ORIGINAL ARTICLE

SALIVA AS AN ACCESSIBLE BIOLOGICAL MATRIX FOR SCREENING OF PSYCHOACTIVE AND THERAPEUTIC DRUGS

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ABSTRACT

BACKGROUND: Saliva functions are becoming increasing uncountable based on recent technology, these functio ranges from lubricant, digestion of food, maintenance of o hygiene, antimicrobial function, ion reservoirs, bufferi capacity, diagnostic tool, hormonal role, taste perceptio DNA and forensic screening. This research focuses on the u of saliva to screen for some psychoactive drugs, with the u of oral screen digital portable machine of motorists and th enhancing highway safety.

MATERIAL AND METHODS: Digital screening devi called Oral Screen was used to screen selected participar saliva to establish those that ingested substances of abu such as motorists. The test detects THC, cocaine, opiat methamphetamine, and their metabolites individually human oral fluids with high sensitivity. The device w complemented with Alco-scan for detection of alcohol.

RESULTS: The findings revealed that significant males 31.07% were positive for alcohol; marijuana was 17.84 while those that combined them were 6.46% males. Th that tested positive for alcohol were highest in Abuja w 37.32%, followed by Benin at 22.75%, Ibadan at 19.07%, a the least was Enugu with 16.61%.

CONCLUSION: The use of digital screening tool with sal is convenient, reliable and time saving methods to enha highway safety by identifying motorists with posi substances of abuse.

Key words; Saliva, psychoactive, therapeutic, drugs

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INTRODUCTION

The use of saliva as tool for screening for drugs and investigation for systemic diseases is becoming increasingly popular. Several documented reports are evident of the role of saliva as tool for screening. Some noticeable examples are, use of saliva in therapeutic drug Monitoring[1,2] (such as antipyrine, caffeine, ethosuximide, Phenobarbital, phenytoin, primidone, and theophylline). Drug concentration in saliva is highlighted by Mucklow J.C et. al., [3] is illustrated with antipyrine, chlorpropamide, meperidine, Phenobarbital, phenytoin, propranolol, and tolbutamide. The use of saliva in the forensic detection of drugs and other chemicals are illustrated with barbiturates, benzodiazepines, cannabinoids, ethanol, lithium, heavy metals, and methaqualone.[4,5]

Researches on drugs of abuse in saliva are well covered by several researchers.[6,7] Major drugs/drug classes discussed are amphetamines, barbiturates, benzodiazepines, cannabinoids, cocaine, methaqualone, opioids, and phencyclidine. The diagnostic uses of saliva, highlighting saliva collection, detecting oral diseases, detecting systemic diseases, clinical analyses, hormone monitoring, drug monitoring, antiviral antibody screening, and viral antigen screening are all encompassing.[8] Laboratory tests for rapid screening of drugs of abuse with salivary ethanol in workplace by Schwartz et al.,[9] are illuminating. Navazesh et al [10] reported on the different methods for collecting saliva such as whole saliva and saliva from individual glands. A study on saliva as an analytical tool in toxicology [1] illustrates the composition, anatomy, and physiology of saliva.

Apart from concern for highway safety, drug screening is of significant value in crime investigation with regards to causative factors assisting in the administration of justice.

To date, testing has been done primarily on blood, plasma, and urine, but alternate biological specimens that can be collected easily, noninvasive and can complement or replace urine and blood are being evaluated, including hair, sweat, and saliva. Each has its own strengths and weaknesses.[10-12] For example, hair may contain a history of a donor's drug use, but, if drugs are smoked in the vicinity of the donor, then the hair is subject to external contamination from touching with the hands and drug vapors. Sweat can be collected using sweat patches, but little is known about interpreting the concentrations of drugs detected in the patches. Un-stimulated saliva can be collected by the draining method, which is performed by allowing saliva to drip from the mouth into a collection container.[10] Several techniques may be used to collect stimulated saliva. The simplest method involves tongue, cheek, or lip movements without the use of an external stimulus.[3,13-16] Following stimulation by one or more of these methods, saliva can be spit out, suctioned, or swabbed from the mouth.[10] Some collection techniques combine stimulation and collection of the saliva using absorbent materials such as cotton balls or cotton rolls.

Some of the most recent advances in drug testing have been the developments in the rapid point-of-collection testing products. There are at least 50 rapid point-of collection-testing (POCT) immunoassay devices currently available. These devices are designed for the commercial market.[14] These POCT devices can be use by police or Federal Road Safety Corps (FRSC) officers to routinely screen impaired driving suspects for illegal drug use and obtain drug test results immediately, as they currently do with alcohol tests. Having immediate

screening results would permit the officer to confront the driver with the drug test result, and make an initial charge. Confirmation testing in a toxicology lab would generally be required.

In traffic law, safety and enforcement, the use of oral screens or urine seems more desirable as a large number of motorists can be screened cost effectively without undue tension and prolonged delay. However, if the driver admits to drug use, additional laboratory testing may not be required for prosecution. A number of these devices have been used successfully by police to test drivers for drug use.[11,12] In a series of studies conducted by the National Institute on Drug Abuse, Jenkins et al [13] demonstrated the feasibility of having police officers use urine testing devices to test DUI suspects for recent use of drugs of abuse. National highway traffic safety administration (NHTSA) has also recently completed a project in which police officers in Houston, Texas and Long Island, New York evaluated five on-site urine test kits (Triage®, AccuSign®, Rapid Drug Screen®, and Testate®) with DUI suspects. The officers participating in this project were certified "Drug Recognition Experts" (DRE) who had been trained in the NHTSA- approved "Drug Recognition and Classification program." GC/MS confirmation of all on-site test positives (and some negatives) indicated that the kits performed well, and the DRE officers participating in the study "favored the use of on-site devices in the enforcement of impaired driving laws".[15-24]

The European Union has recently funded a major drugs / driving study called "ROSITA" (Roadside Testing and Assessment) evaluating rapid urine, sweat, and saliva POCT drug testing devices in eight European nations.[13-16] The principal conclusions of that two-year study were: (1) that roadside drug testing is urgently needed, and (2) that the need is so great that in some countries, police officers would rather use an imperfect device/method than wait for a more suitable one. The device evaluation in the ROSITA project indicated that, while police favoured oral fluids as the preferred matrix, "the present generation of on-site oral fluid tests are insufficiently sensitive and/or specific to give reliable results for most classes of drugs." Sweat testing devices performed poorly. While rapid urine tests are clearly not perfect, they may be suitable for a rapid preliminary screening test. In the ROSITA device evaluations, several urine drug tests satisfied the criteria for accuracy, sensitivity, and specificity when compared to a reference method, although none scored highly for all drug categories. This study focuses on the use of saliva to screen psychoactive drugs and therapeutics in our society.

MATERIALS AND METHODS

This study is a prospective study carried out in some selected cities in Nigeria and these are Abuja, Benin City, Enugu and Ibadan. These cities were selected based on the geopolitical zones of Nigeria. Training workshops were used as a major platform to attract target participants to the screening arena.

The study consisted of the following:

- *Screening of participants for substances of abuse to establish recent drug usage* using the metabolic method with the help of Oral screen (saliva)[Figure 1] or urinalysis to determine those that have used substances of abuse, by measuring the level of metabolism of drugs and absorption in bodily fluid like blood, saliva and urine.[16,17]

- *To confirm some degrees of impairment clinical/ behavioural method was used to identify behaviour or effect of the drugs on the central nervous system that usually occur quickly after drug usage. This is regarded as the Field of Impairment Test (FIT). The first phase (pre-crash) was for those without any accident using saliva, urine and Field of Impairment Test (Structured instruction). The Second phase was post-crashed (after accident) with the use of saliva and urine.*

  Pre-Crash Screening: In the population survey or screening at pre-crash level, 325 volunteers with different background such as, drivers and auto-bike riders, pedestrians and passengers had their saliva and urine screened, using Point of Collecting Testing Devices (POCT).[18,24] The substances of abuse screened were amphetamine, alcohol, marijuana (cannabis), barbiturate, opiates, benzodiazepine, cocaine, MDMA (ecstasy). The screening was by random sampling from the 325 participants. To validate the findings from positive alcohol screening, FIT was also utilized by taking a smaller sample of the population that tested positive to the drug, to enable us establish those that are impaired by making them perform basic skills.

- *Post-Crash Screening:* The second phase was done at the Accident and Emergency Unit of the University of Benin Teaching Hospital and major highways in south-south zone. This phase of the research was considered post-crash screening with significantly lower number of participants. While the pre-crash screening was 325 at each location with Abuja acting as the fulcrum of the north, the post crash screening involved 112 participants with UBTH and Lagos-Benin express highway as point of collection and screening. The cost of consumables made me to restrict this part of the screening to only Benin City (UBTH and incoming highways.

- *The collecting pouch was kept at room temperature prior to being used.* The volunteers being tested were requested to have nothing in the mouth for at least 5 minutes before sample collection, this included water and food. The volunteers were then observed during sample collection, before removing the test device from the foil pouch prior to its use. The oral fluid sample was collected by removing the collector from the package, sliding the plastic hood back and exposing the foam. The volunteers were asked to pucker their mouths and accumulate oral fluid before inserting collector. The entire content of the cassette was introduced into the device and activated by pressing a button and then waited for 15 minutes.

  A negative result is indicated by the continued presence of a red/pink line adjacent to the labeled target drug, while a positive test result was indicated by the absence of a red/pink line at the position indicated for the drug in the test panel. An invalid test result was indicated by the absence of a red/pink line at the control position. Each tested individual had saliva collected three times from the mouth and the presence of any of the abused substances in any two or all of the three samples collected were considered positive and the substances identified in the particular saliva were recorded as positive findings. The same applied for negative and invalid results. The use of various types of POCT devices gave a broad range of psychoactive drugs that tested positive

or negative, but the main emphasis was alcohol and cannabis due to the prevalence of their uses in Nigeria.

RESULTS

Demographic distribution

Demographic distribution of the participants in all the locations is represented in Table 1 and this table illustrates that: Enugu's participants are older at age (27±4.5 years), followed by Ibadan (25±4.8 years), Abuja (24±2.8 years), and Benin (23±4.2 years). The males were more dominant than their female counterparts with Benin City leading at 25[illegible] followed by Enugu 224, Ibadan 218, and Abuja [illegible], while the females were 89, 101, 107 and 111 respectively.

Distribution of screened candidates based on gender

The role of gender in screening is illustrated in table 2. Males who screened positive for alcohol were higher with 31.07%, while the females were 10.76%. Marijuana (cannabis) results followed a similar pattern.

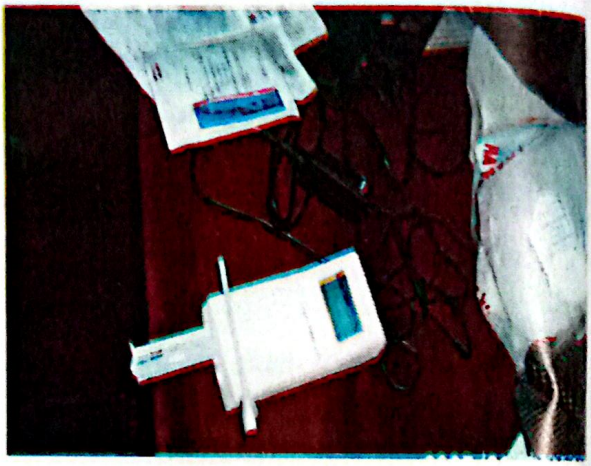
Effect of age on screening

In Benin City, the peak incidence of substance of abuse was in the age range of 15 to 25 years (8.30%) for alcohol only, with marijuana at (9.53%), while those that tested Positive to both were 2.15%. In Ibadan, the peak incident of substances of abuse was 15 to 25 years (7.69%) for alcohol only, followed by marijuana at 8.92%, while those that tested positive for alcohol and cannabis were 2.46% (Table 3).

Distribution of results that tested positive to psychoactive drugs based on locations

Those that tested positive for alcohol were highest in Abuja with 37.32%, followed by Benin at 22.75%, Ibadan at 19.07%, and the least was Enugu with 16.61%. Those that tested positive for marijuana also had Abuja as the highest at 21.55%, followed by Ibadan at 18.75%, Benin at 15.42%, and Enugu at 12.85%. The frequency of those that tested positive to other drugs including benzodiazepines, cocaine, amphetamine, and unclassified drugs was also of similar pattern (Figure 2).

Figure 1: Portable Oral-Screen Digital Machine from Craig Medical Distribution Inc. USA. The test detects THC, cocaine, opiates, methamphetamine, and their metabolites individually in human oral fluids with high sensitivity [15, 18-19]



This study is evidenced based, illustrating the level of participants that tested positive to psychoactive drugs and thus pose greater risk potential to cause traffic accidents similar to previous reports [32-40] that highlighted the role of drivers under the influence of psychoactive drugs.

In all the locations utilized for this study alcohol was the single most used drug of abuse. This finding is in agreement with several global reports that alcohol is the most commonly found substance involved in driving under the influence (DUI).[22-25] The effect of gender on positive screening for alcohol in our results shows considerable significant value in favour of males. In Abuja, it is about 10:1, with a *Kruskal Wallis test P value = 0.1905*.

Table 1: Demographic distribution of participants

n = 1,300	Abuja	Benin	Enugu	Ibadan
Average age (years ± SD)	24 ± 2.8	23 ± 4.2	27 ± 4.5	25 ± 4.8
Range	15 - 48+214	15 - 48+236	15 - 48+224	15 - 48 + 218
Male	(65.84%) 111	(72.61%)	(68.92%) 101	(67.08%) 107
Female	(34.15%)	89 (27.38%)	(31.08%)	(32.92%)

x^2 = 7.242, df = 3, P value = 0.0646 (considered not statistically significant at this p-value)

Table 2: Distribution of screened candidates based on gender

	Male Positives		Female Positives		Total Positives	
	#	%	#	%	#	%
Alcohol	10	31.07	35	10.76	136	41.84
Marijuana (Cannabis)	58	17.84	12	3.69	70	21.53
Both Alcohol & Marijuana	21	6.46	6	1.84	27	8.30

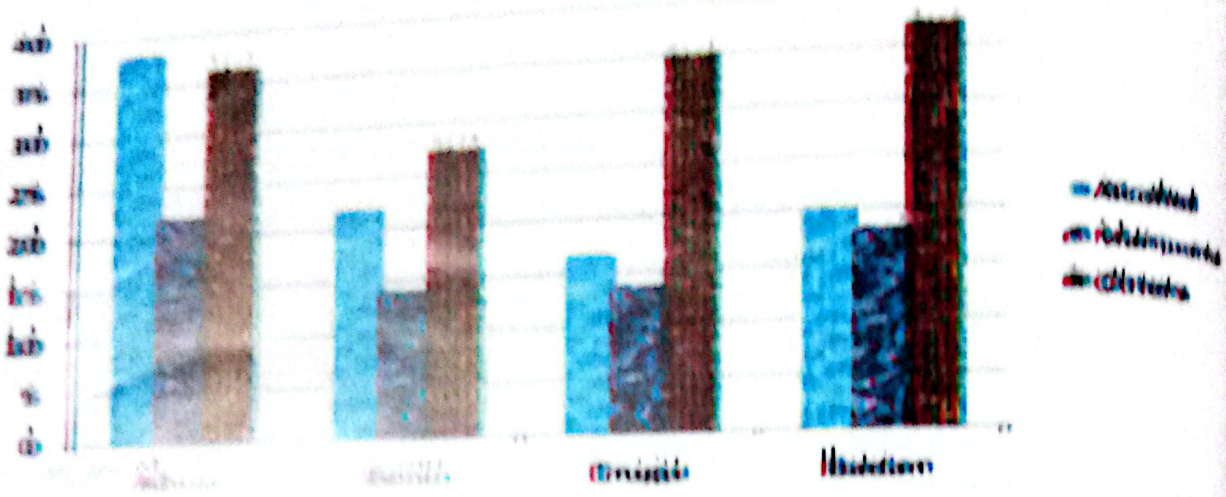
x^2 = 1.946, df= 2 P value = 0.3779 (considered not statistically significant at this p-value)

Table 3: Distribution of screened participants based on age

	15 - 25		26 - 36		37 - 47		48 & Above		Total	
	#	%	#	%	#	%	#	%	#	%
Alcohol	25	7.69	15	4.61	13	4.00	9	2.77	62	19.07
Marijuana (Cannabis)	29	8.92	17	5.23	11	3.38	13	4.00	70	21.53
Both Alcohol & Marijuana	8	2.46	9	2.77	5	1.53	1	0.31	23	7.07

x^2 =4.820 df=6. P value= 05671 (The age variation is not statistically significant at this p-value).

Figure 2: *Percentage distribution of psychoactive drugs that tested positive based on location*



In Benin City, [illegible] result was male to female ratio [illegible] a Kruskal Wallis test of P value 0.26 [illegible]. Abuja to Benin City with a ratio of 1.6:1. A similar pattern was recorded in Benin City and Ibadan. In similar studies by Lowenstein and Koziol-Mclain,[26-28] alcohol was found to have the highest percentage consumed. The observation that significantly more males were users of alcohol and marijuana is similar to previous studies in Nigeria [27-29] and it is also probably due to the fact that driving is essentially male dominated. The fact that as high as 32.53% in this study admitted that their driving skills have been improved after ingestion of psychoactive drugs is worrisome, especially against the efforts by various law enforcement agencies to combat substance abuse. The implication of that high value (32.53%) is that even in a course of rehabilitation, a significant proportion of motorists are likely to sustain use of psychoactive drugs based on their assumed derived benefits. In line with many previous studies in Nigeria and elsewhere in Africa.[29-33] This research found users of drugs or drugs that tested positive to commonly start in adolescence and young adulthood. In our setting, alcohol tends to gain prominence followed by cannabis.

This study indicated that the use of cannabis is very high in all the locations and thus confirms the ease of availability and low cost.[31] Findings in Canada show that cannabis is the most widely used illicit drug and it is certainly higher than cocaine. [32] T prevalence of driving under the influence cannabis has been reported by Walsh et al., with similar results to our study. Laberge a Ward [34] found that a very high value (90%) users were willing to drive after consuming typical dose. In other studies, M donald et [35,36] found that 50% of the tre nent gro drove within an hour of consum g cannab compared to 6% of the genera population This compares to our post-crash finding Benin City with a cannabis use of 16.9% Epidemiological studies have found that t average percentage of injured drivers testi positive for cannabis by urinalysis was abo 11%.[34-36]

Several studies in South Africa compa favourably and also at variance with o studies. In a study over 3 years from 1999 2001, Parry et al,[31] found among 1565 trau patients in 3 major cities, that laborato screening for cannabis showed that across si and over the three time periods, 25% and 5 of patients tested positive for cannabis, a between 7.4% and 35% of patients tes positive for methaqualone and cannabis, so-called white pipo combination. statistically significant interaction was fou between city and year of injury. In Cape To the percentage of patients testing positive w significantly higher in 2001 than in 1999 2000. An interesting debate is the degree which people can compensate for the effects drugs while driving, which renders preval data not to be sacrosanct. Several intervie individuals did admit driving more cautio

after ingestion of licit and illicit drugs. The role of cannabis is well known to be very controversial. [37-40] Previous studies show the deterioration of certain faculties useful in driving under the influence of Cannabis, [38-42] as at now there are no homogenous and robust conclusions proving that the use of Cannabis is a significant factor in accidents. [40-43] Further work on cannabis can be found in reports by Huestis. [41]

The complex metabolism of cannabis is illustrated by the evidence that it becomes more difficult to measure its effect on driving under the influence with any precision. Cannabis involvement in road traffic accident seems over exaggerated especially with Canadian senate special committee on illegal drugs (2002) documenting that there is more caution driving. Their conclusion was that it does not reflect impairment in terms of performance effectiveness. The report from U.K. Dept of environment in (2000) is even less convincing by reporting that—there is no evidence that consumption of cannabis alone increases the risk of culpability for traffic crash fatalities or injuries which lead to hospitalization. [41-43] This study only shows the level of exposure to the psychoactive drugs with the use of these immunoassay devices.

Other psychoactive drugs are amphetamines, cocaine, benzodiazepines, opiates (morphine, heroin, and codeine), or unclassified solutions or concoctions of all shades, and herbal preparations. Some of these products tested positive to POCT with the use of other similar immunoassay techniques in our study. Others constituted the highest positive group with a male to female ratio of about 2:1 in Abuja, while Benin City screening results were lower. These results show that Abuja screened candidates were more likely to try other conventional and unconventional products like solutions and herbal preparations (using both POCT and self-report structured questionnaires).

Amphetamines tested positive but the main focus of alcohol and cannabis in this research overshadow its relevance. Gender analysis shows that males have a considerably higher incidence of use than females whether in Abuja or Enugu, Benin City or Ibadan, and this is similar to previous findings reported. [42-45] The risk of using amphetamines manifests as high speed, diminished or divided attention, inattentive driving, impatience, and other risky behaviours. Driving impairment is attributed to distraction, disorientation, motor excitation, hyper-somnolence, hyper-active reflex, and general cognitive impairment. [44] Similar screenings in Sweden with driving under the influence of drugs (DUID) suspects apprehended is based on the work of Ceder, [45] that had amphetamine as the highest illicit drug identified in blood samples; their findings were 56% followed by THC (cannabis) at 25% and then diazepam.

Gender factors in benzodiazepine showed that it is the most abused drug amongst females in Abuja compared to other individual drugs; except for the unclassified drugs but still lower than its use amongst males. The difference between the males and females was very close compared to others with a ratio of 1.45:1. The work of Mura et al [46-47] in France and the Netherlands shows that benzodiazepines were the most frequently detected drug in 2001 which is above the level found in our study in selected Nigeria cities. Benzodiazepines are commonly prescribed for the rapid relief of anxiety, muscle relaxation, sedation and as anti-convulsants. It is therefore of considerable interest to alert patients of the risk potential in using these drugs.The long acting benzodiazepines (flunitrazepam, clonazepam, and diazepam), show considerable impairment to skills for driving a vehicle and should therefore be prescribed with caution. [46,47] A considerable number of people in Nigeria use the benzodiazepines for sleep disorders and because of lack of enforcement of the relevant laws, these drugs can be purchased without prescription in most parts of Nigeria. They can also be used recreationally, though their use without prescription is illegal in many parts of the world including Canada and the United States (Centers for Addictions and Mental Health, 2006). [48] Our findings in Benin City of its use among our test subjects were 14.7% which is within the range of 6.2% to17.6% reported by Zandstra et al. [49]

In conclusion, this manuscript is evidence based research that saliva is an accessible matrix for screening of several psychoactive drugs that are often abused by motorist in Nigeria. The use of digital screening tool with saliva is convenient, reliable and time saving methods to enhance highway safety by identifying motorists with positive substances of abuse.

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