Hair Testing for Drugs of Abuse and New Psychoactive Substances in a High Risk Population

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ABSTRACT

Hundreds of new psychoactive substances (NPS) have emerged in the drug market over the last decade. Few drug surveys in the United States, however, ask about use of NPS, so prevalence and correlates of use are largely unknown. A large portion of NPS use is unintentional or unknown as NPS are common adulterants in drugs like ecstasy/Molly, and most NPS are rapidly eliminated from the body, limiting efficacy of urine, blood, and saliva testing. We utilized a novel method of examining prevalence of NPS use in a high-risk population utilizing hair-testing. Hair samples from high-risk nightclub and dance music attendees were tested for 82 drugs and metabolites (including NPS) using ultra-high performance liquid chromatography-tandem mass spectrometry. Eighty samples collected from different parts of the body were analyzed, 57 of which detected positive for at least one substance—either a traditional or new drug. Among these, 26 samples tested positive for at least one NPS—the most common being butylone (25 samples). Other new drugs detected include methylone, methoxetamine, 5/6-APB, α-PVP, and 4-FA. Hair analysis proved a powerful tool to gain objective biological drug-prevalence information, free from possible biases of unintentional or unknown intake and untruthful reporting of use. Such testing can be used actively or retrospectively to validate survey responses and inform research on consumption patterns, including intentional and unknown use, polydrug-use, occasional NPS intake, and frequent or heavy use.

INTRODUCTION

Hundreds of new psychoactive substances (NPS) have emerged in the drug market in the last decade, taking advantage of the delay occurring between their introduction into the market and their legal ban. NPS tend to mimic the psychotropic effects of traditional drugs of abuse, but their acute and chronic toxicity, and side-effects are largely unknown. This has led to funneling of resources worldwide to outline the phenomenon, identify the molecules, describe effects, interpret or update existing laws, enact new regulations, and develop appropriate and effective analytical methods for their identification in biological fluids and seized materials (1-9).

Unfortunately, little is known about the current diffusion of NPS among the general population or in high-risk populations. Few surveys query NPS use and there is increasing evidence that much NPS use is actually unknown or unintentional as they are common adulterants in drugs like ecstasy/MDMA/Molly (10-13). Most NPS are also eliminated from the urine, blood, and saliva of users within hours or days, which limits the ability of toxicological confirmation. This is a serious concern, especially in cases of hospitalizations and deaths resulting from intentional or unintentional use. A further challenge to detection of NPS in the biological matrices commonly tested, especially urine, is posed by the extensive, yet insufficiently investigated, metabolic transformation that these substances possibly undergo once introduced in the human body. In practice, most routine analyses do not presently include screening procedures for NPS, preventing clear knowledge of the consumption of these new drugs in the population. As a consequence, a number of unresolved issues are raised, including the number and variety of NPS present in different countries, frequency of their use, and the social features of users.

To circumvent the limitations of urine, blood, and saliva testing, the detection of NPS in hair samples was recently proposed as a practical means to provide preliminary information on the black market penetration of NPS in specific territories and populations (14-18). The keratin matrix incorporates the parent NPS consumed over extended time periods, providing access to a much wider diagnostic window than urine. This feature, combined with the analytical performances of the last generation ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) instruments (8), allows researchers to obtain significant information about past use, even a single intake, of any targeted NPS, with older periods of use corresponding to the hair segments more distant from the hair root (19,20).

In the present study, we considered the specific population of electronic dance music (EDM) nightclubs and festivals attendees in New York City (NYC), as a high-risk population particularly exposed to both intentional and unintentional NPS intake (21,22). Information on NPS prevalence in the US via biological confirmation tends to be limited to drug confiscations (23,24), but we tested hair analysis as a potential tool to gain objective biological drug-prevalence information in the context of an epidemiology study, free from possible biases of unintentional or unknown intake and untruthful reporting of use. Inclusion of both traditional drugs and NPS in the screening allows us to distinguish different consumption patterns, including co-use, occasional NPS intake, and frequent or heavy use. To our knowledge, this is the first large epidemiology study to examine biological hair results of NPS use in this high-risk scene in the US. We believe these biological results with retrospective capability complement both surveys and analysis of biological fluids conducted in EDM festivals population and can help encourage future testing and inform prevention.

EXPERIMENTAL

Study protocol

679 nightclub and festival attendees in NYC were surveyed from July through September of 2015. Participants were surveyed outside of ten different venues (including two dance festivals) over 21 days. Participants were eligible if they identified as age 18-25 and were about to attend the randomly selected party (21). After providing informed consent and taking a survey, participants were asked if they were willing to donate a hair sample to be tested for "new drugs" such as "bath salts". If the participant agreed, the trained recruiter collected the sample by cutting a small lock of hair (~100 hairs) from as close to the participant's scalp as possible. Hair was cut with a clean scissor (wiped with an alcohol-wipe after each use), folded up in a piece of tin foil, and stored in a small envelope labeled with the participant's anonymous study ID number. In some cases, male participants volunteered to have the recruiter to clip or buzz (with an electronic buzzer) body hair from the arm, chest, or leg. Of the 679 participants surveyed, 80 (11.8%) provided a hair sample compatible with the quantity needed for the analysis. This study was approved by the New York University Langone Medical Center Institutional Review Board.

Hair analyses

Hair samples were analyzed in their full length. The average length, calculated for the 80 analyzed samples, was 9.9 cm (median 9.0 cm). Assuming normal hair growth rate (25), the corresponding mean time frame is approximately 1 cm = 1 month. A minimum quantity of 20 mg was needed to perform the analysis. The collected specimens were tested using three previously published and validated methods using UHPLC-MS/MS. One (26) was used to screen each sample for 11 common drugs of abuse or metabolites: morphine, 6-acetylmorphine, codeine, amphetamine (AMP), methamphetamine, 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxymethylamphetamine (MDMA), 3,4-methylenedioxyethylamphetamine (MDEA), cocaine, benzoylecgonine, and Δ^9-tetrahydrocannabinol (THC). The second method (27) was addressed to detect the most expected and common NPS (26 substances) — namely stimulants (primarily synthetic cathinones) and psychedelic substituted phenethylamines: mephedrone, 3-methylmethcathinone (3-MMC), 4-methylethcathinone (4-MEC), methylone, 4-fluoroamphetamine (4-FA), 3,4-methylenedioxypyrovalerone (MDPV), pentedrone, ethcathinone, alpha-pyrrolidinovalerophenone (α-PVP; a.k.a.: "Flakka"), butylone, buphedrone, 25I-NBOMe, 25C-NBOMe, 25H-NBOMe, 25B-NBOMe, 2C-P, 2C-B, 1-(benzofuran-5-yl)-N-methylpropan-2-amine (5-MAPB), 5-(2-aminopropyl)benzofuran (5-APB)/6-(2-aminopropyl)benzofuran (6-APB), para-methoxymethamphetamine (PMMA), para-methoxyamphetamine (PMA), amfepramone, meta-chlorophenylpiperazine (mCPP), and bupropione, plus 5 dissociative drugs, namely methoxetamine (MXE), phencyclidine (PCP), 4-methoxyphencyclidine (4-MeO-PCP), diphenidine, and ketamine. The new designer drug mCPP can be detected in biological fluids as a metabolite of trazodone (28,29); therefore, trazodone was also included in our method in order to discriminate between direct mCPP intake and biotransformation of trazodone. The limits of detection of the analytical methods (26,27) were set as the minimum criterion to identify the positive samples. Hair samples were also screened for synthetic cannabinoids. Our existing method for synthetic cannabinoids (15) was updated by introducing the following compounds: MAM-2201, UR-144, XLR-11, AKB-48,

STS-135, PB-22, AB-PINACA, 5F-AB-PINACA, ADB-PINACA, AB-FUBINACA, ADBICA, JWH-302, AM-2233, CB-13, JWH-016, JWH-098, JWH-147. The total number of targeted analytes in the latter method was 40. Altogether, each sample was screened for 82 drugs and metabolites.

RESULTS

Eighty samples collected from different parts of the body were analyzed, 57 of which detected positive for at least one substance—either a traditional or new drug. Among these, 36 samples were head hair, 3 were leg hair, and in 18 cases the sampling site was not recorded. Among positive specimens, 40.4% (n=23) were provided by female participants. Donors mainly identified as White (*n*=35, 61.4%), while other ethnic groups included Hispanic (*n*=12, 21.0%), Asian (*n*=4, 7.0%) or other/mixed (*n*=6, 10.6%). Among positive samples (*n*=57), 31 tested positive for the exclusive presence of traditional drugs, 2 were positive for NPS only, while in the remaining 24 cases of traditional drugs were also positive for at least one NPS. No sample tested positive for synthetic cannabinoids. Among traditional drugs, THC was detected in 35 samples (range 0.02−1.92 ng/mg, mean=0.34 ng/mg, median=0.11 ng/mg), MDMA in 26 samples (range 0.08−7.17 ng/mg, mean=1.43 ng/mg, median= 0.68 ng/mg), amphetamine in 15 samples (range 0.04−1.68 ng/mg, mean=0.52 ng/mg, median= 0.33 ng/mg), ketamine in 11 samples (range 0.21−11.1 ng/mg, mean=1.90 ng/mg, median= 0.68 ng/mg), and cocaine in 25 samples (range 0.10−33.5 ng/mg, mean=4.13 ng/mg, median= 1.83 ng/mg). MDA, possibly arising from the metabolism of MDMA, was measurable in 18 samples (range 0.01−0.70 ng/mg, mean=0.19 ng/mg, median= 0.14 ng/mg). In all 25 samples that tested positive for cocaine, the benzoylecgonine metabolite was also detected (range 0.01−4.34 ng/mg, mean=0.74 ng/mg, median= 0.38 ng/mg). Two samples positive to amphetamine (0.04 ng/mg and 0.52 ng/mg) were also positive for methamphetamine at similar levels (0.01 ng/mg and 0.34 ng/mg, respectively), suggesting that the former drug was likely present as metabolite of the latter. One sporadic finding of MDEA at the concentration of 0.34 ng/mg was recorded. The remaining analytes considered in the analytical method have never been identified in any of the analyzed samples.

Multiple positivity related to sole use of multiple traditional drugs was detected in 31 cases. Figure 1 presents the percentage of the sample positive for one drug, two, three, and four drugs, respectively, for each class of traditional compounds. More than 30% of participants who tested positive for MDMA or THC appear to have been inclined to use only that single substance. On the other hand, the large majority of participants who used cocaine and ketamine also tested positive for other substances. However, it is unknown whether multiple drugs were used concomitantly or at different points in time.

As shown in Table 1, 26 samples tested positive for at least one NPS. Among these, 9 samples were positive for two or more NPS. Positive specimens were almost equally distributed between males (*n*=14; 53.8%) and females (*n*=12; 46.2%). The most common NPS detected was butylone, which was found in 25 samples, often at high concentrations (range < 7−4900 pg/mg, mean=440 pg/mg, median= 21 pg/mg). Other multiple detection included methylone (5 cases; range <6−98 pg/mg), methoxetamine (4 cases; range 3−19 pg/mg), 5/6-APB (1 case; 82 pg/mg), α-PVP (1 case; 6 pg/mg), and 4-FA (1 case; 29 pg/mg). Multiple positive findings that include at least one traditional drug of abuse and one NPS were detected in 24 cases. Eight samples tested positive for five or more drugs. Traditional drugs detected in association with NPS were MDMA (17 cases),

THC (15 cases), cocaine (15 cases), amphetamine (8 cases), ketamine (6 cases), and methamphetamine (1 case). While butylone was detected in a wide range of concentrations, with 9 samples above 100 pg/mg, the measured levels for the remaining NPS was interestingly below 100 picograms of drug per milligrams of hair, either suggesting sporadic exposure to these substances or low rate of incorporation into the keratin matrix.

DISCUSSION

Our study shows that NPS were detected in a large number of NYC nightclub and dance festival attendees—a high risk population. Methylone, butylone, and methoxetamine were detected exclusively or more often concurrently in 25 out of 26 cases which tested positive for at least one NPS. Our previous work has already shown that many nightclub and dance festival attendees reporting MDMA use tested positive for methylone and/or butylone, with four out of ten self-reported MDMA/ecstasy/Molly users testing positive for these drugs after reporting no use of synthetic cathinones or unknown pills or powders (13). NPS are often identified at festivals as being sold in place of traditional drugs—primarily ecstasy/Molly. The fact that synthetic cathinones are often falsely represented as a pure form of ecstasy to MDMA users has been verified in the past (30). Likewise, ketamine is often replaced or adulterated with methoxetamine. Therefore, it is not unlikely that most of our positive findings would be unexpected by the users and can be attributed to unaware intake of NPS.

Synthetic cannabinoids were not detected in any of the tested hair samples. In the present study, it is possible that the panel of target analytes covered by our method did not fully reproduce the NYC scenario at the time of sample collection. While it is possible that some newly-introduced synthetic cannabinoids were out of the range of the targeted substances, it should also be noted that different groups of synthetic drugs are expected to be used by different social groups. Nightclub attendees and partygoers are likely to use synthetic cathinones, not synthetic cannabinoids, whereas other social groups (31) might resort to synthetic cannabinoids as cheap but more potent substitute of THC to temporarily escape from personal or social problems, such as unemployment, homelessness, and/or incarceration (32).

It should be noted that prevalence of use of specific NPS—particularly synthetic cathinones—is constantly shifting. According to national seizure data, for example, prevalence of confiscations of synthetic cathinones fluctuated greatly between 2013 and 2015 (33). For example, methylone was the most confiscated compound in 2013 (71.8%) and this decreased to 30.7% and 2.3% in 2014 and 2015, respectively. Various other synthetic cathinones (e.g., MDPV) rapidly decreased in prevalence (or virtually disappeared) over the three year period. Therefore, it is possible that other/newer compounds were used and not detected.

CONCLUSIONS

The present study demonstrates that hair testing offers a unique perspective in the investigation of drug consumption, provided that a large panel of target analytes is considered. The extended diagnostic time-window covered by the keratin matrix (unlike urine, blood, and oral fluid) allows retrospective investigation of drug prevalence and diffusion of any targeted psychoactive substance among selected populations. Furthermore, clues about occasional (even sporadic intake)

vs. heavy NPS use are made possible by quantitative results of hair testing. In particular, the present study highlighted the wide occurrence of poly-use and likely unintentional intake of unknown NPS in the NYC area. The prevalent diffusion of synthetic cathinones as recreational drugs, often used in combination with other more traditional drugs, was clearly evident in the data, relative to young consumers attending EDM parties at nightclubs and dance festivals. Butylone was the most frequently detected NPS, followed by methylone and methoxetamine.

In prospect, the limitations concerning the specific population under study will be surmounted by investigating broader groups of young people. Similar occurrences are expected for social groups more inclined to use synthetic cannabinoids, since the composition of "legal highs" sold in the black market is typically variable, implicating that consumers do not have control over the quality of the product they are using, potentially increasing risk of serious health threat. Large NPS screening panels using hair testing and taking into account the timely fluctuation of the market situation can also be applied to other individuals under periodic control (e.g., in workplace control or driving re-licensing) to check how frequently NPS are used in substitution of traditional drugs to escape a positive outcome.

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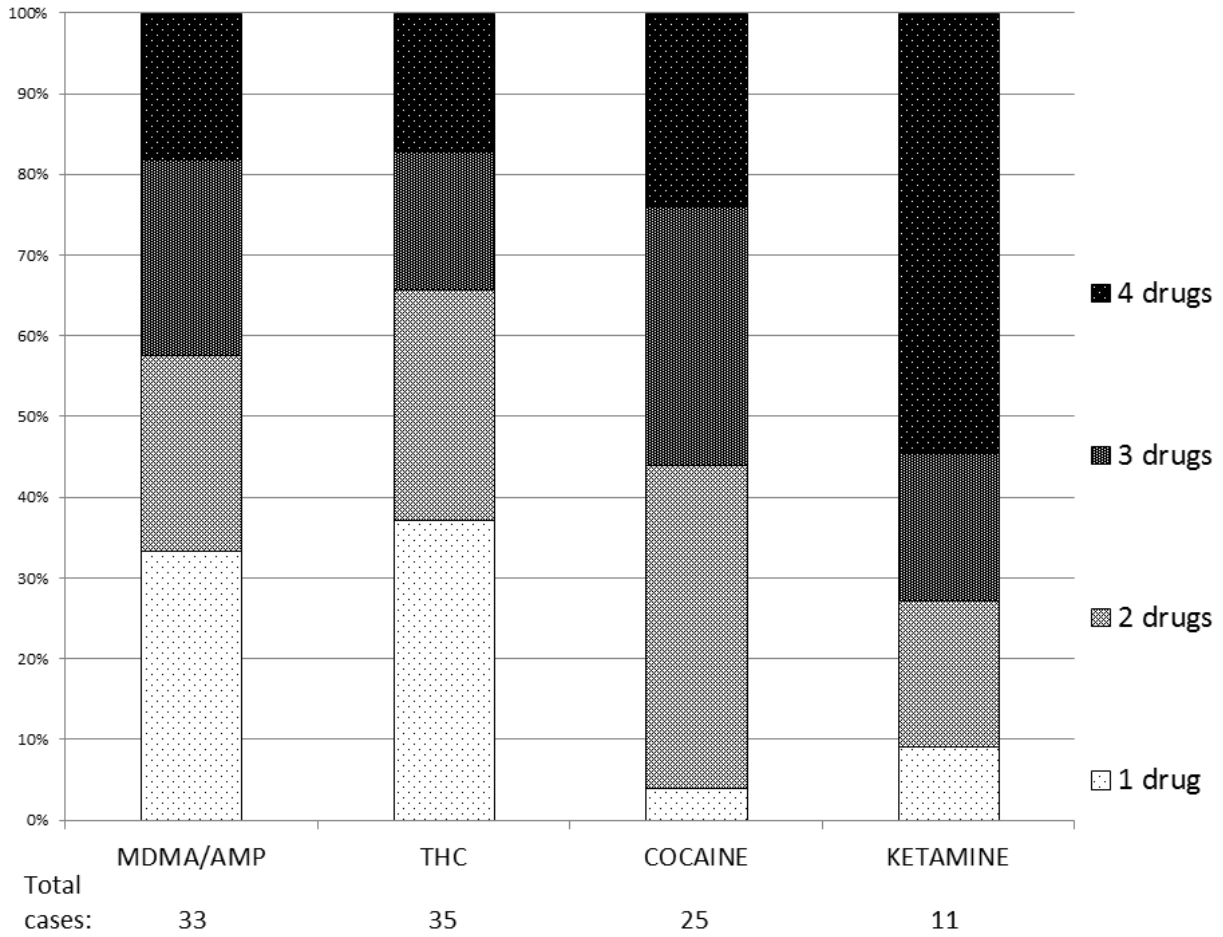

Figure 1. Percentage of the sample positive for one drug, two, three, and four drugs, respectively, for each class of traditional compounds. Positivity related to sole use of multiple traditional drugs was detected in 31 cases.

Table 1. Results from samples which tested positive for at least one NPS (concentrations are given in pg/mg)

Subject					New Psychoactive Substances						Traditional drugs of abuse					
Case	**Sex**	**Ethnic group**	**Hair type**	**Length (cm)**	**Butylone**	**Methylone**	**MXE**	**5/6-APB**	**α-PVP**	**4-FA**	**AMP**	**mAMP**	**MDMA**	**COC**	**KET**	**THC**
1	F	White	head	25				82					1600			
2	M	White	leg	2.5	600	98			6				6300	1200		150
3	M	White	leg	2	100											
4	F	Hispanic	head	12	290								630	5600	680	
5	F	White	head	12	< 7						280			13000		
6	F	Hispanic	head	25	17	8							1040	1800	2050	240
7	F	White	head	12	17		13						160	5200	1400	60
8	M	White	head	12	< 7		19				220		710	9400	1600	80
9	F	Other/mixed	head	7	< 7											
10	M	Hispanic	head	12	4900	10							350			1200
11	M	White	head	8	< 7		3				120		130	1600	310	
12	M	White	head	4.5	< 7								320			
13	M	Other/mixed	head	23	< 7						40	10	1500	5800	420	20
14	F	Hispanic	head	23	< 7											30
15	M	Hispanic	head	8	25									150		40
16	F	Asian	n/a	12	23											850
17	M	Other/mixed	n/a	7	110						950		3100	6200		190
18	F	Asian	head	23	< 7						1700		1900			
19	M	Hispanic	head	12	120						540		540	2600		90
20	F	Hispanic	head	7	200											120
21	M	Hispanic	head	11	< 7								80			
22	M	White	head	4.5	21					29			710	1100		
23	F	Hispanic	head	12	< 7	< 6	5				110		1200	3400		290
24	M	White	head	6	300	< 6							120	1400		
25	F	Hispanic	head	10	190											940
26	M	White	head	4	38									34000		380
		Positive samples			**25**	**5**	**4**	**1**	**1**	**1**	**8**	**1**	**17**	**15**	**6**	**15**