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Review

Drug-induced dysbiosis as a forensic biomarker: Implications for post-mortem interval estimation and forensic diagnostics

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ABSTRACT

Substance use disorders (SUDs) and drug-related deaths represent a growing global burden. Increasing evidence highlights substances ability to reshape the gut microbiome, a highly dynamic and metabolically active ecosystem that contributes to host homeostasis through interactions with neural, endocrine, and immune systems.

Alterations in microbial diversity, depletion of short-chain fatty acid–producing taxa, disruption of epithelial barrier integrity, and systemic inflammation have been consistently associated with substance exposure. While these changes are increasingly characterized in living individuals, their persistence and impact after death remain less explored. In forensic contexts, drug-related fatalities are difficult to interpret due to non-specific autopsy findings, analytical limitations of toxicological methods, post-mortem redistribution, and the absence of clear lethal thresholds. The emerging study of thanatomicrobiome offers a novel avenue to address these challenges, as antemortem dysbiosis may influence post-mortem microbial succession and, consequently, post-mortem interval (PMI) estimation.

This narrative review synthesises current knowledge on substance-induced microbiome alterations across antemortem and postmortem contexts, and extends this perspective to entomotoxicology, highlighting how drug-related microbial changes may influence both insect colonisation and insect-associated microbiomes. It also emphasises current methodological heterogeneity and the need for standardised approaches, outlining opportunities for future forensic applications.

1. Introduction

Drug overdoses are a leading cause of death for many individuals suffering from substance abuse problems or substance use disorders [1]. Drug overdoses can occur accidentally or from taking an inaccurate dose of a substance [2]. In recent years, the United States has seen a growth in substance use (e.g., alcohol and/or illicit or prescription drugs), with many cases beginning in adolescence [3]. Similar patterns are observed in Europe, where substance use often begins during adolescence; for example, around 14% of European students aged 15–16 report having used an illicit drug at least once, and alcohol remains the most commonly used substance among adolescents [4,5]. Many of the substances of abuse fall within drug schedules I and II of the Drug Enforcement Agency's (DEA) five drug schedules. As mentioned, schedules I and II are the main entities abused by people, whereas schedules III-V contain substances used medically to treat pain, anxiety, and/or the common cold. Schedule I and II drugs, such as heroin and cocaine, are classified as substances that are easily abused, with (Schedule II) or without (Schedule I) potential accepted medical use [5]. Notably, opioids (Schedule II) are divided into two categories: natural and semisynthetic opioids and synthetic opioids. The first category is often used as a pain suppressant, but in excess, it can cause death [2]. The second category includes heroin and (illicit) fentanyl, the latter being highly deadly in even the smallest dosage [6]. Other than the previous functions and effects listed, substances of abuse can lead to issues antemortem and postmortem.

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Many of the issues observed within the body antemortem correlate to the symptoms of addiction. Addiction is a chronic, relapsing neuropsychiatric disease that leads from compulsive use of a substance, irrespective of the negative consequences, to loss of motivation for natural rewards, and to long-lasting neurological alterations, with a core role played by the dopamine reward system [7]. An individual can suffer physiological and psychological dependence on substances in the previously listed drug schedules. Schedule II drugs have higher proportions of dependence than the remaining schedules [5].

The use of substances in this schedule, particularly opioids or other forms of hydrocodone, has a negative effect on the body. In particular, drugs tend to activate the mesolimbic dopaminergic pathways [8]. In detail, while acute response to drugs is characterized by the increase in mesolimbic dopamine (Fig. 1), chronic abuse of drugs (and withdrawal states) is characterized by a downregulation of the pathways, with decreased basal and stimulated dopamine [8].

Addiction to substances is extremely frequent, and the prevalence of use disorders depends on the age: most abusers begin use before 17 years of age, and alcohol, cannabis, and opioid use disorders respectively peak in the twenties, at age 18, and in young adulthood [9,10]. The vulnerability of adolescents is likely due to a developmental mismatch between limbic areas (involved in reward) and the prefrontal cortex (involved in judgment, decision-making, and impulse control) [11]. The so-called "gateway substances" (like alcohol, nicotine, and cannabis) are often the first substances to be abused in life, possibly predisposing to the use of other drugs (for instance, nicotine increases the firing rate of the dopamine neurons of the midbrain, thus enhancing the rewards of other substances) [11].

From a forensic point of view, cases of fatal intoxication are common yet often extremely insidious. In forensic toxicology, although chromatography coupled with mass spectrometry is the best screening and confirmation technique (in particular, liquid chromatography coupled with tandem mass spectrometry), immunoassays are still frequently used as screening tests. This fact is due in part to economic factors and the need for trained technicians, but immunoassays are known to have lower specificity and sensitivity, calibration bias, and a risk of cross-reactivity with exogenous and endogenous substances [12]. Moreover, in post-mortem, other issues emerge, like the post-mortem redistribution of drugs [13]. Finally, it is difficult to interpret toxicological data when the blood level of the substance is sub-lethal, or there is no known lethal threshold, since fatal intoxications are usually sudden deaths, and as which differential diagnosis with other causes of unexpected death (as pathogenic variants linked to cardiac disorders) should always be taken into account [14]. Traditional autopsies are not usually informative. In the brain, drug exposure may cause neuroinflammation, promoting the release of local pro-inflammatory cytokines, and dysregulating neuroimmune signaling, thus increasing neurotoxicity and neurodegeneration [15,16]. However, sudden drug-induced deaths lack pathognomonic stigmata: some drugs that cause substantially arrhythmic deaths (such as opioids) do not induce relevant histopathological alterations, whereas others that can provoke acute vascular distress (such as cocaine) may cause only inconstant and non-specific microscopic abnormalities (like ischemic damages) —moreover, with dose-anomaly associations that remain unknown or highly variable [17].

In this review, we explore what is the impact of substances use on human microbiome and how these markers can help the core forensic tasks, i.e., the estimation of time since death and the diagnosis of the cause of the death.

2. Methods

For this narrative review a structured search strategy was deployed

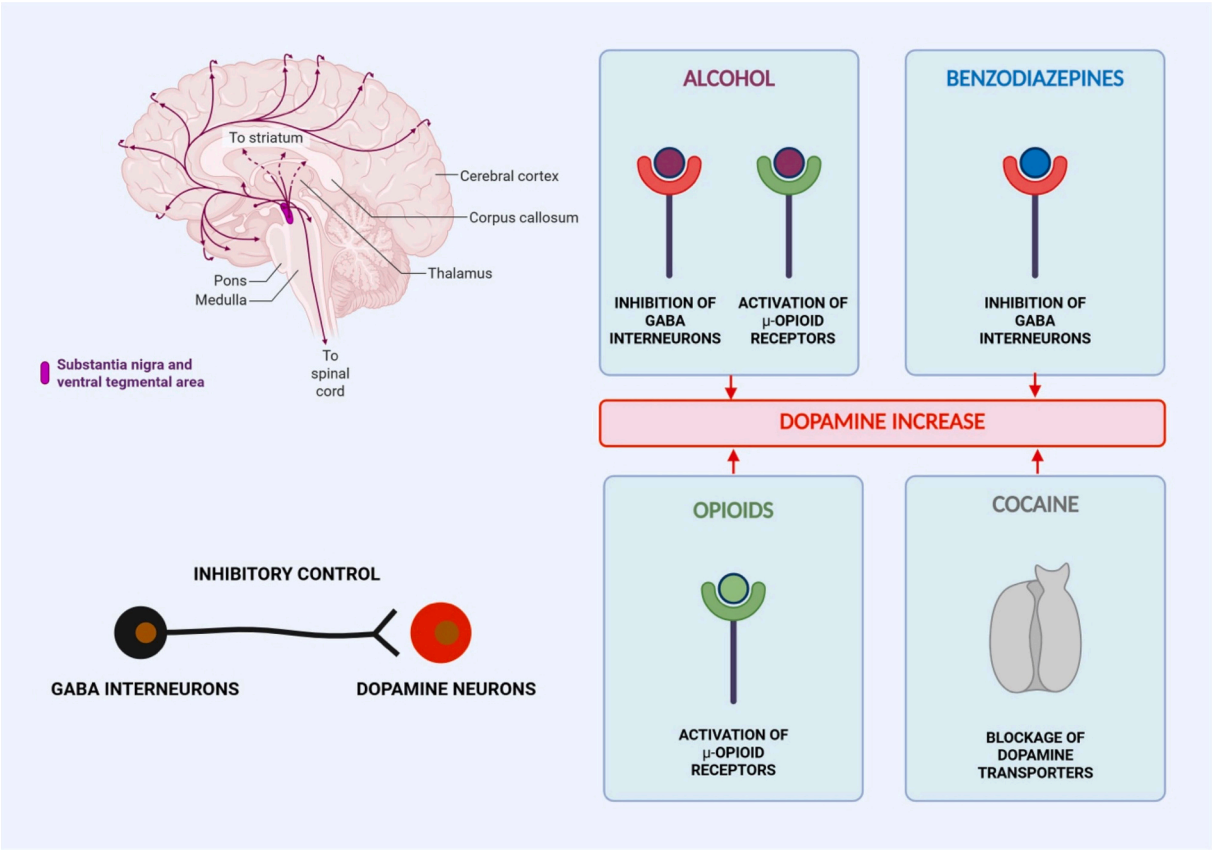

Fig. 1. Addiction is driven by a dopamine-innervated network involving reward, motivation, memory, and inhibitory control circuits, which are differentially modulated by substances like alcohol, stimulants, and opioids. Original illustration produced using Biorender.com.

with literature searches conducted across four primary electronic databases: PubMed/MEDLINE, Scopus, Web of Science, and Google Scholar, covering peer-reviewed articles, clinical trials, preclinical studies, and foundational forensic reports. The search syntax relied on combinations of Medical Subject Headings (MeSH) terms and free-text keywords using Boolean operators (AND, OR), organized into three main thematic blocks: Block 1 (Microbiome & Axis: "gut microbiome" OR "microbiota" OR "dysbiosis" OR "gut-brain axis" OR "thanatomicrobiome" OR "short-chain fatty acids" OR "SCFA"), Block 2 (Substances & Diagnostics: "substance use disorder" OR "SUD" OR "addiction" OR "alcohol" OR "opioid*" OR "morphine" OR "fentanyl" OR "heroin" OR "cocaine" OR "methamphetamine" OR "psychostimulant*" OR "forensic toxicology"), and Block 3 (Post-Mortem & Entomology: "post-mortem interval" OR "PMI" OR "entomotoxicology" OR "necrophagous insect*" OR "Calliphoridae" OR "microbial clock" OR "Wohlfahrtiimonas" OR "Ignatzschineria"). To refine the results, specific eligibility criteria were applied: studies were included if they investigated the impact of psychoactive substances on human/animal microbiomes, post-mortem microbial succession, or entomotoxicological interactions involving drug-induced insect microbial changes. Conversely, publications focusing on general dysbiosis entirely unrelated to substance use or forensic metrics, non-peer-reviewed abstracts, and papers not written in English were excluded. Additionally, the reference lists of the retrieved articles were manually screened to identify further relevant historical or foundational papers ensuring a robust evaluation of the current state of the art.

2.1. The role of the gut-brain axis

The gut microbiome is a densely populated and metabolically active community of bacteria, archaea, fungi, and viruses that inhabit the human gastrointestinal tract, fermenting otherwise indigestible substrates, synthesizing bioactive compounds, assisting the maturation of mucosal immunity, and helping to preserve epithelial barrier integrity. Through metabolites such as short-chain fatty acids (SCFAs), including butyrate, propionate, and acetate, as well as tryptophan indole derivatives and secondary bile acids, the microbiome engages neural, endocrine, and immune pathways, often described as the microbiome-gut-brain axis. Previous studies have determined that substance disorders, including substance abuse, negatively impact the gut microbiome of a living individual [18], causing alterations in its regulation that are able to affect homeostasis in the body and the overall mood of the individual. The idea of the gut-brain axis describes the connection between the brain and gut, where changes to one alter the other [18].

These mechanisms influence reward processing, stress reactivity and executive control [19–21]. The gut-brain axis is a network of integrated functions and bidirectional signals that involves the central and autonomic nervous systems, the enteric nervous system, and the hypothalamic pituitary adrenal axis [22,23]. The autonomic nervous system mediates the bidirectional signals between the central nervous system (brain and spinal cord) and the intestinal lumen and wall through vagal, spinal, and enteric pathways. However, the communication system is not composed of mere electric signals, since it also relies on neuro-immuno-endocrine mediators. For instance, the hypothalamic pituitary adrenal axis regulates the response to stress and pro-inflammatory cytokines, leading to an increase in cortisol, that acts as endocrine mediator with neural and intestinal targets [22,24]. All the signals of the axis are able to influence the enteric nervous system, immune cells, muscle layers, and gut mucosa, thus regulating motility, permeability, immunity, and mucus production [24]. On the other hand, the intestinal targets of the axis are also influenced by the enteric microbiota [24]. This latter contains more than 100 trillion microorganisms (bacteria, archaea, single-celled eukaryotes, helminth parasites, yeasts, and viruses) with a density of bacteria of 10^{11}–10^{12} per milliliter and more than 3 million genes [1,25]. Two phylotypes (i.e., groups of microbes with significant genetic similarity) - *Bacteroidetes* and *Firmicutes* – represent up to the 90% of the microbiota, followed by *Actinobacteria, Proteobacteria, Fusobacteria*, and *Verrucomicrobia* [2,3]. In a healthy individual, microbiota is highly diverse (diverse microbes may grant diverse functions) and with a moderate *Firmicutes/Bacteroidetes* ratio (*Firmicutes* tend to be more efficient in energy extraction from food and thus may be exposed to a higher risk of obesity and metabolic disorders) [4]. The composition of the microbiota is specific to each individual and is neither homogeneous in its anatomical distribution nor consistent over time. For instance, *Bacteroidetes* are mainly localized in the colon (densely populated by anaerobic bacteria and characterized by slow transit times and mild pH), while *Proteobacteria* and *Enterobacteriaceae* prevail in the small intestine (which is a good environment for aerobic bacteria, with short transit times and high bile concentration) [5]. In general terms, many variables influence the composition of the microbiota: gestational age (preterm vs full-term birth), type of delivery (because of the influence of the maternal vaginal microbiota), type of feeding in the infancy, age (bacterial diversity tends to increase with age, but elderlies tend to have impaired diversity due to the weakness of the intestinal and immune systems), medications (antibiotics reduce the bacterial diversity), genetics, ethnicity, BMI, environment, exercise, and lifestyle/diet (high-sugar diet induces intestinal inflammation, while dietary fibers promote the gut barrier function; high-quality and plant-based proteins promote beneficial bacteria; high-fat diet with predominant saturated fat acids causes dysbiosis) [1,6,25]. Microbiota contributes to the maturation of central and enteric nervous systems and to the regulation of intestinal motility, may influence mood, cognition, and learning, and also mediate essential physiological responses, like the adaptation to environmental stress [1,24]. On the other hand, the central nervous system influences microbiota regulating – for instance – intestinal motility, immunological defense, and intestinal secretion [1]. The main communication pathway between microbiota and brain seems to be represented by vagus nerve [1,24]. Vagal and sympathetic afferent fibers are considered the main source of interoception for the brain, i.e., its ability to obtain information about the state of the organism and elaborate responses through the autonomous nervous system and the hypothalamic pituitary adrenal axis, a process that is conditioned by gut microbes and their metabolites [22]. There is not a direct contact between vagus nerve and microbiota, but it is likely that microbes can send inputs through metabolites and modulating enteroendocrine and enterochromaffin cells in the intestinal epithelium [1]. For instance, some microbes can produce neurotransmitters like GABA, serotonin, dopamine, and noradrenaline [1]. In particular, serotonin both tends to increase in response to some spore-forming bacteria and is able to directly influence some specific microbes, like *Turicibacter sanguinis* [1]. Moreover, bacteria ferment dietary fibers (non-digestible carbohydrate polymers) and then produce short-chain fatty acids, that may influence several intestinal functions (motility, secretion, and regulation of inflammation), neuroplasticity, gene expression, and immunomodulation [1,24]. Some microbes may also metabolize bile acids into reabsorbable molecules able to influence gene expression (in particular, activating the nucleus arcuatus of the hypothalamus) [24]. Similarly, they can deconjugate estrogen, that can become reabsorbable [24]. Another possible channel of communication could be represented by immune response. Indeed, bacteria are normally confined on the surface of the mucus layer of the intestinal barrier, where they can form biofilms [24]. If there are not enough dietary fibers to feed them, they start eating the glycans of the mucus layer, thus endangering the impermeability of the intestinal barrier [24]. When the integrity of the intestinal barrier is disrupted, microbes can directly activate dendritic cells and release immune mediators into the system circulation [1]. In (dis)functional terms, dysbiosis relates to abnormal (heightened) responses to stress and has been associated to many diseases like irritable bowel syndrome, inflammatory bowel disease, celiac disease, colorectal cancer, obesity, type 2 diabetes, Alzheimer's and Parkinson's Diseases, hepatic encephalopathy, autism spectrum disorders, anxiety, and depression [1,7,8,25].

Moreover, there is a biunivocal relationship between substances use disorder and gut-brain axis: treatment with antibiotics and feces transplantation have been associated to an improved behavioral pattern, while substances of abuse may alter the intestinal ecology and reduce the local diversity (particularly affecting microbes that produce short-chain fatty acids or/and causing bile acid metabolism disorders), enhance intestinal barrier permeability and pro-inflammatory state [9,10]. Moreover, the low-grade inflammation caused by both dysbiosis and substances like alcohol can enhance substance-related/induced mental disorders like depression [11]. It is also possible that there is a synergistic effect in promoting epigenetic changes: similarly to the effects of drugs, some short-chain fatty acids produced by microbes, such as butyrate, can inhibit histone deacetylase (thus altering chromatin architecture to promote transcriptional activation) [10]. Finally, social contact allows the transmission of microbes, and this can be impaired in drug abusers (since they tend to social isolation) and, globally, social factors (like early life adversity) may cause abnormal stress responses and induce a prolonged pro-inflammatory state, thus predisposing to both substance use disorders and dysbiosis [8].

While humans can be exposed to both prescription and illicit drugs throughout their lifetime, childhood [12] and pregnancy [13] are two of the most critical periods for establishing and maintaining a healthy microbiome. Any drug exposure during these critical periods can lead to serious drug-microbiome interactions [14]. Since drugs can influence the microbiome, and the microbiome can influence drug functionality, pharmacomicrobiomics investigates microbiome-drug interactions [15]. It has been demonstrated that drug usage impacts the abundance, diversity, and function of the microbiome in living individuals [14], with echoing consequences postmortem. While drugs of abuse are commonly associated with cases of overdose and death, proton-pump inhibitor (PPI) medications should also be considered, as they were found to significantly affect the gut microbiome, by decreasing the bacterial diversity even more than antibiotics [16,17].

2.2. Gut microbiome and drug addiction

The microbial community structure is plastic and responds to many stimuli, including the introduction of substances of abuse in the body. Drug exposure can reshape the microbial composition and function, and the resulting dysbiosis feeds back into immune, endocrine, and neural signaling in ways able to influence vulnerability to use, craving, withdrawal severity, and relapse. A scoping review that synthesized 75 clinical studies reported a robust difference in beta diversity (i.e., a metric that quantifies the variation in species composition between different communities or groups) between people who use alcohol, opioids, stimulants, or cannabis and non-using control counterparts, with preliminary longitudinal evidence suggesting a partial recovery of the microbiome during abstinence. The review also highlighted substantial methodological heterogeneity and substance-specific trajectories [18]. A complementary narrative synthesis collated preclinical and clinical findings that link substance-associated dysbiosis to shifts in short-chain fatty acids, indole and tryptophan metabolites, and bile acids. These shifts affect blood-brain barrier integrity, immune activation, neurotransmission, and gene expression, all of which are relevant to addiction biology [20].

2.2.1. Alcohol use disorder

Human studies consistently associate chronic alcohol exposure with lower microbial diversity, impaired intestinal barrier function and systemic inflammation. These features correlate with neuroinflammation and with risk of relapse [19,20]. A recurrent finding is the depletion of short chain fatty acids-producing taxa, providing a plausible bridge between peripheral dysbiosis and central adaptation. Reduced butyrate weakens epithelial and blood brain barrier tight junctions, alters microglial activation, and perturbs stress circuitry that sustains negative effect and craving [20,21]. Reports in translational journals also implicate the mycobiome. Overgrowth of *Candida albicans* may generate neuroactive metabolites that potentiate alcohol consumption, which suggests that fungal communities can contribute to the vulnerability to alcohol use disorder [21]. t Serious adverse events during six months of follow up were fewer in the transplantation arm [22]. A prospective clinical study in twenty 25 reported favorable safety and reductions in systemic inflammation after transplantation in alcohol related disease, which supports the feasibility of microbiome restoration as an adjunct to standard care [23].

2.2.2. Opioid use disorders

Opioids, such as morphine and hydromorphone, slow intestinal transit and induce constipation and nausea [24]. They are associated with changes in the alpha microbial diversity (i.e., the number and distribution of different species within a specific individual), however results on alpha diversity are not always consistent between different studies [25–28]. A shift in the gut microbiome composition (beta diversity) has also been reported in literature [29]. Due to the limited number of studies available on the topic and to the potential inter-individual variability, particularly for human studies, it is difficult to get a complete understanding related to specific trends (increase of decrease) of specific bacterial taxa in association with specific opioid substances. However, it seems that the Bacteroidetes/Firmicutes ratio is reduced in murine models treated with morphine, resulting in raising of inflammation levels, barrier dysfunction and endotoxemia [25,30]. The decrease of taxa such as *Lactobacillus* and the increase of taxa such as *Clostridium* and *Enterococcus* overall lead to the effects described above [27–29,31]. In fact, these changes amplify peripheral cytokine signaling that can affect mesolimbic and prefrontal circuits. Reduced availability of short chain fatty acids affects the maintenance of epithelial and blood brain barrier integrity, and these patterns are consistent with barrier and immune-to-brain signaling routes outlined above [20,32,33]. Preclinical morphine models consistently show restructuring of the main phyla together with metabolomic profiles that indicate inflammation and barrier impairment, which aligns with the proposed mechanism [32].

Heroin use can affect alpha diversity values [34], overall indicating a disruption of the healthy gut system; however, in this case, specific Firmicutes tend to decrease, and Enterobacteriaceae, *Lactobacillus,* and *Clostridium* cluster XIVa tend to increase, partially in contrast with some of the findings reported for morphine users [34].

Methadone users have been found to have increased levels of Actinobacteria, such as Bifidobacteriaceae, and reduced levels of Verrucomicrobia [35].

Preliminary studies suggest that fentanyl exposure is associated with measurable alterations in gut microbial composition and inflammatory responses. Animal models have reported bidirectional interactions between fentanyl self-administration and gut microbiota profiles, while more recent studies have observed microbiome dysbiosis accompanied by inflammatory and metabolic changes in fentanyl-dependent subjects [36,37]. However, the effects of fentanyl on the microbial community have not yet been fully characterized.

2.2.3. Psychostimulants

Exposure to psychostimulants, such as cocaine and methamphetamine, has been associated with alterations in gut microbiome composition. In rodent studies, cocaine reduces beneficial genera such as *Mucispirillum* and increases pro inflammatory taxa such as *Barnesiella*. These changes track with inflammatory tone and with demand for the drug. Methamphetamine can increase overall diversity while depleting taxa that are associated with the production of short-chain fatty acids and while altering metabolic output. These features align with anxiety-like behaviors and with cognitive deficits [38]. Emerging evidence now supports a causal relationship: depletion of the microbiome using non-absorbable antibiotics enhances cocaine sensitization and conditioned place preference, whereas supplementation with short-chain fatty acids reverses these effects. This directly implies microbial metabolites in

stimulant addiction phenotypes [39]. In human datasets, gut microbial features discriminated methamphetamine use disorder from controls and identified signatures of early withdrawal, which suggests utility for stratification and for monitoring [40].

2.2.4. Cannabinoids

The literature on cannabinoids is more nuanced. Editorial syntheses propose that phytocannabinoids can exert anti-inflammatory effects, in part, by modulating the microbiome and metabolome. This includes effects on short-chain fatty acids, bile acids, and indole pathways. The direction and the magnitude of these effects appear to depend on dose, duration, and host baseline [20]. These observations raise the possibility that microbial pathways contribute to both therapeutic effects and adverse effects of cannabinoids on affect, motivation and stress buffering. A comparative synthesis of substance-specific microbiome alterations and associated mechanistic routes is provided in Table 1.

2.2.5. Nicotine and tobacco smoke

Smoking has been associated with lower microbial diversity, with perturbed mucosal immunity and with inflammatory dysbiosis that may only partly reverse after cessation. Mechanistic reviews describe nicotine and smoke constituents as modulators of mucus composition, epithelial tight junctions and immune signaling. In this way smoking reshapes microbial ecology and metabolite profiles. These changes could plausibly influence withdrawal trajectories and cardiometabolic recovery during quit attempts [41].

Mechanistic routes linking dysbiosis to addiction largely converge on the gut–brain axis pathways described above. In addition, the microbiome may alter the pharmacokinetics and pharmacodynamics of addiction therapeutics via direct drug metabolism and regulation of host enzymes and transporters, supporting inclusion of microbiome covariates in dose-optimization models [42].

Progress in this field has been slowed by heterogeneity in sampling sites, sequencing modalities, and control of confounder factors such as diet and medications. Methodological consensus on collection, on metadata, and on analytical pipelines, together with longitudinal and multi-omics designs that embed behavioral and neuroimaging endpoints, is required to clarify causality and to identify actionable targets. Resources from the Human Microbiome Project and from the Earth Microbiome Project provide reference frameworks that support harmonization and interpretation [43–45].

2.3. Microbiome in death

Postmortem changes in the body are widely studied and are critical for determining the post-mortem interval (PMI) and cause of death. Previous studies by [12–14], have identified the growing importance of post-mortem microbial communities or the thanatomicrobiome. The thanatomicrobiome, "microbiome of death," describes the progression of microbes in the human body post-mortem, aiming to understand the important role of microbes in the process of decomposition [14]. Through evaluating the relative abundance of certain taxa, PMI can be estimated [13,15]. However, SUDs or substance abuse can alter the observed proliferation of specific bacterial taxa. For example, alcohol exposure has been associated with increased abundance of Proteobacteria and reduced levels of Bacteroidetes, whereas opioid use has been linked to decreases in Bacteroidetes and Firmicutes together with increased Proteobacteria. Similarly, psychostimulants such as cocaine have been reported to alter microbial community composition, including shifts in Bacteroidetes abundance (Mutlu et al., 2012; Wang et al., 2018; Hofford et al., 2019b; Meckel and Kiraly, 2019). These substance-specific alterations may persist after death and potentially influence thanatomicrobiome succession patterns. More research is needed to understand the impact of SUDs and substance abuse on the body postmortem. Current studies targeting this area have suggested some crucial findings in understanding the impact of SUDs on the body postmortem. Multiple studies [16–18] have noticed specific gene expression patterns from tissues affected by SUDs that either signaled apoptosis or delayed the cellular process. Another study [19] noted that mRNA degradation pathways were expressed in the tibial artery, esophageal mucosa, and the thyroid. More research is needed to evaluate the implications that SUDs have on the thanatomicrobiome and postmortem changes to the body.

While the gut-brain axis discoveries are increasingly important for the medical field in understanding different diseases and triggers, knowing how the microbiome is affected by the presence of different substances of abuse can lead to a better understanding of the postmortem microbiome. This in-depth view can have repercussions not only for the microbial postmortem "clock", but also for the necrophagous insect microbiome and on the insect-decomposed tissue interactions. For example, the presence of alcohol in patients undergoing colonic biopsies showed a higher rate of Proteobacteria and a lower rate of Bacteroidetes [54], observed to be a cause of decreased bacterial diversity in postmortem samples as well [55]. Like alcohol, opioids influence gut motility, thus impacting the gut microbiome [18]. For instance, during morphine treatment, levels of Bacteroidetes and Firmicutes decrease, while those of Proteobacteria increase [56]. Stimulants, such as cocaine, have also been found to alter the gut microbiome, and in certain cases, this results in increased levels of Bacteroidetes [32,57].

After death, the microbial patterns and composition can change in

Table 1
Summary of addiction-related gut microbiome changes, key mechanism routes, and supporting evidence described. Cross-drug mechanisms (i.e., effects of polydrug assumptions) are also treated.

Substance class	Impact on gut and microbiome	Mechanistic implications	Reference
Alcohol	Lower microbial diversity; impaired epithelial barrier; systemic inflammation; depletion of butyrate-producing taxa; possible *Candida* overgrowth	Reduced butyrate weakens tight junctions; microglial activation; increased relapse vulnerability; fungal metabolites may modulate craving	[8,19,20,46–49]
Opioids	Alteration of Bacteroidetes/Firmicutes ratio; barrier dysfunction; endotoxemia	Cytokine-driven effects on mesolimbic and prefrontal circuits; reduced SCFA signaling; increased stress reactivity and withdrawal complications	[32,46,50]
Psychostimulants (cocaine, methamphetamine)	Cocaine: ↓ *Mucispirillum*, ↑ pro-inflammatory taxa (*Barnesiella*); Methamphetamine: ↑ overall diversity but ↓ SCFA-related taxa; metabolic disruption	SCFA depletion increases cocaine sensitization; dysbiosis contributes to anxiety-like behaviors and cognitive deficits; microbial features discriminate methamphetamine use disorder from controls	[39,51,52]
Cannabinoids	Context-dependent microbial changes; modulation of SCFAs, bile acids and indole pathways	Potential anti-inflammatory effects mediated by microbial/metabolomic shifts; influence on affective and motivational domains	[20]
Nicotine / Tobacco smoke	Lower diversity; mucosal immune perturbation; inflammatory dysbiosis	Changes in mucus, epithelial junctions, immune signaling; potential influence on withdrawal trajectories	[41]
Cross-substance mechanisms	SCFA depletion; tryptophan–indole axis disruption; bile-acid signaling alterations; barrier dysfunction; endotoxemia	Increased neuroinflammation; altered dopamine/glutamate signaling; altered stress responses; changes in drug PK/PD	[8,39,42,46,53]

predictable ways as a result of variation in environmental factors [58–60]. Drug ingestion before death and postmortem redistribution will likely cause a disequilibrium in the bacterial composition. Therefore, investigating microbial patterns and their dynamics as a result of drug exposure can lead to a better understanding and determination of a common temporal succession to be used for postmortem interval (PMI) estimations.

For the past decade and with the advancement of next-generation sequencing technologies, the microbiome has shown the potential to be used as a forensic tool in establishing microbial "clocks" to aid in PMI estimations, individual identification, and even geolocation [61]. Two large-scale projects have helped advance understanding of the microbiome – the Human Microbiome Project [43,44] and the Earth Microbiome Project [45]. These projects, together with the latest research papers focused on the postmortem microbiome, have led to an expansion of the forensic microbiology definition that was initially focused on bioterrorism / identification of biological weapons [62,63] to a broader one that includes the use of microbiome as a forensic tool in criminal or civil cases [64]. For example, skin microbiome specificity can be helpful in forensics when investigating microbial transmission to other individuals or objects [65]. On the other hand, when investigating the postmortem microbiome in cases where different substances or poisons are involved, the investigation should focus on the gastrointestinal tract, as this is the primary location for the decomposition process [66].

In addition to interpersonal variation, environmental factors, drug type and concentration, overall health, polydrug use, and geographical location, the presence of different substances will not only lead to changes in the microbial patterns, but also to changes in insect colonisation, when temperature thresholds allow for insect presence and activity.

The forensic branch that deals with investigating substances and insect species is called entomotoxicology [67,68], and it can be regarded from two perspectives – 1) the use of insect samples as an alternative matrix to decomposing tissues for toxicological investigations [69], and 2) understanding drug-insect interactions for estimating the PMI [67,70]. For the first category, drugs in insect tissues can be identified using various methods, including gas chromatography–mass spectrometry [71], liquid chromatography–mass spectrometry [72], or high-performance liquid chromatography [73]. Regarding PMI estimation, special consideration must be taken when drugs are present in the feeding environment, as certain substances can shorten or prolong the development cycle, while others with no effect on the time length will cause changes in the insect physiology related to length and weight, introducing errors in PMI estimations. Although forensic entomotoxicology primarily focuses on identifying drugs and poisons from insect samples, understanding the effects of drugs at the microbial level will help better interpret insect-drug interactions.

Approximately 400,000 people die every year [74,75] from the ingestion of different drugs or poisons. Most cases involve drugs of abuse, and their intentional or unintentional misuse can have serious consequences, from overdoses to hundreds of thousands of deaths worldwide annually [74,76].

During warmer months, a body will be colonized by insects relatively fast, depending on the environment (outdoors vs indoors), and the level of containment (exposed, covered with different materials, buried, or concealed in a suitcase) [77]. Drugs will also influence insect colonisation. Nevertheless, the first to be affected and influenced by the presence of drugs will be the individual's microbiome, as once ingested, injected, or inhaled, the parent drug will begin to break down into metabolites affecting bacterial composition both ante and postmortem [10,18]. The onset and progression of this process will be dependent on the type of drug, its concentration, polydrug use, and the individual's overall health and medical history [42,50]. This aspect is important, as after death, the microbial and chemical cues will act as attractants for the necrophagous insect species to colonize the body [58,60,78]. Once the internal microbiome is affected by the presence of certain drugs, it is to be expected that the insect microbiome will be affected as well, not only by the consumption of drug-containing tissues, but also by the microbial exchange between insect and decomposed tissues [79,80]. Regardless of the tissue type under investigation, insect or human, the method of collection and preservation will be critical for the success of the toxicological investigation and microbiome analysis [69,81,82]. While different drugs and poisons such as amitriptyline [83], morphine [84], diazepam [85], codeine [72], malathion [86], have primarily been detected from Calliphoridae species, there is a gap of experimental studies focusing on the investigation of insect microbial changes as a result of drug exposure.

As previously demonstrated, drug identification from insect samples appears to be a viable alternative, given the stability of drugs in insect tissues compared to those in decomposing human tissues [87]. Nonetheless, information on the pharmacokinetics of drugs and their bioaccumulation in the insect tissue, as well as their elimination must be understood and taken into consideration when analyzing insect evidence [69,72]. A study published more than a decade ago [88] showed that the drug metabolism takes place in the insects' Malpighian tubules via cytochrome P450 and glutathione transferase enzymes. This site of metabolism is available during the larvae stages, as in the pupae stage the tubules are degraded, resulting in different drug concentration corresponding to each developmental stage. At the same time, continuous exposure to drug-containing substrate should be considered. The same study [88] showed that temperature influences not only the insect activity and development time, but also the gut motility as it relates to the absorption and excretion of drugs. Moreover, the presence of drugs can act as a stress stimulus affecting the overall insect microbiome composition. During death investigations, the collection of specimens for entomotoxicological investigations is of paramount importance, as is information regarding the collection site on the body, since drugs will be distributed throughout the body based on their physicochemical properties and redistributed postmortem [89,90], influencing the concentration of drug/metabolites in the insect samples.

It is generally accepted that forensic entomotoxicology has proven its importance during numerous cases involving human remains [87,91–93], as on several occasions insect specimens were the only source of evidence for toxicological investigations.

With more and more researchers and practitioners requesting standardised methods, procedures, and validated protocols for both experimental research and casework, an international consortium formed by toxicologists and entomologists could be established to draft the baseline for the minimum accepted standards to be followed by researchers and practitioners, considering smaller laboratories, reduced financial capabilities, access to animal models and ethical committees' requirements.

As the presence of drugs affects insect physiology and the chronological duration of their development cycle, it will equally affect their microbiome. The insect gut microbiome studies have grown in interest due to their potential biotechnological applications [79], such as obtaining antimicrobial components, probiotics and even enzymes. Representatives of Enterobacteriaceae, Bacillaceae, Pseudomonadaceae, Staphilococcaceae, and Enterococcaceae are often identified as insect-associated bacteria [94]. The insect gut microbiome will be influenced by different factors, including seasonal variation, metamorphosis and diet [79]. Once necrophagous insect species feed on decomposed organic matter, they produce enzymes to help degrade the substrate, acquire bacteria from the feeding environment and leave behind their own specific microbiome signature. The question is: to what extent does the human microbiome, affected by drugs ante and postmortem, in turn affect the necrophagous insect microbiome? Could this have repercussion for the PMI estimation?

The insect microbiome has been investigated in relation to the degradation of various pesticides, by studying resistance mechanism [95], but more data is needed for forensic investigations, as there is a gap in understanding the microbiome of necrophagous insects species

[80]. A previous study [80] demonstrated that insect-specific bacteria like *Wohlfahrtiimonas* and *Ignatzschineria* are transferred from the insects to the decomposed tissues, highlighting both taxa as potential bacterial markers for insect identification in their absence. Studies investigating the influence of drugs on the insect microbiome are underrepresented. There is a growing need for more studies in this niche area as a more recent study [96] revealed critical changes in the microbiome of insects reared on flunitrazepam spiked liver tissues compared to the control specimens, including increased relative abundance of Pseudomonadota and the emergence of Actinomycetota.

Investigating insect-microbiome changes as an effect of drug exposure will lead to a better understanding of growth rates and development time, resulting in a more accurate PMI estimation.

3. Conclusions

SUDs are increasingly recognised as systemic conditions characterized by complex interactions between the gut microbiome and host biological processes. Evidence indicates that substance-induced dysbiosis contributes to alterations in host gene regulation through microbial metabolites, immune signaling, and epigenetic mechanisms, with downstream effects on neurobiology and behaviour.

Importantly, microbiome alterations established during life may persist into the postmortem period, shaping microbial succession patterns collectively referred to as the thanatomicrobiome. These findings support the potential of microbiome-based signatures to inform the interpretation of drug-related deaths and to contribute to forensic applications, including postmortem interval estimation and, potentially, cause-of-death assessment. In addition, drug-induced microbial changes may influence insect colonisation dynamics, further extending their relevance within entomotoxicological frameworks. These interactions also highlight the need to consider microbiome-driven variability when interpreting toxicological and entomological evidence in forensic casework.

Despite these advances, significant limitations remain, including inter-study variability, limited causal inference, and a lack of standardised methodologies. The molecular pathways linking substance exposure, microbial dynamics, and postmortem processes are not yet fully elucidated, and their translation into forensic practice requires robust validation. Future research should prioritise controlled, longitudinal, and standardised approaches to establish reproducible microbiome-based markers suitable for forensic applications. Unlike previous reviews that primarily focus on the role of the microbiome in addiction and human health, this review provides a forensic-oriented perspective by integrating evidence from addiction biology, thanatomicrobiome research, and forensic entomology within a unified framework. By highlighting how substance-induced dysbiosis may influence postmortem microbial succession and insect-associated microbial communities, we identify previously underexplored mechanisms that could affect PMI estimation and the interpretation of drug-related deaths. This integrative perspective may support the future development of microbiome-informed tools for forensic investigations and contribute to advancing evidence-based practice within the criminal justice system.

CRediT authorship contribution statement

Gulnaz T Javan: Writing – review & editing, Writing – original draft, Funding acquisition, Conceptualization. **Michele Di Nunzio:** Writing – review & editing, Writing – original draft, Investigation, Formal analysis. **Simone Grassi:** Writing – review & editing, Writing – original draft, Investigation, Formal analysis. **Lavinia Iancu:** Writing – review & editing, Writing – original draft, Investigation, Formal analysis. **Noemi Procopio:** Writing – review & editing, Writing – original draft, Project administration, Funding acquisition.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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