2014 study revealed that microbial samples from different surfaces within the same household exhibited significantly greater similarity to each other than to samples from the same type of surface across different households[58]. Subsequent research further demonstrated that even brief contact during a conference allows location-specific environmental microbial communities to influence the microbial assemblages associated with the attendees[59]. An individual not only exchanges microorganisms with the environment but also deposits a unique microbial "fingerprint" on contacted items[60]. This transfer dynamic facilitates the establishment of an evidential network interconnecting people, objects, and locations, thereby providing a scientific basis for directly linking a suspect to a specific crime scene or key piece of evidence. Empirical support for this concept is provided by multiple studies: microbial communities recovered from phone surfaces can specifically discriminate between contact with hands and faces, while those from shoe soles exhibit significant geographic variation. However, these investigations remain confined to the taxonomic level, which imposes inherent limitations on their resolution and definitiveness[59,61]. Achieving precise forensic associations necessitates moving beyond taxonomic composition to the resolution of microbial strains and genes. A seminal study on the skin microbiome highlighted the potential superiority of genetic-level analysis for human identification. The research revealed that while identification based solely on species-level profiles achieved a mere 52.5% accuracy, constructing a panel of SNPs from *Cutibacterium acnes* and applying a machine learning model dramatically increased the accuracy to 97.5%[62]. Further validating this approach, researchers developed the "hidSkinPlex+" panel, which comprises 365 highly discriminatory and reliably detectable SNP loci curated from hand, sternum, and foot samples. In a blind test of 225 samples, this method achieved 96% identification accuracy [Matthews correlation coefficient (MCC) = 0.954]. These findings collectively indicate that SNP-based analysis of bacterial genomes effectively overcomes the instability inherent in community-level profiling, pointing towards a potentially reliable and highly accurate pathway for forensic identification from microbial traces[63].

KEY TECHNOLOGIES AND BIOINFORMATICS

The paradigm shift of redefining microorganisms from mere "taxonomic labels" to "event recorders" imposes exceptionally high demands on analytical technologies. Traditional approaches such as 16S rRNA gene sequencing or shallow metagenomics are no longer sufficient. It is imperative to rely on a progressive

technological framework comprising deep metagenomics, microbial single-cell genomics, and artificial intelligence (AI)-driven integrative analysis to transform microscopic "genomic scars" into forensic evidence characterized by high resolution, specificity, and interpretability.

Ultra-deep metagenomics overcomes the technical limitations of traditional metagenomics in detecting low-abundance species (relative abundance < 0.1%), resolving genomes at the strain level, and reconstructing complex microbial community structures by implementing ultra-deep sequencing (typically exceeding 50 Gb per sample) and integrating hybrid assembly strategies that combine long-read and short-read sequencing technologies[64,65]. It not only enables the assembly of a large number of high-quality, nearly complete metagenome-assembled genomes (MAGs), but also systematically uncovers extrachromosomal MGEs (such as plasmids and bacteriophages), thereby providing a foundational framework for understanding the species composition, functional potential, and ecological interactions of microbial communities. Building upon this foundation, microbial single-cell genomics elevates the resolution to the level of individual cells. It not only reveals intercellular heterogeneity in chromosomal genomes but also directly links specific MGEs to their host cells[66]. For example, it can precisely identify which specific strain or single cell harbors an antibiotic resistance plasmid, thereby accurately defining the carrier of functional genes. This is crucial for tracing the transmission pathways of functional genes within complex communities and identifying rare cells with key phenotypes (such as antibiotic resistance or unique metabolic capabilities). It provides direct evidence for enabling a shift from answering "who is there?" to "who is doing what?" However, the application of single-cell genomics to forensic samples, which are often characterized by low biomass and DNA degradation, poses significant challenges for isolating intact single cells. To overcome this limitation, proximity-ligation-based methods, such as meta-Hi-C (metagenomic chromosome conformation capture), offer a powerful complementary or alternative approach. This technology captures the physical proximity of DNA molecules within a cell, enabling the direct association of plasmids and phages with their host chromosomes even in complex samples where single-cell isolation is not feasible[67,68]. Integrating such techniques would significantly enhance the reliability of linking event-specific genomic scars to the responsible microbial actors.

Ultra-deep metagenomics and single-cell genomics generate vast, multidimensional, and highly complex datasets. These data encompass not only multi-layered information such as species, genes, and functional pathways but also structural variations in chromosomes and MGEs, single-cell heterogeneity, and spatiotemporal dynamics. Traditional bioinformatics approaches face significant challenges in processing such high-dimensional, nonlinear, and noise-laden association networks, making it difficult to distinguish biologically meaningful and forensically valuable causal signals from complex correlations[69]. AI technologies, particularly machine learning and deep learning, provide revolutionary tools for addressing the complexity of microbiome data. By constructing unified analytical frameworks that integrate multi-omics data, AI can automatically identify key microbial biomarkers, gene modules, or combinations of MGEs most relevant to specific forensic phenotypes - such as geographic origin, individual characteristics, and more - from vast microbial genomic datasets, thereby achieving dimensionality reduction and feature extraction[55,70-72]. Furthermore, AI constructs potential interaction and transmission networks among microorganisms, between microorganisms and host genes, and of MGEs within communities. This approach moves beyond simple species abundance correlations to reveal the underlying ecological and evolutionary dynamics driving changes in microbial community structure. Ultimately, by applying advanced analytical frameworks such as causal discovery algorithms and structural equation modeling, AI provides critical support for reconstructing the temporal sequence and logical chain of events in forensic science[73,74].

CHALLENGES AND OUTLOOK

The paradigm of "microbial genomic scars" offers a potential pathway for forensic science to transition from identity association to behavioral reconstruction. Furthermore, a holistic approach that integrates the analysis of genomic scars with traditional community-level taxonomic shifts is crucial for building a robust interpretive framework. Genomic adaptations (the "cause") and subsequent changes in community composition (the "effect") are two sides of the same coin in a microbial ecosystem's response to a stressor. For instance, identifying a specific antibiotic resistance plasmid (a genomic scar) within a bacterial strain, and concurrently observing the dramatic rise in that strain's abundance, provides a powerful, two-layered line of evidence. This integrated analysis not only strengthens the causal inference but also creates a more resilient and comprehensive narrative of the forensic event, moving beyond what either data type could reveal in isolation. However, its translation from a theoretical concept into reliable and admissible evidence in court faces three core challenges.

First is the fundamental challenge of signal discrimination. A critical hurdle is distinguishing genuine event-driven genomic signatures from the vast background noise generated by daily living and random genetic drift. This challenge begins at the crime scene itself; standardized pre-analytical protocols - from sampling methods and swab types to DNA preservation buffers - are crucial to ensure that detected genomic signals are true reflections of an event, not artifacts of evidence collection. Furthermore, the intensive sample preparation steps, such as whole-genome amplification and library construction, can introduce their own procedural artifacts, including polymerase chain reaction (PCR)-induced mutations or sequencing errors, which could be mistaken for genuine genomic scars. This risk is magnified in low-biomass or degraded forensic samples. Therefore, ensuring signal integrity requires not only standardized collection but also the implementation of ultra-high-fidelity methods and rigorous controls throughout the analytical process. Subsequently, the "genomic scars" of a forensic event must be robustly distinguishable from the vast background "noise" generated by both daily living and random genetic drift. For instance, individual-specific factors, such as personal hygiene habits (from fastidious to infrequent), diet, or medication use (e.g., a proton pump inhibitor), can introduce significant confounding effects. The strong signal induced by a sterilizer represents one end of a spectrum, while the subtle impact of a brief visit to a new location may be far more difficult to detect. Consequently, a unified statistical model applicable to complex communities is currently lacking to precisely distinguish genotypes driven by specific forensically relevant stimuli from this complex background of variation. This requires capabilities beyond mere variant detection, extending to the analysis of their allele frequency dynamics, genomic distribution patterns, and whether they exhibit signatures of a selective sweep.

Second is the challenge of establishing causality. Current research is largely limited to phenomenological descriptions, whereas the evidentiary value of this approach hinges on the critical leap from correlation to causation. There is an urgent need to construct a rigorously validated causal knowledge base of "stimulus-specific genomic scars" through controlled experiments. This would systematically elucidate the reproducible and predictable adaptive genomic changes induced by different stimuli.

Finally, a significant void exists in standardized evidentiary quantification. Unlike the mature probabilistic frameworks used in traditional DNA fingerprinting, evidence from microbial scars lacks a quantitative assessment system. Consequently, it cannot answer key questions regarding its evidential strength, the probability of its random occurrence, its degree of specificity, or the confidence level of temporal inferences.

To address these challenges, a concerted, multi-pronged effort is required in three key directions. First, developing novel algorithms that integrate evolutionary theory with machine learning. This involves combining population genetics models with deep learning to model mutation and selection processes at the

strain level, thereby isolating genuine signatures of directional selection from background noise. Second, launching a large-scale, standardized "Microbial Forensic Stress-omics" initiative. Through international collaboration, this program would systematically chart a comprehensive atlas of "forensic stimulus-genomic response" profiles, establishing an open-access causal association database to provide a robust foundation for interpretation. Third, establishing a likelihood ratio evaluation framework and validation system for microbial evidence. Drawing on best practices from forensic genetics, this would involve building reference databases, developing statistical models to quantify evidential strength, and performing large-scale empirical validation through blind trials and retrospective case studies to establish scientific standards for admissibility in court.

Furthermore, it is imperative to contextualize microbial evidence within the broader ecosystem of forensic investigation. Forensic scenes are inherently complex, and no single piece of evidence, microbial or otherwise, can provide a complete picture. Therefore, the insights derived from genomic scars should not be interpreted in isolation. Instead, they must be integrated with traditional physical evidence (e.g., fingerprints, DNA profiles), chemical analyses, and circumstantial evidence. This multi-modal approach, where microbial findings corroborate or challenge other evidentiary lines, is essential for building a robust and legally defensible case. The ultimate goal is to weave microbial narratives into the larger tapestry of the investigation, using them as a powerful auxiliary tool to enhance, rather than replace, established forensic methodologies.

CONCLUSION

In summary, the ultimate success of this paradigm hinges on a rigorous and systematic resolution of the serial bottlenecks from signal discrimination and causal establishment to evidentiary quantification. Through interdisciplinary integration and methodological innovation, this field holds the promise of providing judicial practice with "microscopic time capsules" and "molecular narrators" capable of reconstructing the sequence of events.

DECLARATIONS

Authors' contributions

Conceptualization, writing - original draft, visualization, formal analysis, funding acquisition: Dou S
Writing - review & editing, investigation, resources: Ma G
Conceptualization, writing - review & editing, supervision, funding acquisition: Li S
All authors have read and agreed to the published version of the manuscript.

Availability of data and materials

Not applicable.

AI and AI-assisted tools statement

During the preparation of this manuscript, the AI tool Google's Gemini (version 2.5 Pro) was used solely for language editing. The tool did not influence the study design, data collection, analysis, interpretation, or the scientific content of the work. All authors take full responsibility for the accuracy, integrity, and final content of the manuscript.

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Conflicts of interest

Li S is an Editorial Board Member of *Legal Medicine Research*. Li S was not involved in any steps of editorial processing, including reviewers' selection, manuscript handling, and decision-making. The other authors declare that they have no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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