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Review

Applications of microbiology to different forensic scenarios – A narrative review

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ABSTRACT

In contrast to other forensic disciplines, forensic microbiology is still too often considered a "side activity" and is not able to make a real and concrete contribution to forensic investigations. Indeed, the various application aspects of this discipline still remain a niche activity and, as a result, microbiological investigations are often omitted or only approximated, in part due to poor report in the literature. However, in certain situations, forensic microbiology can prove to be extremely effective, if not crucial, when all other disciplines fail. Precisely because microorganisms can represent forensic evidence, in this narrative review all the major pathological forensic applications described in the literature have been presented. The goal of our review is to highlight the versatility and transversality of microbiology in forensic science and to provide a comprehensive source of literature to refer to when needed.

1. Introduction

Like other forensic sciences, microbiology has undergone developments and innovations over the years[1]. However, to this day, the discipline remains an intensely debated topic, and forensic microbiological analyses are often considered merely "ancillary" to those routinely performed in other forensic disciplines[2]. Nevertheless, microorganisms can be a type of physical evidence in many different circumstances. They can be found almost anywhere and are certainly present in any habitat relevant to humans. Therefore, they can be collected and analyzed in various scenarios of forensic interest and provide valuable information for investigative purposes. Although microorganisms are ubiquitous, they are not found everywhere, and like many other types of evidence, some microorganisms are restricted to specific environments. Another element that makes microorganisms potentially useful for forensic purposes is the ability of many of them to adapt to the environment by changing their structures to survive over very long periods of time. Therefore, microbial profiles isolated from various substrates can acquire the status of potential "forensic indicators" and can be used as evidence in numerous circumstances[3,4]. In any case, they can serve as a complement to traditional investigative methods[5–7]. Recognising that the full potential of microbiology for forensic investigations has not been adequately explored and is often underappreciated, perhaps because it is poorly understood, in this narrative review we provide a comprehensive and up-to-date overview of the various areas of forensic application in which microbiology has been shown to make an important contribution.

2. Material and methods

We conducted a literature search that included all studies dealing with the applications of microbiology in forensic science by selecting the titles and abstracts of relevant articles. The literature search was performed in the most popular electronic databases (PubMed, Scopus, Medline, Google Scholar, and Web of Science) using the following combination of text protocols "post-mortem microbiology" combined individually and randomly with the Boolean operator "and": "forensic," "microbiome," "tanatomicrobiome," "necropsy," "cause of death,"

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"nosocomial diseases," "toxicology," "drowning," "communicable diseases," "biological fluids," "soil analysis," "post-mortem interval," "lethal food toxins," "child abuse," "bioterrorism," and "personal identification". Only full-text English-language articles were considered, and dated publications were also used. All bibliographies of the selected articles were revised to include additional relevant articles.

3. Forensic application areas of microbiology

3.1. The role of microbes as indicators of the manner and cause of death

Some studies have focused on analyzing the microbial community of bodies from different countries that died in different ways to assess the potential utility of microorganisms to identify possible biomarkers associated with them[8,9]. Although such studies are promising, they are limited by sample size and require the implementation of a large database before their practical application in forensics. On the other hand, in determining the cause of death, isolation of a single bacterial species from cadaveric material is usually considered to indicate infection in the body. In contrast, detection of mixed species is generally considered to be the result of postmortem transmigration and contamination. However, microbial growth and species identification are not sufficient for the correct interpretation of a postmortem culture examination. A quantitative assessment of the vital microbial load must also be performed to determine its pathognomonic significance, especially in cases of mixed or multiple growths. These aspects are critical from an evidential standpoint, as direct causality between isolation of the *noxa* from cadaveric cultures and death must be demonstrated[10]. Combining histologic examination of various organs with microbiologic evidence allows more reliable differentiation between intravitam acquired infection and postmortem contamination. Histology may show signs of vital reactions (neutrophilic or lymphocytic inflammatory infiltrates, fibrin reticulum, edema, necrosis, and the presence of hemorrhagic infiltrates) in response to the presence of infectious pathogenic *noxae*[11]. Clinical or postmortem biochemical data related to inflammatory indices can certainly also play a role in the assessment of causation. The correct interpretation of these different aspects is crucial. Several factors such as agonal transmission of bacteria, time interval between death and autopsy, as well as evisceration and intestinal manipulations before microbiological sampling may affect the reliability of microbiological examinations at autopsy[12].

These critical issues are the main reason that postmortem microbiology (PMM) remains controversial to this day. Another element that must be considered is the possible antibiotic therapy administered intravitam. In this regard, the general guideline in forensic medicine is not to exclude the possibility of isolating a potentially lethal pathogen by performing tests anyway[13,14]. Many antibiotics have a bacteriostatic effect rather than a bactericidal one. They do not eradicate the bacterial infection completely, but limit it and leave the healing to the host's immune system. In addition, it should always be kept in mind that the concentration of the administered antibiotic in the focus of infection may be below the minimum inhibitory concentration and thus not a limitation for isolating the microorganism in question. To properly pursue the diagnosis of some causes of death, the contribution of PMM may be essential. We report below the most important of them.

3.1.1. Sudden Infant Death Syndrome (SIDS)

SIDS is the sudden and unexpected death of apparently healthy infants under one year of age who were not suffering from any disease and were in good health[15]. In such cases, even after a careful series of investigations, including a thorough autopsy (comprehensive of histologic analysis), a complete review of medical records, and a careful examination of the scene of death, it is imperative to perform microbiologic investigations because of the unexplained cause of death[16]. Therefore, exhaustive samples of biological fluids and viscera (especially heart blood, cerebrospinal fluid, spleen, and lungs) should be collected during the autopsy to identify bacterial or viral infections, either congenital or acquired, that may have played a role as a cause of death[3]. Some studies suggest that underlying bacterial infectious diseases may be falsely identified in SIDS[17–19], although interpretation of culture tests in these cases is often complex. Whether viral infections play a role in SIDS remains a controversial topic. The microorganisms associated with increased risk of death in SIDS are *Neisseria meningitidis*, *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Bordetella pertussis*, *Staphylococcus aureus*, Epstein-Barr virus, *cytomegalovirus*, respiratory syncytial virus, adenovirus, and human metapneumovirus[20]. Recent evidence suggests that a possible cause of SIDS is the interaction between the infectious agent and the immature immune system of infants[21]. However, to date, the pathogenesis of SIDS remains unclear.

3.1.2. Sepsis

Post-mortem diagnosis of sepsis is not always straightforward because clinical history may be lacking, cultures may be contaminated, and macro- and microscopic findings are nonspecific[22]. Serologic inflammatory markers such as procalcitonin and C-reactive protein can help confirm the diagnosis. In general, the most common causes of septic shock are bacterial infections (both Gram + and Gram -), although they can also be caused by fungal, viral, and protozoal infections. The anatomic sites from which sepsis most commonly originates are the lungs, abdomen, and urinary tract. In 10–30% of sepsis cases, blood cultures cannot detect a causative microorganism. The likelihood of detecting organisms in the blood is inversely proportional to the duration of intravitam antibiotic therapy[23]. However, their isolation alone may not be sufficient to diagnose sepsis as a cause of death[24]. Therefore, recent studies have aimed at an integrated approach based on a combination of cultural studies (especially for infectious foci observed at autopsy), microscopic analysis, and biochemical and immunohistochemical investigations of blood and pericardial fluid samples[25].

3.1.3. Food toxic infection

Deaths from food poisoning are due to ingestion of food or water contaminated with infectious agents (mainly bacteria, but also viruses, fungi, and parasites) or natural toxins[26,27]. The bacteria responsible for these infections have a high adaptability. They are mainly *Salmonella* (dairy products, pork, poultry, beef, and vegetables)[28], *Campylobacter* (poultry), *Escherichia coli*, *Shigella*, and *Vibrio* (raw seafood)[29], and *Listeria monocytogenes* (prepackaged food)[30]. Some bacteria (*Salmonella*, *Shigella*, *Campylobacter*, and *Yersinia*) act directly by invading the intestine and colonizing mucosal cells, resulting in ulceration; others (*Clostridium botulinum* and *Staphylococcus aureus*) act through preformed toxins or some others (*Vibrio* spp, *Clostridium perfringens*, *Shigella*, and toxin-producing *Escherichia coli*) through toxins produced in the intestine[27]. Severe gastro-enterocolitis with potentially fatal outcome usually occurs because of dehydration, hydro-electrolyte imbalance, shock, intestinal perforation, and/or disseminated intravascular coagulation[31]. Food-related deaths raise many medicolegal issues, including complex questions about exposure to the infectious agent of concern, the type of food contaminated and consumed, the composition of the food, and toxicological analysis to characterize it. Given the variability of lethal mechanisms that may underlie such cases, they also require a thorough autopsy, which must be supplemented by microbiological studies of gastrointestinal viscera, feces, and blood samples. However, molecular diagnostics is playing an increasingly important role in this field, either in the detection of single microbial pathogens such as *Clostridium difficile* or in the simultaneous search for the presence of multiple pathogens using multitarget multiplex and array systems[32].

3.1.4. Other infectious diseases

Other diseases in which the contribution of microbiological examination can be very helpful are pneumonia, mycobacterial infections, fungal infections, infective endocarditis and meningitis.

- *Pneumonia* is one of the most commonly overlooked diagnoses in life, with the first diagnosis being made at autopsy[33]. However, even when clear and known cases of pneumonia end up on the autopsy table, lung specimens should always be obtained and analyzed. This would not only prove the cause of death but also identify the microorganisms that resisted medical therapy and contributed to death. In general, microbiologic examination is the only means to elucidate the etiopathogenesis of infection because pneumonia can be caused by a variety of microorganisms, including mycobacteria and fungi, which are frequently fatal, especially in children[34]. The contribution of autopsy to understanding the pathogenesis of emerging respiratory pathogens was evident in the recent SARS-CoV-2 virus pandemic, which was claimed COVID -19 and caused millions of deaths[35]. Autopsy revealed marked pulmonary involvement with findings of diffuse exudative and proliferative alveolar damage, vasculitis and microthrombosis of alveolar capillaries, pulmonary emboli, pulmonary hemorrhage, focal or diffuse bronchopneumonia and emphysema, and frameworks of mucosal tracheitis[36]. These autopsy findings have been crucial for the development of appropriate therapeutic strategies. An indispensable diagnostic tool in viral infections is usually the PCR technique, which can detect even small amounts of nucleic acids in tissue samples[37].
- *Tuberculosis* has different incidences in different countries, and although it can be suspected macroscopically at autopsy and mycobacterial infection demonstrated histologically, specimens for culture should be performed. Although not easy to perform, such procedures are essential to confirm the cause of death while investigating resistance profiles to a specific therapy. As for environmental non-tuberculous mycobacteria, they are ubiquitous and frequent interaction with humans takes place. Although they have been recognized as a cause of human infections, the epidemiological characteristics of such diseases remain largely unclear[38].
- *Fungal infections* typically occur in patients with predisposing factors such as an impaired immune system, hematologic cancers, AIDS and organ transplantation[39]. Fungal infections can also be fatal in individuals who appear to have no risk factors[40] or who experience unusual complications during surgery[41]. These forms of infection are difficult to diagnose intravitam, especially in immunocompromised individuals whose picture is often unclear, whose symptoms are nonspecific, and whose diagnostic tools are insensitive. In addition, intravitam cultures are often negative, especially when antifungal therapy is ongoing or when substrates of sufficient size for analysis cannot be collected. Therefore, fungal infections often remain undiagnosed, and their true incidence and pathogenesis depend largely on autopsy studies. Several researches have reported changing trends in fungal infections in autopsy studies[42], and the most commonly isolated species recently include *Candida albicans*, *Zygomycetes*, and *Penicillium* species. The various *Aspergillus* species responsible for localized or systemic forms of aspergillosis represent a nearly constant autopsy finding over time.
- *Infective endocarditis* of bacterial or fungal origin is a quite common disease that is still life-threatening despite recent diagnostic and therapeutic advances. The most commonly affected valves are the mitral and aortic ones. However, it is also possible to develop endocarditis derived from infected venous vessels by *Staphylococcus aureus*[43]. Nontheless, there is evidence that a large proportion of infective endocarditis is not diagnosed intravitam and the first diagnosis is not made until autopsy[44]. For this reason, macroscopic signs of valve infection (in both native and artificial valves), such as vegetations, ulcerations, or thickening, must be looked for at the autopsy. If infection is suspected, the best postmortem diagnostic approach is to combine blood cultures, valve samples, and histologic examination of valve sections to look for bacteria.
- *Meningitis* is a disease that is usually correctly diagnosed in symptomatic patients admitted to the hospital. When medical therapy fails and the affected person dies, microbiological diagnosis can be made in a minimally invasive manner by PCR of blood, CSF, and skin lesions. However, meningitis can also cause sudden death in children and adults, and these victims may be then examined by a forensic pathologist. Usually, macroscopic brain findings and sometimes visceral findings are very informative about the cause of death in fulminant meningococcemia[45]. However, microbiologic examination is essential to identify the pathogen involved (usually *Neisseriae meningitidis*, *Haemophylus influenzae*, *Streptococcus pneumoniae*, and *Legionella*) in the interest of the victim's close contacts and the general community to protect public health.

3.1.5. Death by drowning

The forensic diagnosis of drowning, after ruling out the other causes of death[46], is based on the concordance of a set of data[47] that can be obtained by laboratory analysis. Traditionally, diatoms (*Bacyllariophiceae*) have been used to improve the diagnosis of drowning because they spread to different organs after inhalation during cardiovascular activity[48,49]. However, the reliability of this test is controversial. Further studies have begun to analyze bacterioplankton (a bacterial component of plankton) as a possible substrate to confirm the diagnosis of drowning death. The cadaveric substrates successfully tested were liver, kidney, and lung samples[50] and blood[51] analyzed by a molecular PCR assay using fluorophore-labeled TaqMan oligonucleotide probes[52]. This technique also allows rapid and accurate detection of bacterial components in samples of drowning fluids, both hypotonic (freshwater with detection of *Aeromonas* and *Plesiomonas shigelloides*) and hypertonic (seawater with detection of *Vibrio* and *Photobacterium*)[53].

3.2. Role of bacteria in suspected child abuse and shaken baby syndrome

It is known that some natural infectious diseases can cause external lesions on the body that resemble traumatic injuries in type and shape[54]. For example, in pediatric victims, the differential diagnosis between skin hemorrhages in streptococcal sequelae of toxic shock syndrome and physical abuse can be particularly problematic. Toxic shock syndrome is a systemic infectious disease that can manifest as erythematous lesions and necrotizing skin caused by either exotoxin-producing strains of *Staphylococcus aureus* or erythrogenic toxin-producing strains of *Streptococcus pyogenes*[55]. However, in these cases, the forensic pathologist may have reasonable suspicion that the crime is one of child abuse. Therefore, microbiologic examination (autopsy cultures), flanked by histologic examination, plays an essential role because it allows identification of all microorganisms consistent with the observed picture and determines the final diagnosis. The microbiological approach is also crucial in properly evaluating suspected shaken baby syndrome (SBS). Indeed, especially in cases without typical trauma findings (e.g., fractures), the possibility that skin lesions rather than subdural and retinal hemorrhages have a natural cause must be considered. For this purpose, it is necessary to perform bacterial and viral microbiological analysis on different substrates: blood, urine, cerebrospinal fluid, brain, throat, lung, heart, spleen, and kidney. Each isolated microbial profile should be accompanied by histologic findings for vital reactions, C-reactive protein, and ante-mortem clinical information[56]. Only an integrated approach, necessarily based on microbiology, can rule out natural simulation conditions and determine whether a traumatic criminal event is indeed involved. Microbiology can also play a critical role in the assessment of child sexual abuse. In particular, the diagnosis of sexually transmitted infections such as *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Trichomonas vaginalis*, syphilis, or HIV in these individuals would strongly suggest that sexual abuse has occurred because they are transmitted almost exclusively by sexual practices[57,58].

3.3. Role of microbes in sexually transmitted infections

In forensic science, great importance is attributed to so-called "reckless transmission," i.e., infections acquired after sexual assault. In these cases, genotyping of their bacterial flora can prove that intimate contact occurred between the assailant and the victim of violence[59,60]. International forensic studies that have identified genetic markers for *Neisseria gonorrhoeae* and *Chlamydia trachomatis*[61] make it possible to link the perpetrator to his victim[59]. If the bacterial strain is rare, its rarity would be a possible clue[62]. However, in viral infections, such as HIV, this test is not helpful because of the high mutation rate of viruses[63].

3.4. Microbes in toxicological-forensic investigations

Bacteria can metabolize and degrade drugs and medications ingested by victims by colonizing and feeding on cadaveric tissue. Therefore, their postmortem activity may affect autopsy results regarding the consumption of drugs and poisons before death and consequently alter the interpretation of the results[64]. Indeed, microbial metabolism/degradation of antidepressants[65], antipsychotics, cannabinoids, cocaine, and heroin, as well as new formation of other metabolites, has been documented during decomposition of cadavers. For example, benzodiazepines are converted to their metabolites by the action of nitroreductase produced by various bacterial species such as *Bacillus cereus*, *Staphylococcus epidermidis*, *Streptococcus faecalis*, *Clostridium perfrigens*, and *Bacteroides fragilis*[66]. Morphine is also converted by hydrolysis to morphine-3-glucuronide (M3DG) by glucuronidase enzyme-producing bacteria of normal intestinal flora (*Escherichia coli*). In fatal alcohol intoxications, positive detection of ethanol in bacterial and yeast samples[67] from well-preserved cadavers is considered indicative of antemortem abuse. Nevertheless, there are interpretive difficulties in cadavers that are in an advanced stage of transformation because microorganisms ferment glucose to ethyl alcohol[68]. Therefore, the origin of postmortem ethanol can be attributed to either antemortem consumption or postmortem microbial production[69], and to this end, analysis of ethyl glucuronide (ETG), a metabolite of ethanol, would allow discrimination between the two substances. Gamma-hydroxybutyric acid (GHB) is a "recreational drug" commonly associated with alcohol and has been used in several cases of drug-related sexual assault[70]. It has been shown that some microbial species (*Pseudomonas aeruginosa* and *Clostridium aminobutyricum*) can convert GABA to GHB, leading to potential postmortem microbial production of GHB[71], which has been detected in biological fluids such as blood and urine. Finally, when toxins such as cyanide are metabolized, a decrease in postmortem concentrations has been observed in blood and tissues (lung, brain, liver, and kidney) of sacrificed experimental animals[72,73]. The observed decrease is thought to be related to the conversion of cyanide to thiocyanates, but the bacteria involved in this process are still unknown[74].

3.5. Microbes in medical malpractice and nosocomial infections

Hospital-acquired or transmitted infections (healthcare-associated transmission), i.e., infections that occur during hospitalization or after patient discharge (without prior clinical manifestation or incubation), are common and serious complications that occur in healthcare settings. Most of these morbid forms are due to opportunistic or less invasive germs (*Escherichia coli*, *Staphylococcus epidermidis*, *Pseudomonas*, *Aspergillus*, *Candida*, *rotavirus*, *adenovirus*), whose introduction, multiplication, and initiation of the disease state correlate with the patient's decreased resistance[70]. Nosocomial infections are usually associated with allegations of medical negligence leading to transmission of unintentional infections due to improper cleaning and maintenance of buildings, equipment, and supplies. However, proving whether the infection originated solely in the hospital, was due to noncompliance with aseptic techniques, or was an unavoidable event even though appropriate standards of care were applied is a challenge that often remains unresolved. In this context, DNA sequencing methods have in some cases provided sufficient means to obtain information about the source of contamination or to rule out its involvement. Overall, however, the results of microbiologic genetic testing remain controversial because they cannot capture all possible sources of nosocomial infections[75]. No other applications of microbiology to the topic of physician liability management and nosocomial infections are available to date.

3.6. Microbes as indicators of biological fluids

A biological finding is not always associated with a criminal incident, but can also be the result of "innocent" activity or contamination[76]. To further investigate how it was deposited, it is helpful to determine from which part of the body it originated. To this end, microbes in forensic samples can be considered indicators of different body fluids[77], although they do not consistently show absolute specificity. Moreover, in crimes, the body fluids involved are often mixed, which is why they have been the subject of studies that have attempted an identification approach using microbial markers[78,79]. In sexual assaults, for example, one usually deals with mixed body fluids[80], which in the past often could not be distinguished with certainty. Today, thanks to the evaluation of genital microbiomes, it is possible to determine the male and female participants[81] by finding predominantly *Lactobacillus* strains in the fluid, which is presumably vaginal secretion, using qPCR assays for 16S ribosomal RNA genes[82]. Notably, the vaginal bacterial microbiome has been found to exhibit interindividual variability that also reflects ethnicity[83]. Specifically, among white, black, Hispanic, and Asian women, the proportions of each bacterial group are statistically significantly different[84]. Therefore, the vaginal microbiome not only allows correct diagnosis of vaginal fluid[85] but can also be considered a potential new marker for personal forensic identification[86]. As for saliva, among the most important and unique bacteria forming its microbiome are *Streptococcus salivarius* and *mutans*[87]. They can be effectively identified on forensic substrates of various types (including objects)[88] by PCR with specific GTF (glucosyltransferase) primers or microarrays[89]. Searching for and identifying these bacteria may allow discrimination of the origin of a blood sample from the oral cavity[88]. It is also possible to distinguish a blood sample from the oral cavity from a blood sample from the nose by looking at the different microbiome, with the latter area predominantly colonized by *Firmicutes* and *Actinobacteria*[90]. Similarly, traces of menstrual blood can be successfully detected[91,92]. Another approach to detection is based on the technique of reduced metagenome sequencing, which has shown promising results[93]. In a recent study[94], biological fluids exposed to controlled environmental conditions for 30 days were shown to retain their microbial "signature" of the site of origin.

3.7. Microbes as indicators of the skin

Human skin also harbors a variety of microbes that can be transferred to surfaces ("tactile microbiome"), and these microorganisms can be considered as forensic markers such as "tactile DNA"[95]. Some studies have examined the interaction between these microorganisms and various objects: in particular, it has been investigated whether the microbiota of the fingers of the hand can be detected on the keys of PC as well as on a computer mouse[96] and on the telephone, including quantification of the bacterial communities shared by telephones and human fingers[97]. Other studies have successfully examined microbial communities transferred from hands to different types of textiles (cotton and polyester) so that their correspondence could be reconstructed[98]. Similarly, this approach has been applied to items used at indoor crime scenes (e.g., doorknobs, faucets, computers, and medical equipment), and postmortem skin microbiomes have been shown to be stable for up to 60 h with repeated sampling[99]. However, the study noted that it is difficult to determine when a person last touched an object, which can significantly affect forensic considerations. Indeed, microbial communities may change and lose their properties over longer periods of time. Finally, some items may be made of materials that prevent or inhibit microbial colonization.

3.8. Microbes in personal identification

The development of biomolecular techniques has made it possible to identify the microbial components of the human body, leading to the Human Microbiome Project (HMP)[12]. The possibility of identifying a human individual by the microbial communities that inhabit it is currently being discussed in the scientific community. Such a technique could make an important contribution to doubtful cases in which analysis of remains by anthropological and genetic forensics cannot be performed satisfactorily. Initial protocols for obtaining human DNA for the identification of human microbial profiles[100] require further investigation. It is not known whether the microbial communities of different individuals exhibit sufficiently significant and stable variation over time to uniquely identify an individual. In this regard, the most promising studies have focused on smaller taxa[101] using multiple body sites[102] but have not yet yielded useable results for forensics. However, there are initial approaches to unambiguously identify human individuals in the population based on microbial profiles that originate from a particular environment[103] and permanently colonize their bodies[104]. The limitation here, however, is that comparative accuracy in identifying individuals decreases as the number of comparison individuals potentially sharing the same environment, lifestyle, and microbial patterns increases[105,106]. To this end, the establishment of biobanks of microbiomes from large populations specifically for human forensic identification could be helpful[107]. Biological sex differentiation using the human tanatomicrobiome has yielded encouraging results in several studies by allowing observation of differential tissue distribution of bacterial communities in deceased individuals of the opposite sex[108]. Other studies have theorized that the absence of different bacterial skin types may also be related to the use of cosmetic products[109]. Current data show excellent accuracy in estimating sex, whereas prediction of other personal characteristics has been less successful[109]. Therefore, these studies are only a first step, and further research is needed before their results can be validly applied in forensics.

3.9. Microbes as forensic indicators of soil and a specific place

Soil represents an excellent and increasingly recognized potential for forensic investigation because it harbors unique microbes that may have specific physical and chemical characteristics[110] that indicate the specificity of the crime scene[111]. In fact, it can be used as evidence because its traces adhere under fingernails, shoe soles, tire treads, weapons, and clothing[112]. Soil can also be "misplaced" during the commission of a crime, and on this basis geolocation[113] allows the origin of a soil sample of unknown origin to be indicated, a specific search area to be narrowed down (urban environment, rural environment, different cities)[114], and a suspect to be "linked"[115] to a specific crime scene[116], as well as whether a victim was moved from one location to another where he or she was later found[112]. Various soil microbial samples were analyzed as a function of distance[117] and were found to differ with increasing distance, suggesting that both soil type and geographic location are important factors in microbial community composition. The latter was correctly classified by site of origin in 95.4% of bacterial soil profiles[118,119]. Soils associated with crime scenes exhibit altered bacterial communities due to decomposition of a corpse in the open, resulting in a significant change in available nutrients, biochemistry, and microbiome composition of the local community in addition to that of the corpse[120]. These specific soil changes can also be used as circumstantial evidence of the location of illegal burials[121]. In the context of identifying the origin from a specific geographic location, the Forensic Microbiome Database (FMD) was created in 2016[122], which contains microbiome data from various sites in the human body from 35 different countries[123]. Microbiome and DNA studies of soil bacteria are on the rise[115,124,125] and have proven to be reliable forensic tools in certain circumstances. Other research has focused on the plant microbiome from plant fragments found at crime scenes and/or autopsies. The latter aspect needs to be explored in further studies but could provide important insights into the origin of the fragments and their seasonal/temporal nature[125].

3.10. Microbes in the determination of PMI (Post mortem-interval)

Estimation of PMI is a complex forensic assessment, and differential flux in the succession of colonizing microbial communities in major organs has been studied in animal models of pigs and mice[126,127] to improve the accuracy of PMI estimation. Such sequences of exogenous and endogenous bacterial species have been shown to be useful for PMI estimation[128] and to allow assignment to a specific time interval. The first microorganisms microbiologically isolated from the cadaver belong to *Staphylococcus* species, followed by coliform organisms and various *Candida* species, whereas the last microorganisms colonizing the cadaver belong to anaerobic species[129]. In this context, *Clostridium* sp. was confirmed to be most abundant in long PMI[130]. Recently, models built at the taxonomic level of classes or strains have been shown to provide more accurate information about PMI[131]. The use of human samples for forensic microbial studies is still in the early stages of research. To date, only a few studies have been performed, but they have provided interesting data. For example,[132] pyrosequencing was used to measure the richness and diversity of microbial communities from the mouth, rectum, and internal organs of two cadavers in the pre-emphysematous and post-emphysematous phases. The abundance of microorganisms was found to increase from the upper to the lower gastrointestinal tract. Recently, a study was conducted to determine microbial succession at various body sites of human cadavers in an outdoor setting[133]. Finally, Johnson et al. examined the skin microbiome of decomposing human cadavers and developed an algorithm to estimate PMI[134]. An international database containing all information on microbial succession as a function of body sites examined, type of death, and place of death or discovery needs to be created[135] to significantly advance reliable estimation of PMI based on microbiological data.

3.11. Microbes in paleopathology

A special application of microbiology in forensic science is paleomicrobiology, i.e., the study of pathogens in skeletonized and mummified corpses[135]. These studies make an important contribution to the molecular evolution and phylogenetic relationships of various microorganisms and allow comparative analysis of modern and ancient microbial sequences[136]. The development of molecular tools that allow the extraction and identification of pathogenic DNA from human remains has, over time, enabled the identification of a variety of pathogens[137] responsible for serious bacterial diseases (tuberculosis, cholera, leprosy, bubonic plague, typhoid fever, and syphilis), viral (influenza, hepatitis B, human poxvirus, human papillomavirus, human lymphotropic virus T -HTLV-1-, and human immunodeficiency virus -HIV-), and parasitic (malaria, leishmaniasis, and Chagas disease). Whole genome sequencing of microorganisms from bone material, including older material, provides information on the mechanisms of pathogen evolution and adaptation and is critical for understanding emergence and reinfection[138]. In addition, it can be used to study the impact of environmental factors, the spread of disease from its point of origin, which is often related to human migration, the difficulty in eradicating certain diseases in certain parts of the world compared with others, and, last but not least, the impact of the introduction of antibiotic therapy. Therefore, the extraction of microbial DNA from human skeletal remains is an important research tool and a historical source of new insights into host genomics and provides information about possible past infections in the body.

4. Conclusions

For years, microorganisms have been used as forensic evidence in criminalistics, but microbiology is an ever-evolving science in which new applications are constantly being developed, many of which have great potential. Some applications are already more widespread than others, so only preliminary results are available to date. In this narrative review, we have compiled all relevant information on the various forensic applications of microbiology to provide a comprehensive and versatile overview of this fascinating subject. The contribution that microbiological studies can make to cadaveric material collected during autopsy examination has proven to be fundamental. Undoubtedly, further research is needed to explore the efficacy of microorganisms in general and their full applicability for forensic purposes, but in the meantime there is no doubt that microbiology stands out as one of the fundamental disciplines in forensic investigations.

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Authors' contributions

TS and MF equally contributed to this work: writing – original draft, writing – review & editing, conceptualization, investigation, methodology, data curation. GG, BM, BP and MM contributed to investigation and methodology, to literature research and editing. RZ guarantor of the project and directed the study, devised the main conceptual idea of the article.

Declaration of competing interest

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