Microbes as Biomarker for Drugs of Abuse: A Narrative Review

Shivani Sharma¹, *Shivam Dwivedi²

¹Msc.Forensic science student, Chandigarh University Gharuan, Mohali, Punjab ²Assistant Professor, Chandigarh University Gharuan, Mohali, Punjab <u>¹sharmashivani0567@gmail.com</u>, ²shivamdwivedi204@gmail.com

Abstract

The undiscovered microscopic world is a key to advancement in multidisciplinary fields like Medicine, Toxicology, Forensic science, and Microbiology etc. The microbial forensics is one of such advancing field helps in decoding the identification of illicit drugs from abusers by taking advantage of microbial metabolic pathways of drug. The microbes metabolize illicit drugs like cocaine into its metabolites (ecgonine methyl ester, benzoic acid, and methanol) indicating pre mortem administration of drug. The unexplored microbiome can aid in future crime scene investigation techniques like Biosensors using bacteria metabolic pathway to ascertain drug identification. The microbial approach has advantage over other techniques as it is easily modulated, large sampling according to experimentation without involving animals. However, microbes of soil and sewage water also construct a forensic epidemiological approach to reveal the drug operation of clandestine laboratory. The future research will aid the control of drug trafficking and identification of illicit parent drug.

Key Words: Illicit drug, Post-mortem Toxicology, Biosensor, Forensic epidemiology, Bioterror

Introduction

The drug abuse has been a growing concern worldwide for community health problems and criminal cases. The differentiation of illicit drug and licit use is still a borderline concept especially when designer drugs are made licit in name of "Research Drug" or recreational drug in clandestine black market [1]. The illicit drugs are being defined by the United Nation Office on Drugs and Crime (UNODC) as the way in which they are formulated, manufactured, and distributed through drug trafficking by avoiding the legal norms of controlled substances considered as illicit drugs. The norms of controlled substances schedule 1 do not have any medical use for example methylation of amphetamine (Methamphetamine), Di acetylation of Morphine (Heroin) is considered as illicit drugs[2]. The identification of drugs of abuse with sensitive and specific techniques because of biotransformation and degradation of illicit drugs in biological system have become topic of future research. The degradation of parent drug is aided by the microbiota of gastro- intestinal tract, bio transforming parent drug into metabolites. The metabolites can be detected by spectroscopic methods or mass spectroscopy for specifications.

The identifications of metabolites give chemical clue to the parent drug thus giving cause of death and pre mortem drug administration in post mortem toxicological samples, illicit drugs like Cocaine are bio transformed by *Rhodococcus spp*. bacteria to ecgonine methyl ester and benzoic acid and Pseudomonas *maltophila* cocaine to benzoylecgonine and methanol[3]. The forensic epidemiology is also a growing research to identify location of clandestine laboratory manufacturing of illicit drugs through soil and waste water having contaminants of metabolites of drugs of abuse. The biofilm samples of water were used to identify drugs in environmental matrices which showed presence of Cocaine and Mephedrone in highest transformation by bacteria [2,4,5]. The developing techniques for identification of illicit drugs on the crime scenes without using any sophisticated instrument is also aided by microbes in biosensors for example *Staphylococcus aureus* have nanopore which releases alpha-hemolysin ultimately degrading cocaine into its metabolite ecgonine methyl ester [6]. The microbial forensics is an evolving field which aids in multidisciplinary approach of forensics like post mortem toxicology , bioterror identification and nanotechnology giving specific and desired results for identification purpose[7].



Figure 1. The figure is depicting applications of microbes as biomarker in Forensic chemistry and toxicology[7–9].

Microbes in Post mortem toxicology

The post mortem toxicology revelation of drugs of abuse by aid of microbes' biotransformation of drugs in different biological matrices like Blood/Plasma preferred for all types of drugs, Bile specifically accurate for Morphine, Tramadol and MDMA (ecstasy) and Fat is preferred for analysis of tetrahydroxy cannabinol THC, vitreous humor preferred in post mortem alcohol estimation [10].

a. Stimulants:

The stimulants like cocaine, amphetamine and methamphetamine are biotransformed by gastro intestinal tract bacteria or post mortem microbiome as *Rhodococcus spp*.as cocaine to ecgonine methyl ester and benzoic acid, *Pseudomonas maltophila* converts cocaine to benzoylecgonine and methanol, *Pesudomonas fluorescene* hydrolyse cocaine into ecgonine which further hydrolyzed to pesudoecognyl. The *Aspergillus niger* is another microbe for transformation of cocaine into ecgonine methyl ester (EME)[3,11].Amphetamine and Methamphetamine are reduced by gastro intestinal bacteria *Enterobacterium, enterococcus, Lactobacillus, and clostridium* to N- demethylation metabolite[12,13].

b. Depressants:

Ethanol and Benzodiazepines are the most abused depressants and Bio transformed by bacteria in post mortem specimens. The Ethanol is transformed by *saccharomyces* and *candida* in urine samples, *S.aureus*, *E.coli* and *K.pneumonia* are the predominant bacteria responsible for digesting alcohol and thus decreasing the blood alcohol volume in estimation. The studies have suggested that alcohol estimation in post mortem blood and urine is a challenging task for qunatification thus the presence of EtG(Ethyl glucuronidase) and EtS(Ethyl sulphate) is the most accurate confirmation of presence of alcohol in pre mortem stage .Among the viscera sample vitreous humor is considered as the most preferrable specimen for alcohol estimation in long post mortem interval. The presence of specific bacteria like *Corynebacterium*, *Lactococcus garviae* are the indication for consumption of alcohol [2,14,15].The benzodiazepines are the other type of depressants among which diazepam is mostly abused, the diazepam degradation and transformation performed by microbe *Corious versicolor* and *Beauveria arsiana*, the other benzodiazepines like clonazepam, nitrazepam liver samples had biotransformation by *B.cereus*, *Staphylococcus epidermidis and B. fragilis*.The flunitrazepam is specifically transformed by *C. perfringens*, *Staphylococcus aureus* [16].

c. Narcotics:

The Heroin among the narcotics is highely abused and post mortem specimens of liver, prostate reveals the shift in gut microbiota of daily abuser. The techniques like next generation sequencing,RT-PCR and 16SrRNA gene sequencing reveals the dominance of bacteria in dail abuser and non-abuser of heroin[17]. The *D.polymorpha* oxidation stress is increased in the bivalve when heroin is administered. *Pseudomonas putida* converts the 14- Hydroxymorphine to dihydroxymorphine,*Pesudomonas testosteronii* converts morphine to morphinone[3,11]. The gut microbiota reveals decrease of beta diversity predominant in non-abuser and abusers having high diversity of *Ruminococcaceae*. The methadone is having specific bacteria for transforming it to metabolites by *Bifidobacterium,Lactobacillus and Fusciatenibacter*[16,18].

d. Hallucinogens:

Delta Tetrahydrocannabinol (THC) is a hallucination causing drugs of abuse and many strains of bacteria can help to determine the abuse of THC as *Rhodococcus, Mycobacterium, Gordonia* and *Dietza*[3,11].

e. Other drugs:

The gamma hydroxy butyric acid which is a Drug facilitated sexual assault DFSA drug or Date rape drug also have biotransformation in post mortem specimens like blood and urine by *clostridium strains*,*P.aeruginosa* post mortem production which converts Gamma amino butyric acid to Gamma hydroxy butyric acid helps to estimate the pre mortem consumption of

GHB [7,8,15,19]. The 4 methyl meth cathinone 4MMC is highly abused designer drug found in putrified biological matrices biotransformed by *Staphylococcus aureus, Kleibsiella pneumonia* to its degraded products 2-hydroxy-1-(4-methylphenyl propan-1-one)HMP[20].

Forensic Epidemiology in Identification of Clandestine Laboratory Drugs of Abuse

The illicit drugs/street drugs/Recreational drugs are the interchangeable terms for drugs synthesized, manufactured, and distributed by clandestine labs in the absence of legal norms. The specification of location of clandestine labs can be estimated by soil and water contaminants of illicit drugs by environmental matrices analysis. The sewage water contains millions of microbes which transform the drug into metabolites and soil or dirt also have microbiome which transforms the illicit drugs into its metabolites. The dust examination is dominated by phyla Proteobacteria, Firmicutes and Actinobacteria but drug synthesis changes the microbiome due to vapor phase exposure of drug and bacteria causing increased abundance of *Pseudomonas* which degrades the toluene and *Staphylococcus* degrades the naphthalene, benzene analyzed by metagenomics 16SrRNA sequencing revealed drug operation [21]. The biomarker microbe for waste water or sewage forms biofilms having metabolites of drugs specifically Cocaine and Mephedrone reveals the location of drug operation laboratory and type of illicit drug manufacturing[22]. The water testing is very useful in determining distribution of drugs in particular area like amphetamine and MDMA is having a new precursor hiding from law 1-phenylpropan-2-one(BMK) which is revealed by the biomarker in water for example metabolite of methadone is found as 2-Ethylidone-1,5-dimethyl-3,3-diphenyl pyrrolidine (EDDP) by LC-MS/MS[23]. The species of bacteria is found in water and soil according to the resistance developed by different strains towards the illicit drugs. The resistance for degradation of drugs is in the order 1Benzyl-3of Methylnaphthalene>Methamphetamine>N-formylmethyl methamphetamine>Pseudoephed Rine[24,25]. The Psychoactive substances in Water based environment of opioids are transformed by Chloroflexi, Planctomycetes and for tramadol Xantobacter, Sphingobacterium[26].

Microbes in Biosensors

The increase in the demand of fast and reliable techniques for the crime scene investigation has been a topic of research. The drug abuse being a major concern all over the world requires on site testing of illicit drugs for proper functioning of justice system. The microbes metabolize parent drugs by various pathways of oxidation, reduction, hydrolysis, condensation to form metabolites giving positive test results for the primary confirmation of drug of abuse. The *Staphylococcus aures* have nanopores releasing the metabolite of Cocaine with the help of alpha hemolysin enzyme thus confirming the presence of cocaine [6].

Bioterror/Bio crime

The silent weapon of biological origin is a dangerous threat to community public health for a country. The microbes with modified strains are used as bioterror to release them in a community. The *anthrax* used as bioweapon in past caused destruction of lives thus law enforcement agencies also have eye on bioweapon being released and specific techniques to sequence their strains by 16SrRNA sequencing targeting V3 and V4 region to timely neutralizing the bioweapon[27,28].

Sr No.	Drug	Microbes	Biotransformation	Sources
1.Post-mortem				
Toxicology				
Stimulants				
	Cocaine	Rhodococcus spp	cocaine to ecgonine methyl ester and benzoic acid	[3,11]
		Pseudomonas	cocaine to - benzoylecgonine and methanol	
		maltophila	cocaine - ecgonine to pesudoecognyl.	
		Pesudomonas fluorescene	cocaine into ecgonine methyl ester (EME)	
		Aspergillus niger		
	Amphetamine Methamphetamin e		N- demethylation metabolite	[12,13]
		Enterobacterium , enterococcus, Lactobacillus		
Depressants				

Table No. 1. The table shows illicit drugs and their metabolites by the aid of biomarker microbes

	Ethanol	saccharomyces	EtG(Ethyl	[2,14,15]
		candida	glucuronidase) and	
		,S.aureus	EtS(Ethyl sulphate)	
		,E.coli		
		K.pneumonia		
		-		
			Oxazepam,	
	Benzodiazepines		desmethyldiazepam	[16]
	1	Corious	temazepam	
		versicolor	1	
		Beauveria		
		arsiana		
		B cereus		
		Stanhylococcus		
		enidermidis		
		R fragilis		
		D. fragins C nerfringens		
		Stanhylococcus		
		Suphylococcus		
		uureus		
Narcotics				
	Heroin	D polymorpha	14Hvdroxymorphin	[3,11]
		Pseudomonas	e to	[0,11]
		nutida	dihydroxymorphine	
		puuuu		
			converts morphine	
	Morphine		to morphinone	
	1	Pesudomonas	I	
		testosteronii		[16,18]
				[10,10]
	Methadone			
		Rifidohacterium		
		Lactobacillus		
		and		
		Fusciatenihacter		
Hallucinogens	Delta	Rhodococcus	CYP2C	[3 11]
Tunuemogens	Tetrahydrocannah	Mycohactorium	CYP3A	[2,11]
	inol (THC)	Gordonia		
		Dietze		
		Dieiza.		

Other drugs	Gamma hydroxy butyric acid GHB	clostridium strains, P.aeruginosa Staphylococcus aureus, Kleibsiella pneumonia	2-hydroxy-1-(4- methylphenyl propan-1- one)HMP.	[20]
2.Forensic				
Epidemiology	Soil Cocaine Sewage Water	Pseudomonas Staphylococcus	Toluene Naphthalene, benzene	[22]
	MDMA	Chloroflexi, Planctomycetes	1-phenylpropan-2- one(BMK)	[24,25]
	Methadone	Xantobacter, Sphingobacteriu m	2-Ethylidone-1,5- dimethyl-3,3- diphenyl pyrrolidine (EDDP)	[26]
3.Biosensors	Cocaine	Staphylococcus aures	alpha hemolysin enzyme	[6]

Advantages of Microbial Approach in Illicit Drug identification

The quantitative and qualitative information of xenobiotics (illicit drugs) related to their metabolic pathways, extent and regions gives metabolic profiling of illicit drugs in correct manner. The biotransformation pathways are well established and gives strong inferences of metabolites with regio and stereospecificity. The prior optimization of samples is not needed compared to traditional approaches and scaling up is affordable for wide ranges of specimen [29]. The microbial approach along with nanoparticle advancements is building specificity and target metabolite identification for illicit drugs[30].

Future Perspective

The microbial approach is an advancement over traditional approaches which were nonspecific but future database for microbial species having regio and stereospecificity of metabolites. The major problem is contamination during analysis and sampling which can be avoided by advancement of fully automated machines involving least human involvement. The are specific for each type of illicit drug which can be used to manufacture biosensors based on the metabolic pathways of parent drug assisting as novel tool in the crime scene investigation process.

Conclusion

The illicit drugs are a concern worldwide in safeguarding of young generation and community public health. The identification of drugs trafficked by clandestine laboratory must be precise and specific. The microbial and nanoparticle approach in forensics aid to precisely gather information of parent drug and its metabolite. The examination of soil and sewage water also reveals the forensic epidemiology of drug trafficking. The new nano techniques involving microbes for biotransformation of drugs reveals the identity of drug. The future research needs reliable database of microbial species associated with specific illicit drug transformation pathways, making microbes as biomarker for illicit drug identification.

References

- [1] Govindarasu P. Illicit drugs-environmental occurrence, fate and toxicity. 2016.
- [2] Whitacre DM, editor. Reviews of Environmental Contamination and Toxicology Volume 210. vol. 210. New York, NY: Springer New York; 2011. https://doi.org/10.1007/978-1-4419-7615-4.
- [3] de Campos EG, de Almeida OGG, De Martinis ECP. The role of microorganisms in the biotransformation of psychoactive substances and its forensic relevance: a critical interdisciplinary review. Forensic Sci Res 2023;8:173–84. https://doi.org/10.1093/fsr/owad025.
- [4] Kates LN. Chemical profiling and environmental modelling of waste from clandestine methylamphetamine laboratories. 2013.
- [5] Liu Y, Xu C, Dong W, Yang X, Zhou S. Determination of a criminal suspect using environmental plant DNA metabarcoding technology. Forensic Sci Int 2021;324. https://doi.org/10.1016/j.forsciint.2021.110828.
- [6] Majeed MI, Nawaz H, Arshad FN. Detecting the Presence of Illicit Drugs Using Biosensors. 2020.
- [7] Oliveira M, Amorim A. Microbial forensics: new breakthroughs and future prospects. Appl Microbiol Biotechnol 2018;102:10377–91. https://doi.org/10.1007/s00253-018-9414-6.
- [8] Charaya N. Microbial Forensic's Microbes as a part of Forensic Investigation. Journal of Advanced Medical and Dental Sciences Research 2016;4:32–7. https://doi.org/10.21276/jamdsr.2016.4.4.8.
- [9] Rawal R. Microbial Fingerprinting-An Emerging Tool in Forensics: A Review. vol. 7. 2022.
- Kaushik KS, Kapila K, Praharaj AK. Shooting up: The interface of microbial infections and drug abuse. J Med Microbiol 2011;60:408–22. https://doi.org/10.1099/jmm.0.027540-0.

- [11] Sandeep K Vaishnav, R. D. Ahirwar, Rajesh Mishra. Decoding the chemical clues: The role of advancements in forensic toxicology. Magna Scientia Advanced Research and Reviews 2023;9:063–71. https://doi.org/10.30574/msarr.2023.9.2.0157.
- [12] Fernández-Rodríguez A, Burton JL, Andreoletti L, Alberola J, Fornes P, Merino I, et al. Post-mortem microbiology in sudden death: sampling protocols proposed in different clinical settings. Clinical Microbiology and Infection 2019;25:570–9. https://doi.org/10.1016/j.cmi.2018.08.009.
- [13] Zhao X, Zhong Z, Hua Z. Estimation of the post-mortem interval by modelling the changes in oral bacterial diversity during decomposition. J Appl Microbiol 2022;133:3451–64. https://doi.org/10.1111/jam.15771.
- [14] Drummer OH. Postmortem toxicology of drugs of abuse. Forensic Sci Int 2004;142:101–13. https://doi.org/10.1016/j.forsciint.2004.02.013.
- [15] Dinis-Oliveira RJ, Magalhães T. Forensic toxicology in drug-facilitated sexual assault. Toxicol Mech Methods 2013;23:471–8. https://doi.org/10.3109/15376516.2013.796034.
- [16] Li Q, Chen S, Liu K, Long D, Liu D, Jing Z, et al. Differences in gut microbial diversity are driven by drug use and drug cessation by either compulsory detention or methadone maintenance treatment. Microorganisms 2020;8. https://doi.org/10.3390/microorganisms8030411.
- [17] Yekafore S, Shado A. POSTMORTEM MICROBIOME OF INTERNAL ORGANS OF SUBSTANCE ABUSE DISORDER CRIMINAL CASES. n.d.
- [18] DeBruyn JM, Hauther KA. Postmortem succession of gut microbial communities in deceased human subjects. PeerJ 2017;2017. https://doi.org/10.7717/peerj.3437.
- [19] Li H, Jia W. Cometabolism of microbes and host: Implications for drug metabolism and drug-induced toxicity. Clin Pharmacol Ther 2013;94:574–81. https://doi.org/10.1038/clpt.2013.157.
- [20] Uruena MT, York R, Philp M, Kuzhiumparambil U, Wei Z, Yun K, et al. Identification of unique 4-methylmethcathinone (4-MMC) degradation markers in putrefied matrices. J Anal Toxicol 2020;44:803–10. https://doi.org/10.1093/jat/bkaa041.
- [21] microbial community and clandestine labs n.d.
- [22] Talukdar S, Maitra A. Analysis of an Aerosol Environment in an Urban Region and Its Impact on Regional Meteorology. Energy, Environment, and Sustainability, Springer Nature; 2020, p. 143–64. https://doi.org/10.1007/978-981-15-0540-9_7.
- [23] Testing_The_Waters_III_2017_Abstract_book n.d.
- [24] Pal R, Megharaj M, Kirkbride KP, Heinrich T, Naidu R. Biotic and abiotic degradation of illicit drugs, their precursor, and by-products in soil. Chemosphere 2011;85:1002–9. https://doi.org/10.1016/j.chemosphere.2011.06.102.
- [25] Pal R, Megharaj M, Naidu R, Klass G, Cox M, Kirkbride KP. Degradation in soil of precursors and by-products associated with the illicit manufacture of methylamphetamine: Implications for clandestine drug laboratory investigation. Forensic Sci Int 2012;220:245–50. <u>https://doi.org/10.1016/j.forsciint.2012.03.011</u>.

- [26] de Campos EG, De Martinis ECP, De Martinis BS. Forensic Analysis of Illicit Drugs and Novel Psychoactive Substances in Wastewater A review of toxicological, chemical and microbiological aspects. Brazilian Journal of Analytical Chemistry 2022;9:15–34. https://doi.org/10.30744/brjac.2179-3425.RV-19-2021.
- [27] UNIVERSITA' DEGLI STUDI DI MILANO SCUOLA DI DOTTORATO IN TERRA, AMBIENTE E BIODIVERSITA' DIPARTIMENTO DI BIOSCIENZE DOTTORATO DI RICERCA IN BIOLOGIA ANIMALE CICLO XXVII TESI DI DOTTORATO DI RICERCA PHARMACEUTICALS AND ILLICIT DRUGS AS NEW ENVIRONMENTAL CONTAMINANTS: ECOTOXICOLOGICAL EFFECTS AND NEW DEPURATION METHODOLOGIES BIO/07 STEFANO MAGNI Matricola: R09876 n.d.
- [28] Graham KA. FORENSIC APPLICATIONS OF DNA SEQUENCING TO COMBAT DRUG TRAFFICKING AND BIOTHREATS. 2022.
- [29] Salter R, Beshore DC, Colletti SL, Evans L, Gong Y, Helmy R, et al. Microbial biotransformation-an important tool for the study of drug metabolism. Xenobiotica 2019;49:877-86. https://doi.org/10.1080/00498254.2018.1512018.
- [30] Rudramurthy GR, Swamy MK, Sinniah UR, Ghasemzadeh A. Nanoparticles: Alternatives against drug-resistant pathogenic microbes. Molecules 2016;21. https://doi.org/10.3390/molecules21070836.