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POSSIBILITIES OF USING MICROBIOME ANALYSIS IN FORENSICS

Summary

The article takes a closer look at the applicability of forensic microbiology in cases other than biohazard and bioterrorism. Applications have been limited to microorganisms present virtually everywhere, in distinctive communities, called microbiomes. The issue of personal identification is presented and extended to the analysis of “bacterial fingerprints,” i.e. bacterial communities characteristic of each person. The potential for locating crimes, including typing the locations of hidden corpses based on soil microbiome studies, is discussed. The related possible ways of eliminating suspects are characterized, and a method for estimating the time of death based on microbiome studies of cadavers and soil in the immediate vicinity is described. The review paper aimed to compile the most common foreign studies conducted on the use of microbial communities in forensic practice, and attempt to present promising perspectives in the pages of a Polish forensic journal, as the topic seems insufficiently explored in our country.

Keywords: microbiome, microorganisms, personal identification, scene, time of death

Introduction

Microbiology is a science that is juxtaposed with forensic science much less frequently than other fields (such as genetics or chemistry). Its most widespread and probably first large-scale application in the criminal sciences relates to combating bioterrorism, with the 2001 incident in the United States particularly cited. Packages containing anthrax bacilli as biological weapons have been sent to several locations. These actions resulted in the deaths of five people and infected seventeen, and as a result, the FBI opened an investigation and sought the help of the Genomic Research Institute. The Institute of Genomic Research) in Rockville to analyze microbial traces. The

spores tested were linked years later to a scientist working at a government lab in Maryland, who committed suicide as soon as he became suspicious¹. In contrast, in standard investigations and prosecutions, forensic microbiology is giving way to fields that have become much more useful and informative on this front, such as the genetics mentioned at the beginning. The essence of the application of a discipline in forensic science is the possibility of obtaining information through it that can be used in legal proceedings. The diagnostic value and subsequent evidentiary value are indicated here. For the proposed research method to have a high diagnostic value, it should be characterized by relevance and reliability, which is established based on experimental results. Relevant methods are those that provide the desired information, and reliable methods are characterized by accuracy. Forensic expert reports using methods of high diagnostic value and approved by the justice system are, from the point of view of forensic science, studies of high evidentiary value². Despite its lesser popularity, microbiological testing, due to its promising evidentiary value, may prove significantly more useful in the future.

Leaving the topic of bioterrorism, the forensic use of microbiology can be referred to as the concept of the microbiome. This term was first used by Joshua Lederberg. With his help, he described the ecological system of microorganisms residing in the human body³. Today, the microbiome is called the total of microorganisms inhabiting a given environment. These include bacteria, fungi, viruses, and archaeons. It is also formed by the interrelationships and interactions with the environment in which the microorganisms reside, as well as their characteristic genomes and proteomes. This is determined by the evolutionary colonization of newer and newer ecological niches by microorganisms, which are thus characterized by an individual set of microflora. The diversity of the microbiomes of humans and other animals is fostered by the direct interaction of individual species with each other, as well as with the terrain in which they reside⁴.

¹ National Institute of Justice, *The Forensic Microbiome: The Invisible Traces We Leave Behind*, June 7, 2021, nij.ojp.gov, <https://nij.ojp.gov/topics/articles/forensic-microbiome-invisible-traces-we-leave-behind> (accessed: 30.01.2023 at 2 p.m.).

² A. Domin-Kuźma, *Wartość diagnostyczna i wartość dowodowa badań DNA*, "Przegląd Bezpieczeństwa Wewnętrznego" 2012, no. 4(7), pp. 77–78.

³ J. Lederberg, A.T. McCray, 'Ome Sweet 'Omics – A genealogical treasury of words, "The Scientist" 2001, no. 15(7), p. 8.

⁴ Z. Gliński, K. Kostro, *Mikrobiom – charakterystyka i znaczenie*, "Życie Weterynaryjne" 2015, no. 90(07), p. 446.

Speaking of humans, one cannot refer to the entire body as a single ecological niche. Its various parts and internal organs create radically different conditions that favor the colonization of distinct microbial species. Typically, the microbiome of the skin, oral cavity, respiratory system, gastrointestinal tract, and genitourinary tract are distinguished. The very skin on certain parts of it is characterized by a kind of diversity. The presence of selected species is determined by anatomical differences, moisture levels, or the distribution of sebaceous glands. Areas such as the groin are characterized by higher temperatures and humidity, which is conducive to microorganisms that thrive in conditions typically associated with Gram-negative bacilli or *Staphylococcus aureus*. On the other hand, the skin of the face and back, rich in sebaceous glands, is an environment for lipophilic bacteria, such as *Propionibacterium* spp. It is also worth mentioning that parts of the body that are “exposed” and susceptible to various types of weathering, namely the arms and legs, are not as rich in microorganisms⁵.

The article discusses the potential of microbiomes in the context of expanding forensic investigations, focusing on personal identification, locating crime scenes based on microbial traces, and a method for estimating the time of death. The study aimed to list the most frequently mentioned applications of microbiome analysis in forensic science in foreign literature and to try to popularize them in Poland since this type of research is not conducted on a large scale in our country.

Bacterial fingerprint

In forensic terms, special attention should be paid to the skin microbiome, which is characteristic of every person⁶. This fact may promote the expansion of research leading to even more effective individual identification. The most widespread tests of this type are genetic tests; within them, individual identification is based on the analysis of short DNA fragments, so-called microsatellite sequences (STRs), located in autosomal chromosomes. Analysis of X or Y chromosome variation and mitochondrial DNA is also routinely performed⁷. At the scene of an incident, a potential perpetrator often leaves behind biological traces in the form of genetic material,

⁵ M. Malinowska, B. Tokarz-Deptuła, W. Deptuła, *Mikrobiom człowieka*, “Postępy Mikrobiologii” 2017, no. 56(1), p. 34.

⁶ P. Tozzo, G. D’Angiolella, P. Brun, I. Castagliuolo, S. Gino, L. Caenazzo, *Skin microbiome analysis for forensic human identification: What do we know so far?*, “Microorganisms” 2020, no. 8(6), p. 2.

⁷ B. Hołyst, *Kryminalistyka*, 13th edition, PWN Scientific Publisher, Warsaw 2018, pp. 460–462.

which can be uncovered, secured, and examined by experts, and then used in a case. The leaving of microbiological traces is done on a similar basis. The ecological niche that is human skin mixes with the environment. For example, after touching a surface at the scene of an incident, microbes previously residing on the body of the potential perpetrator stay on the surface in question, and those previously remaining on the scene are transferred to his body. This was demonstrated, among other things, by Meadow et al. comparing samples taken from test subjects with samples obtained from subjects in the classroom⁸. This mechanism is reminiscent of Edmond Locard's principle of exchange and even bookends it.

Differences in the microbiome between people are due to personal characteristics such as age and gender, for example, the dissimilarity between men and women is due to different types of hormones secreted by their bodies⁹. The presence or absence of disease and overall health are also cited as important factors¹⁰. In addition to an individual's predisposition, environmental conditions, namely diet, interpersonal relations, a person's occupation, cosmetics used, or clothing worn, also have an impact. Attention is also paid to the medications taken, primarily antibiotics, which have bactericidal and bacteriostatic effects¹¹. As mentioned earlier, a bidirectional effect has been shown in diversification; while environmental microbes can affect the microbiome of people spending time in a particular place, people also leave microbes living on the surface of the body in the surrounding environment, changing its microbiome in the process¹².

Given this rationale, microbiological analysis can allow people to be differentiated based on their microbiomes and contribute to individual human identification. Research on microbial communities associated with humans, such as the *Human Microbiome Project*¹³, can help with this. Even

⁸ J.F. Meadow, A.E. Altrichter, S.W. Kembel, M. Moriyama, T.K. O'Connor, A.M. Womack, G.Z. Brown, J.L. Green, B. Bohannan, *Bacterial communities on classroom surfaces vary with human contact*, "Microbiome" 2014, no. 2(7), <https://link.springer.com/article/10.1186/2049-2618-2-7> (accessed: 05.02.2023 at 3.40 p.m.).

⁹ M. Malinowska, B. Tokarz-Deptuła, W. Deptuła, op. cit., p. 34.

¹⁰ R.C. Casarin, A. Barbagallo, T. Meulman, V.R. Santos, E.A. Sallum, F.H. Nociti, P.M. Duarte, M.Z. Casati, R.B. Gonçalves, *Subgingival biodiversity in subjects with uncontrolled type-2 diabetes and chronic periodontitis*, "Journal of Periodontal Research" 2013, no. 48, pp. 30–36.

¹¹ M. Malinowska, B. Tokarz-Deptuła, W. Deptuła, op. cit., p. 34.

¹² S. Fujiyoshi, D. Tanaka, F. Maruyama, *Transmission of airborne bacteria across built environments and its measurement standards: A review*, "Frontiers in Microbiology" 2017, no. 8, p. 2336.

¹³ P.J. Turnbaugh, R.E. Ley, M. Hamady, C.M. Fraser-Liggett, R. Knight, J.I. Gordon, *The Human Microbiome Project*, "Nature" 2007, no. 449(7164), pp. 804–810.

monozygotic twins have been shown to harbor fundamentally different microbial communities¹⁴, suggesting that the collective genomes of microbes inhabiting the human body may allow for more specific identification than individual DNA. However, the degree of diversity in the skin microbiome depends on the taxonomic selectivity of the analysis performed. In all people, the characteristic clusters found on the skin can be identified, which is not particularly helpful in terms of distinguishing between individuals. However, when the genus, species, and strain of the population are taken into account, the individualization is high enough to equal that of fingerprint examinations¹⁵.

Over the years, researchers have tried to demonstrate the possibility of linking a suspect to evidence in an investigation based on molecular analysis of microbiological material taken from a typed person and from the surface of the object examined. As demonstrated by Ziemińska-Buczyńska and Kraśnicki, in 9 out of 14 cases they studied, microbiome samples taken from the mobile device were similar to those obtained from the epidermis of their users (Dice ratio >70%)¹⁶. Similar experiments were conducted by Fierer et al. who described a high similarity between the bacteria present on the keyboards used by the test subjects and on their fingertips. They also proved that an object belonging to its owner (in this case, a computer mouse) is colonized by bacteria that are primarily inherent to it. To do this, they used a database of microbiomes from the hands of 240 people; of the 9 samples taken from the subjects, all showed the greatest similarity with samples taken from their owners¹⁷.

Forensic microbiological analysis may carry more potential benefits than fingerprint analysis or DNA sequencing, e.g., a fingerprint trace may not always be valuable evidence, and sometimes it is not present at all (use of gloves, obliteration of traces). Blurred or only partial prints sometimes hinder analysis, and can even derail it altogether. For similar reasons, microbiome analysis can complement classical genetic testing. When only low

¹⁴ P. J. Turnbaugh, M. Hamady, T. Yatsunenko, B.L. Cantarel, A. Duncan, R.E. Ley, M.L. Sogin, W.J. Jones, B.A. Roe, J.P. Affourtit, M. Egholm, B. Henrissat, A.C. Heath, R. Knight, J.I. Gordon, *A core gut microbiome in obese and lean twins*, "Nature" 2009, no. 457, pp. 480–484.

¹⁵ M. Oliveira, A. Amorim, *Microbial forensics: New breakthroughs and future prospects*, "Applied Microbiology and Biotechnology" 2018, no. 102, pp. 10385.

¹⁶ A. Ziemińska-Buczyńska, K. Kraśnicki, *Application of the PCR-DGGE method in forensic microbiology*, "Problems of Forensic Science" 2016, no. 292, pp. 15–21.

¹⁷ N. Fierer, C.L. Lauber, N. Zhou, D. McDonald, E.K. Costello, R. Knight, *Forensic identification using skin bacterial communities*, "Proceedings of the National Academy of Sciences" 2010, no. 107(14), pp. 6477–6481.

biomass or degraded samples are available, making it impossible to obtain a full STR profile, it may be easier to isolate bacterial DNA from a potential offender than its DNA. Assenmacher, Fields, and Crupper proved in their study that trace amounts of bacterial DNA from fingerprints are sufficient to analyze the microbiome¹⁸.

Microbiological traces are a promising source of speculation to identify the person who came into contact with the scene. As mentioned earlier, the microbiome is influenced by a number of both environmental and individual factors. This fact can be used to pick out the person in contact with the surface from which the sample was taken. Ying et al. showed that it is possible to infer age, gender, and whether a person lives in a rural or urban environment from samples taken from various parts of the subjects' bodies¹⁹.

The information gathered in this way could help rule out suspects and put the investigation or inquiry on the right track. The prospect of developing a new identification technique that is as reliable as the currently trusted tests is an important issue for both the justice system and forensic science itself.

Environmental microbiome

Research in environmental microbiology has shown that many microorganisms are common in water and soil, and their species and strains vary by region²⁰. Soil analysis, which involves recognizing its composition in terms of both inanimate and animate matter, can provide a wealth of valuable evidence. This is because the advantage of soil is its ability to adhere to and hold onto many surfaces, such as shoes, tires, and tools. As with human samples, soil can be studied in terms of its microbiome profile, the structure of which is determined by several factors, such as soil type, seasonal variation, vegetation cover, and environmental conditions²¹.

¹⁸ D.M. Assenmacher, S.D. Fields, S.S. Crupper, *Comparison of commercial kits for recovery and analysis of bacterial DNA from fingerprints*, "Journal of Forensic Sciences" 2020, no. 65(4), pp. 1310–1314.

¹⁹ S. Ying, D.-N. Zeng, Y. Tan, C. Galzote, C. Cardona, S. Lax, J. Gilbert, Z.X. Quan, *The influence of age and gender on skin-associated microbial communities in urban and rural human populations*, "PloS one" 2015, no. 10(10), e0141842, <https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0141842> (accessed: 09.02.2023 at 8 p.m.).

²⁰ J. Zhang, W. Liu, H. Simayijiang, P. Hu, J. Yan, *Application of microbiome in forensics*, "Genomics, Proteomics & Bioinformatics" 2022, <https://www.sciencedirect.com/science/article/pii/S1672022922000961> (accessed: 10.10.2023 at 8:20 a.m.).

²¹ N.S. Grantham, B.J. Reich, K. Pacifici, E.B. Laber, H.L. Menninger, J.B. Henley, A. Barberán, J.W. Leff, N. Fierer, R.R. Dunn, *Fungi identify the geographic origin of dust samples*, "PloS one" 2015, no. 10(4), e0122605. <https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0122605> (accessed: 10.10.2023 at 10:20 a.m.).

Finley et al. showed that the soil microbiome is altered in the vicinity of decomposing cadavers. The taxonomic diversity of soil samples for corpses decomposing on the surface increases exponentially, while the same trend for a sample from the soil in which the corpse was buried takes a U-shape. In addition, these researchers confirmed that *Proteobacteria* are the most abundant cluster in all grave soil samples, while in surface samples, as decomposition progresses, the abundance of *Acidobacteria* decreases, while *Firmicutes* increases²². These changes can be used as biological clues in locating potential burial sites by examining the soil in a selected area. Consequently, a better understanding of the structure of soil microbial communities and their changes during decomposition may be important for forensic science.

Research also indicates that samples from different locations can be distinguished from each other based on their microbiomes. In recent years, Habtom et al. used terminal restriction fragment length polymorphism (TRFLP) to characterize microbial DNA profiles in three soil types, at local and regional scales (2 m – 260 km). They showed that DNA profiles of soil microorganisms allow inferences to be made about the geographic location of the sample source to at least 25 m regardless of soil type and environmental conditions. They also found that geographic location is more important than soil type for microbiome diversity²³. Similar studies also suggest that soil microorganisms can provide clues to the source of secured samples. Yang et al. using 16S rRNA gene sequencing in 529 samples collected from 61 districts of 10 major cities in China determined microbiome profiles, which they then assigned to specific districts and cities with an accuracy of 66.7% and 90%, respectively²⁴. Such findings are extremely promising for expanding forensic research. For example, taking swabs from a shovel that may have been used as a tool in the criminal burial of a corpse will yield a microbiological trace; this, in turn, when analyzed, will be linked to where the body was hidden.

Creating multiple databases of the soil microbiome and developing machine learning algorithms could provide much more effective results.

²² S.J. Finley, J.L. Pechal, M.E. Benbow, B.K. Robertson, G.T. Javan, *Microbial signatures of cadaver gravesoil during decomposition*, "Microbial Ecology" 2016, no. 71, pp. 524–529.

²³ H. Habtom, Z. Pasternak, O. Matan, C. Azulay, R. Gafny, E. Jurkevitch, *Applying microbial biogeography in soil forensics*, "Forensic Science International: Genetics" 2019, no. 38, pp. 195–203.

²⁴ T. Yang, Y. Shi, J. Zhu, C. Zhao, J. Wang, Z. Liu, X. Fu, X. Liu, J. Yan, M. Yuan, H. Chu, *The spatial variation of soil bacterial community assembly processes affects the accuracy of source tracking in ten major Chinese cities*, "Science China Life Sciences" 2021, no. 64, pp. 1546–1559.

The Earth Microbiome Project (EMP), which aims to standardize testing methods and collect data on samples from different soil types collected around the world, appears promising²⁵. In contrast, an example of the application of machine learning is the research of Grantham et al. who used the DeepSpace algorithm to analyze a database containing more than 1,300 dust microbiome profiles. They related their research to the considerations of the aforementioned Edmond Locard, who was already interested in this issue in the 1930s. By analyzing only the fungi in the dust, the algorithm they presented selected the location of the sample country with an accuracy of almost 90%²⁶.

A review of previous research work focused on the environmental microbiome and linking it to databases leads to the conclusion that it offers new opportunities as to the location of crimes of all kinds.

Estimating the time of death

In cases involving the death of a human being, important from a forensic point of view, in addition to recognizing the event and generally answering all the “seven golden questions” (what? where? when? how? why? by what means? who?), is to determine the time that passed between the death and the finding of the corpse. This information is one of the key findings of the investigators’ version and is dealt with primarily by forensic medicine. A number of respected and standardized methods are used to determine this interval. Measurement of cadaver temperature, examination of precipitation stains, and postmortem concentration are leading the way. The above-mentioned methods are applicable shortly after death, because over time it becomes impossible to make these analyses or the information resulting from them is completely useless²⁷. The reason is that corpses gradually decompose under the influence of bacteria, generally saprophytic. This process is categorized as a late postmortem mark and referred to as putrefaction. At the same time, gases that are products of the decomposition process (carbon dioxide, hydrogen sulfide, methane, ammonia) gradually accumulate in the tissues. Recognizing the time of death in the late stages of decay can be significantly

²⁵ J.A. Gilbert, J.K. Jansson, R. Knight, *The Earth Microbiome project: Successes and aspirations*, “BMC Biology” 2014, no. 12, p. 69.

²⁶ N.S. Grantham, B.J. Reich, E.B. Laber, K. Pacifici, R.R. Dunn, N. Fierer, M. Gebert, J.S. Allwood, S.A. Faith, *Global forensic geolocation with deep neural networks*, arXiv, 2019, 1905.11765, <https://arxiv.org/abs/1905.11765> (accessed: 13.02.2023 at 10 a.m.).

²⁷ R. Prabucki, *Czas zgonu w kontekście nauk penalnych*, “Zeszyty Naukowe Ruchu Studenckiego” 2016, no. 1, pp. 27–36.

difficult or even impossible²⁸. Another obstacle is the unsuitability of other techniques for estimating the time of death, such as forensic entomology when a corpse is found in an environment where insects are not present due to unfriendly conditions²⁹. This raises the need to isolate other methods that can complement or even replace those used so far.

It has been observed that the amount of putrefactive bacteria and products of the decomposition process changes over time according to certain patterns, which makes it possible to estimate the moment of death based on the composition of the microbiome of the corpse and the soil in the immediate vicinity³⁰. Among mammals, they are somewhat similar and reproducible³¹, hence most of the work uses animal models to conduct the study. Among others, they were used by Metcalf et al. who used mice as models. They collected microbiome samples from the animals' bellies, backs, and heads, as well as from the soil in their immediate vicinity. Thus, using microbial analysis, they estimated the time of death with an average absolute error of about three days over 48 days. They obtained the lowest error for samples taken from the scalp³². Another study, this time on four human cadavers, was conducted by researchers DeBruyn and Hauther, who sampled the cecum several times at intervals. Sequencing of the 16S rRNA gene amplicon revealed that over time the number of bacterial species increased significantly, while their diversity decreased and specific species dominated. The results showed that the abundance of microorganisms from the order *Bacteroidales* decreased over time, contrary to the occurrence of representatives of the order *Clostridiales* and the class *Gammaproteobacteria*, which were more numerous³³. A similar relationship had already been observed by Hauther, Cobaugh, Jantz, Sparer, and DeBruyn. Using gut microbiome samples from

²⁸ G. Teresiński, *Medycyna sądowa*, vol. 1, PZWL, Warsaw 2019, pp. 128–129.

²⁹ H. Volckaert, *Current applications and limitations of forensic entomology*, "Themis: Research Journal of Justice Studies and Forensic Science" vol. 8, Article 4, pp. 9–10.

³⁰ J.L. Pechal, T.L. Crippen, M.E. Benbow, A.M. Tarone, S. Dowd, J.K. Tomberlin, *The potential use of bacterial community succession in forensics as described by high throughput metagenomic sequencing*, "International Journal of Legal Medicine" 2014, no. 128, pp. 193–205.

³¹ J.L. Metcalf, *Estimating the postmortem interval using microbes: Knowledge gaps and a path to technology adoption*, "Forensic Science International: Genetics" 2019, no. 38, pp. 211–218.

³² J.L. Metcalf, L. Wegener Parfrey, A. Gonzalez, C.L. Lauber, D. Knights, G. Ackermann, G.C. Humphrey, M.J. Gebert, W.V. Treuren, D. Berg-Lyons, K. Keepers, Y. Guo, J. Bullard, N. Fierer, D.O. Carter, R. Knight, *A microbial clock provides an accurate estimate of the post-mortem interval in a mouse model system*, "elife" 2013, no. 2, e011104, <https://elifesciences.org/articles/11104> (accessed: 13.02.2023 at 12.03).

³³ J.M. DeBruyn, K.A. Hauther, *Postmortem succession of gut microbial communities in deceased human subjects*, "PeerJ" 2017, 5, e3437, <https://peerj.com/articles/3437/> (accessed: 13.02.2023 at 2 p.m.).

12 cadavers, they showed that the abundance of *Bacteroides* and *Lactobacillus* decreases exponentially as body decomposition progresses, thus proving the potential usefulness of quantitative studies of bacteria in this genus for estimating the time of death³⁴.

The prospect of the research cited seems promising, but the postmortem microbiome may be affected by several factors that have not yet been fully studied, including the presence of insects feeding on the corpse³⁵ or the season³⁶. In their conclusions, the researchers agree that this method of estimating the time of death still needs to be clarified and further analyzed. This does not change the fact that microorganisms, like insects feeding on corpses, can be a good indicator in estimating the time of death. Definitely among the advantages are their ubiquity regardless of the season and survival in extreme conditions.

Summary

Microbes accompany us throughout our lives and have been “witnesses” to every crime; this fact favors their use by humans. In the end, whether we want it or not, as humans we strive to adapt our environment to our advantage. It is natural to develop in this direction. Microbial research and the prospect of implementing it into everyday forensic practice is encouraging, but its widespread use requires more work from researchers around the world.

The results of the ongoing research work presented statistically and the evidence that the “bacterial fingerprint” can be used to distinguish individuals suggest that in the future it will be possible to use these profiles for forensic investigations. However, microbiome-based personal identification should never replace traditional DNA profiling or fingerprinting techniques; it can only help expand existing evidentiary options. The case is similar to analyzing the environment in this regard or estimating the time of death. Forensic microbiology cannot replace physicochemical, traseological, or classical forensic methods. It should be considered an extension of current procedures, which in itself already has a lot of advantages. As you know

³⁴ K.A. Hauther, K.L. Cobaugh, L.M. Jantz, T.E. Sparer, J.M. DeBruyn, *Estimating time since death from postmortem human gut microbial communities*, “Journal of Forensic Sciences” 2015, no. 60(5), pp. 1234–1240.

³⁵ J.L. Pechal, T.L. Crippen, M.E. Benbow, A.M. Tarone, S. Dowd, J.K. Tomberlin, op. cit., pp. 193–205.

³⁶ J.L. Pechal, T.L. Crippen, A.M. Tarone, A.J. Lewis, J.K. Tomberlin, M.E. Benbow, *Microbial community functional change during vertebrate carrion decomposition*, “PloS one” 2013, no. 8(11), e79035, <https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0079035> (accessed: 13.02.2023 at 7:30 p.m.).

– the more corroborating and concordant studies, the more credible the version presented. Substituting proven methods would be justified, and even helpful, only in the absence of other traces.

Once the nature of the microbiome has been thoroughly explored and effective methods have been developed, it would be appropriate to think about putting it into practice. This will require significant investment in the standardization and implementation of techniques for analyzing microbiome profiles, as well as the development of techniques for revealing and securing microbiological traces that could automate or accelerate profiling in this way. For comparative studies, constantly updated databases would need to be created. Although there is a lot of work to be done, the introduction of a new type of research may prove to be an effective method of increasing the rate of cleared cases and reducing the number of crimes in Poland.

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No

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