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Prevalence of Vaginal Pathogens Associated with Genital Tract Infections in Ogun State, Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Author OOB designed the study, performed the statistical analysis, wrote the first draft of the manuscript and managed the literature searches. Author s OOM and TKB assisted in the collections and transportation of samples. Author OOM and MOE managed the analyses of the study. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Aims: The occurrence of vaginal pathogens associated with genital tract infections [GTIs] was investigated in this study.
Study Design: Over a three-month period, 106 High Vaginal Swab [HVS] samples were obtained from women with genital tract infections [GTIs] within the ages of 15 – 50 years attending In- and Out- patients clinic at General Hospital, Ijebu-Ode, Ogun State, Nigeria and percentage frequencies of isolates were determined comparatively.
Place and Duration of Study: Collections of samples were made at General Hospital, Ijebu-Ode, Ogun State while microbiological analyses on samples were carried out at the Department of Microbiology and Parasitology, Olabisi Onabanjo University Teaching Hospital [OOUTH], Sagamu, Ogun State, between August and November, 2011.
Methodology: Samples were screened for the presence of vaginal pathogens using conventional microbiological techniques. Potato dextrose agar [PDA] was employed to isolate and enumerate *Candida* species. Chocolate agar was used for the isolation of *Neisseria gonorrheae,* while Columbia agar base in 10% CO_2-enriched atmosphere was

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employed for the isolation of *Gardnerella vaginalis*. Microscopic examinations of smears were carried out to determine the presence of *Trichomonas vaginalis.* Paired sample t-test was employed to analyze results statistically.

Results: *Candida* species recorded the highest prevalence of 58 [54.7%], followed by *Trichomonas vaginalis* 27 [25.5%], *Gardneralla vaginalis* 12 [11.3%], while *Neisseria gonorrhea* recorded the least prevalence of 09 [8.5%]. Among the *Candida* isolates obtained, *Candida albicans* had the highest prevalence of 39 [67.2%], followed by 11 [19%] *Candida tropicalis*, 6 [10.3%] *Candida parapsilosis* while the least occurred was *Candida krusei* with 2 [3.5%]. Results also showed that the incidence of *Candida* species was highest within the age group of between 21 and 30 years except *Candida tropicalis* which recorded highest incidence within the age range of 15 – 20 years. Statistical analyses established that there was no significant difference between the incidence of *Candida* sp and other vaginal pathogens.

Conclusion: Vaginal pathogens are directly associated with genital tract infections and this is on the high side among women in the developing world like Nigeria. This calls for commitment to routine evaluation and appropriate intervention in antenatal clinics.

Keywords: Vagina; Candida; pathogens; women; prevalence; GTIs.

1. INTRODUCTION AND LITERATURE REVIEW

The genus Candida and species C. albicans was described by a botanist - Christine Marie Berkhout - in her doctoral thesis at the University of Utrecht in 1923 as reported by Bodey [1]. Over the years, the classification of the genera and species has evolved. Obsolete names for this genus include Mycotorula and Torulopsis. The species have also been known in the past as Monilia albicans and Odium albicans. The current classification is nomen conservandum, which means the name is authorized for use by the International Botanical Congress [IBC] [2]. The genus Candida includes about 150 different species. However, only a few are known to cause human infections. C. albicans is the most significant pathogenic species [3,2]. Other Candida species pathogenic to humans include C. tropicalis, C. glabrata, C. krusei, C. parapsilosis, C. dubliniensis and C. lusitaniae [2]. Candida species are frequently part of the human bodies' normal oral and intestinal flora. The yeast is a common commensal of the gastrointestinal tract. Most Candida infections are opportunistic occurring in debilitated persons [2].

The clinical diagnosis of vaginal candidiasis is unreliable [4,5,6,7], and laboratory confirmation is needed. More recently, however, it has been shown that symptomatic candidiasis is usually associated with higher numbers of vaginal yeasts than those found in asymptomatic carriers [6,8,9]. Unfortunately, conventional mycological laboratory techniques for yeast quantification involve serial dilutions [8], and dip slides or broth media do not allow for quantification [10,11,12].

Candidiasis has been associated with prolonged broad spectrum antibiotic therapy. Many different clinical forms of candidiasis are known, involving primarily the mucosal surfaces [thrush], gastrointestinal or urogenital tract, and deep-seated infections such as candidemia or meningitis. Candida vaginitis is a common infection during pregnancy [13]. Infection of the vagina or vulva may cause severe itching, burning, soreness, irritation, and whitish or whitish-gray cottage cheese-like discharges, often with a curd–like appearance. These symptoms are also present in the more common bacterial vaginosis [14,15,16].

However, some other groups of microorganisms have also been indicted as being involved in vaginal infections. Gardnerella vaginalis is a facultative anaerobic Gram-variable rod that can cause bacterial vaginosis in some women as a result of a disruption in the normal vaginal microflora. The resident anaerobic Lactobacilli population in the vagina is responsible for the acidic environment. Once the anaerobes have supplanted the normal vaginal bacteria, prescription antibiotics with anaerobic coverage may have to be given to eliminate the G. vaginalis and allow the balance to be restored [17,15,16]. While typically isolated in genital cultures, it may also be detected in other samples from blood, urine, and pharynx. G. vaginalis is sexually transmitted. Although G. vaginalis is a major species present in bacterial vaginosis, it can also be isolated from women without any signs or symptoms of infection. Symptoms include vaginal discharge, vaginal irritation, and a "fish like" odor. In the "amine whiff test" 10% KOH is added to the discharge, a positive result indicated if a fishy smell is produced. These and other tests can be used to distinguish between vaginal symptoms related to G. vaginalis from those caused by other organisms, such as Trichomonas and Candida albicans, which require different treatment [18]. A selective medium for G. vaginalis is colistin-oxolinic acid blood agar [18].

Gonorrhoea is a common sexually transmitted infection caused by the bacterium - *Neisseria gonorrheae* [also called Gonococcus, which is often abbreviated as "GC" by clinicians]. In both men and women, if gonorrhea is left untreated, it may spread throughout the body, affecting joints and even heart valves. The infection is transmitted from one person to another through vaginal, oral, or anal sexual relations; transmission occurs rarely with safe sex practices of condom usage with lubricants. The incubation period is 2 to 30 days with most symptoms occurring between 4–6 days after being infected. A small number of people may be asymptomatic for a lifetime. Between 30% and 60% of people with gonorrhea are asymptomatic or have subclinical disease [19].

Trichomonas vaginalis [TV] is a protozoan that causes Trichomoniasis. It is a common cause of vaginitis in women and occasionally, urethritis in men. The condition is localized and considered to be sexually transmitted in the majority of patients. The parasite *T. vaginalis* can survive outside the body in a moist environment; extracorporeal transmission by fomite is a theoretical possibility. A proportion of women, especially in the older age group, are asymptomatic carriers; in others, the vaginitis ranges from mild to severe. In men, *Trichomonas vaginalis* is not commonly recognized. This may be due to the infection being mild or asymptomatic or because the parasite is not regularly looked for even in the presence of urethritis. And it is also possible that T. vaginalis is commonly inhibited from colonizing the male urethra for any length of time [20]. In infected female patients, symptoms include: vaginal discharge, vulvar pruritis, dysuria and dyspareunia. Classical green, frothy, foul-smelling discharge occurs in 10% of the women, and the red granular ‘strawberry’ cervix can be identified by the naked eye in less than 2% of patients [21,22]. However, up to 50% of the infected female patients are asymptomatic, depending on the study population, selection criteria and diagnostic methods used.

There is lack of simple, cheap and reliable diagnostic tools for the evaluation and management of GTIs. Available clinical approaches have low sensitivity and/or specificity and do not appear to serve in all settings. Genital tract infections affect pregnancy outcome adversely. The effective controls of GTIs have both individual and public health relevance. Interventions that have been proven to affect disease rates include effective treatment with appropriate antibiotics and efficient case-finding through improved diagnosis. Therefore, the aim of this study is to determine the prevalence of vaginal pathogens with special reference to Candida species

2. MATERIALS AND METHODS

2.1 Sampling

A total number of 106 patients with genital tract infections attending in- and out- patients' clinic of General Hospital, Ijebu-Ode, Ogun State, Nigeria were investigated.

2.2 Collection of High Vaginal Swabs [HVS]

A high vaginal swab was collected using sterile swabs. The sterile swabs were obtained ready for use. Gloves were worn and broken skin or wounds were covered with Elastoplast during the handling of urogenital specimens to prevent contamination. High vaginal swabs were collected using a vagina speculum. The cervix was first examined, including the vaginal walls, and colour, consistency, and the source of the discharge were noted. Two test tubes were used, marking one tube "HVS.", and leaving the other tube unmarked. The speculum was inserted into the fully opened vagina, and a bright lamp was used to examine the cervix and the vaginal walls. In the collection of HVS specimens, the sterile swab from the tube marked "HVS" was taken and passed high into the posterior fornix of the vagina [the highest part of the vagina] and rolled for a few seconds to collect the secretions. The swab was returned into the test-tube. The specimens were then taking into the laboratory without delay for processing and examination.

2.3 Examination of Specimen

2.3.1 Microscopic examination

Each sterile swab was labeled with patients' laboratory number using a grease pencil. Glass slides were cleaned using gauze and were also labeled with the patients' laboratory number. A drop of physiological saline was dropped and smeared on the slide. This was properly mixed using the swab stick and covered with a cover-slip. The slide was examined under the microscope using the oil immersion lens, x10 objective lens and x 40 objective lens for a more detailed structure.

2.3.2 Culturing method

Each swab was streaked on the appropriate culture medium. Potato dextrose agar [PDA] [Oxoid] was employed to isolate and enumerate Candida species. Growth on PDA was identified by cultural characteristics, morphotyping, microscopy and battery of biochemical tests. On PDA, cream-coloured pasty colonies with characteristic yeast smell whose Grams reaction appears large [3-6m by 6-10m] positive cocci were subsequently confirmed to be yeast cells [23]. Any multiple colonies were separately identified. This was incubated for 3 – 5 days at 37ºC to allow development of yeast colonies. Chocolate agar [Oxoid] was used for isolation of Neisseria gonorrhoeae and sub-culturing was made on modified Thayer-Martin plate warmed to room temperature using a sterile loop. Cultures were incubated for 18 - 24 hours at 35 - 37ºC in the candle jar and plates examined for the presence of typical growth. Cultures showing no growth or atypical growth were re-incubated and examined again after an additional 24 hours. Plates with typical growth were evaluated by testing for the production of oxidase. Oxidase positive cultures were smeared, stained and examined microscopically. Columbia agar base was employed for isolation of Gardnerella vaginalis in 10% CO_2-enriched atmosphere with incubation temperature of 37ºC for 1 - 2 days. The

morphological characteristics of colonies allowed initial diagnosis of the organisms causing disease symptoms [13].

2.4 IDENTIFICATION OF VAGINAL PATHOGENS

2.4.1 Cultivation on the selective medium [Chrom agar]

Chrom agar is selective medium, which indicates an increase in the number of non-albicans species based on a colour change. It can be used for identification of individual non-albicans species, as well as *C. albicans*, if germ tube test was not characteristic. All pure cultures of yeasts from PDA was cultivated on a Chrom agar. This was incubated at 37ºC for 48 hrs and identification of yeast was performed based on a colony colour. Using this method, individual non-albicans species: *C. tropicalis* [blue colonies, wet], *C. krusei* [light pink colonies, dry], *C. parapsilopsis* [cream colonies, wet] and *C. albicans* [green colonies, wet] were identified.

2.4.2 Yeast assimilation test [API test]

This test represents the safest identification of non-albicans species. Test is based on the ability of yeast to assimilate coal hidra [organic compounds]. Premade factory tests of high sensitivity and precision [API YZC AUX] was used. This test was performed with the 'pure cultures' obtained on PDA. Due to the reliability of this method, we tested all non-albicans species using this test, confirming the results obtained by cultivation on Chrom agar.

Assimilation test, germ tube test and pseudohyphae tests were all carried out for the identification of Candida species according to the methods described by Ochei and Kolhatkar [24]. Sugar assimilation procedures were conducted using minimal sugar-free nitrogen-containing basic agar, inoculated with highly turbid suspension of overnight subculture of each yeast-like cells. The preparation was allowed to set, dried and previously sugar-impregnated-discs were placed on the plates and incubated overnight. A milky halo formation around each disc corresponds to diffusion zone of that particular sugar and hence its assimilation. Where necessary, organisms were also checked for urease production, nitrate assimilation, and ascospore formation [25]. Other tests carried out for the identification vaginal isolates include sugar fermentation test, capsule staining with Indian ink, Gram's staining and other biochemical tests in accordance with the descriptions of Cheesebrough [26] and Ochei and Kolhatkar [24].

2.5 Statistical Analysis

Paired sample t-test was performed to analyze results statistically using the SPSS version 15.

3. RESULTS AND DISCUSSION

In this study, 106 females with age ranging from 15 – 50 years, who had been diagnosed clinically for the presence of one or more vaginal pathogens were recruited to participate. Tables 3.1a and 3.1b showed the biochemical characterization of Candida species and 2 bacterial genera, respectively. The morphological characteristics of Trichomonas vaginalis was shown in Table 3.1c. The identified Candida include *C. albicans, C. krusei, C. tropicalis* and *C. parapsilopsis* [Table 3.1a]

Table 3.2 showed the distribution of vaginal pathogens obtained among selected age range of 15 and 50 years. The highest prevalence of vaginal pathogens was recorded within the age range of 21 – 30 years. It was, however, amazing that the age range of 15 – 20 years recorded the second highest prevalence rate of vaginal pathogens despite the smallest class size out of four groups. *Gardnerella vaginalis* had the lowest occurrence in the age range of between 31 – 40 years. However, in all, Candida species recorded the highest incidence of 54.7% while *Neisseria gonorrheae* recorded the lowest prevalence of 8.5% [Table 3.2].

This study also put into cognizance the distribution of the vaginal pathogens among In-patients and Out-patients. Out of the total of 106 patients screened, 74 were out patients while 32 were in patients. It was, however recorded that the incidence of the vaginal pathogens was higher among the out-patients, except the bacterium - *Gardnerella vaginalis* – whose incidence was higher among the in-patients. It can also be pointed out that the incidence of *Neisseria gonorrheae* was lowest among the in-patients [3.13%]; and among the out-patients*, Gardnerella vaginalis* and *Neisseria gonorrheae* possessed lowest percentage frequency of 10.8% [Table 3.3].

Studies were further conducted to determine the distribution of Candida species within the age range of 15 – 50 years. It was discovered that out of the 58 Candida species, 39 [67.2%] were *Candida albicans*, followed by 11[19%] *Candida tropicalis*, 6[10.3%] *Candida parapsilosis* and 2[3.5%] *Candida krusei*. It was, however, noticeable that the occurrence of Candida species was highest within the age group of between 21 and 30 years except *Candida tropicalis* which recorded highest within the age group of 15 – 20 [Table 3.4]. This information gives an insight on the distribution of Candida species that are associated with genital tract infections aside the popularly indicted *C. albicans*.

It was statistically established that there was no significant difference between the paired observations performed on Candida sp Vs. *Gardnerella vaginalis*, Candida sp Vs. *Trichomonas vaginalis*, Candida sp Vs. *Neisseria gonorrhea* and *Gardnerella vaginalis* Vs. *Neisseria gonrrrhea*. In the contrary, however, the mean difference in the incidence of *Gardnerella vaginalis* Vs. *Trichomonas vaginalis* was significant [$P < 0.05$] [Table 3.5].

Comparative studies of genital tract colonization [asymptomatic] by Candida species with microbiological evidence are difficult. Many studies examine tertiary hospital populations and may include or target only those with symptoms of vaginitis. Worldwide estimates of genital Candida colonization range from 17% in Turkey to up to 30% in a U.S. study of asymptomatic young women followed over 12 months [27-33]. These estimates, reported by the authors, were less compared with 54.7% incidence rate obtained in this study [Table 3.3]. These results also showed higher incidence rate of *C. albicans* [40%] [Table 3.3] compared with a study of 1009 women in New Zealand where C. albicans was isolated from vagina of 19% of apparently healthy women [34]. The proportion of genital infections by *C. albicans* in symptomatic women ranged from approximately 65% in Belgium [30], Turkey [31], and Saudi Arabia [35] to approximately 90% in U.S. and Australian samples [28,32].

Candida yeasts are commonly present in humans, and their growth is normally limited by the human immune system and by other microorganisms, such as bacteria occupying the same locations [niches] in the human body [36]. Douches or internal disturbances [hormonal or physiological] can perturb the normal vaginal flora such as lactobacilli and result in an overgrowth of Candida cells causing symptoms of infection, such as local inflammation. Pregnancy and uses of oral contraceptives have been reported as a risk factor [37], while the

roles of engaging in vaginal sex immediately and without cleansing after anal sex and using lubricants containing glycerin remain controversial.

In conclusion, this study showed that incidence rate of GTIs among women in the developing Countries like Nigeria is on the high side and calls for commitment to routine evaluation and appropriate intervention in antenatal clinics. The changes in sexual behavior can be inferred from changing incidence rates in Western countries. The greatest gains are achieved when all elements are included in an integrated strategy. However, the complexity of the interrelationship of these disparate factors means that a lower rate of disease attained by one means may be offsetted by a decline in compliance in another effector arm. For example, improved case-finding through better diagnostic tests and instigation of proper treatment regimens may provide gains which are offset by deleterious behavioral change, and vice versa.

Table 3.2 showed the distribution of vaginal pathogens obtained among selected age range of between 15 and 50 years. *Candida* species recorded the highest prevalence of [54.7 %] while *Neisseria gonorrheae* recorded the least prevalence of [8.5%].

Table 3.3 showed the distribution of vaginal pathogens in In- patients and Out-patients examined in this study. It was recorded that the incidence of the vaginal pathogens was higher among the out-patients, except the bacterium - *Gardnerella vaginalis* – whose incidence was higher among the in-patients.

Table 3.4 showed the distribution of *Candida* species within the age range of 15 – 50 years. *Candida albicans* recorded the highest incidence of 67.2 % in all cases while *C. krusei* recorded the least prevalence of 3.5%. Occurrence of *Candida* species was highest within the age group of between 21 and 30 years except *Candida tropicalis* which recorded highest within the age group of 15 – 20.

The calculated P-value is greater than 0.05 in paired comparison tests performed on *Candida* sp Vs. *Gardnerella vaginalis, Candida* sp Vs. *Trichomonas vaginalis, Candida* sp Vs. *Neisseria gonorrhea* and *Gardnerella vaginalis Vs. Neisseria gonrrrhea* indicating that the mean differences between the paired observations were not significantly different. However, the mean difference between *Gardnerella vaginalis Vs. Trichomonas vaginalis* was significant [$P < 0.05$] [Table 3.5].

Table 3.1a. Biochemical reactions of *Candida* isolates

Assimilation						Fermentation					Other Tests			
No of isolates	Glucose	Maltose	Sucrose	Lactose	Dulcitol	Glucose	Maltose	Sucrose	Lactose	Urease	Pseudohyphae	Germ tube test	India ink	*Candida* spp
39	+	+	+	-	-	AG	AG	AG	-	-	+	+	-	*C. albicans*
2	+	-	-	-	-	AG	-	-	-	-	+	-	-	*C. krusei*
11	+	+	+	-	-	AG	AG	AG	-	-	+	-	-	*C. tropicalis*
6	+	+	+	-	-	AG	-	-	-	-	+	-	-	*C. parapsilopsis*

Keys: AG = Acid and Gas; + = Positive; - = Negative

Species of Candida identified include C. albicans, C. krusei, C. tropicalis and C. parapsilopsis

Table 3.1b. Biochemical characteristics of bacterial isolates

No. of Isolates	Gram's stain	Oxidase test	Catalase test	Methyl-red test	Indole Test	Starch hydrolysi	Motility test	Glucose	Maltose	Sucrose	Lactose	Fructose	Growth on nutrient agar at	Growth on blood agar at 35^0C	Most probable Isolate
9	Gram –ve diplococci	+	+	-	-	-	+	+	–	–	–	–	–	+	*Neisseria gonorrheae*
12	Gram variable rod	-	-	NA	NA	+	-	+	+	+	NA	NA	NA	+ [β-haemolyses]	*Gardnerella vaginalis*

Keys: + = present/positive; - = absent/negative

Two bacterial isolates were obtained in this study and these include Nesseria gonorrheae and Gardnerella vaginalis.

Table 3.1c. Characteristics of *Trichomonas vaginalis*

No of Isolates	Shapes	Flagella+ Undulating	Axostyle	Motility	Flagella	Infective
27	Pear-shaped/ Ovoid	+	+	+ Jerky/Rapid	3-5 anterior; 1 posterior	Trophozoite

Key: + = present/positive

Twenty-seven [27] isolates were identified by their shapes, flagella with undulating membrane, axostyle, and jerky/rapid motility as Trichomonas vaginalis.

Table 3.2 Distribution of vaginal pathogens among selected age range

Age [yrs]	N	*Candida* species	*Gardnerella vaginalis*	*Trichomonas vaginalis*	*Neisseria gonorrhea*
15 – 20	32	15 [46.9%]	05 [15.6%]	10 [31.3%]	02 [6.3%]
21 – 30	47	30 [63.8%]	04 [8.5%]	10 [21.3%]	03 [6.4%]
31 – 40	20	08 [40%]	03 [15%]	05 [25%]	04 [20%]
41 – 50	07	05 [71.4%]	0 [0%]	02 [28.6%]	0 [0%]
Total	106	58 [54.7%]	12 [11.3%]	27 [25.5%]	09 [8.5%]

Keys: n [%] = Percentage of Patient infected; N = Number of Patient Tested

Table 3.3 Occurrence of vaginal pathogens in In- and Out-patients

Departments	N	*Candida* species n [%]	*Gardnerella vaginalis* n [%]	*Trichomonas vaginalis* n [%]	*Neisseria gonorrhea* n [%]
In – patients	32	20 [62.5%]	04 [12.5%]	07 [21.9%]	01 [3.13%]
Out – patients	74	38 [51.4%]	08 [10.8%]	20 [27.0%]	08 [10.8%]
Total	106	58 [54.7%]	12 [11.3%]	27 [25.5%]	09 [8.5%]

Keys: n [%] = Percentage of Patient infected; N = Number of Patient Tested

Table 3.4. Distribution of *Candida* species by age

Age [yrs]	N	*Candida albicans*	*Candida parapsilosis*	*Candida tropicalis*	*Candida krusei*
15 – 20	15	08 [53.3%]	02 [13.3%]	05 [33.3%]	01[6.7%]
21 – 30	30	24 [80%]	02 [6.7%]	04 [13.3%]	0 [0]
31 – 40	08	05 [62.5%]	01 [12.5%]	02 [25%]	0 [0]
41 – 50	05	02 [40%]	01 [20%]	0 [0]	01 [20]

Keys: n [%] = Percentage of Patient infected; N = Number of Patient Tested

Table 3.5. Determination of the relationships among vaginal pathogens using Paired Samples Test

Paired isolates		Paired Differences					t	df	Sig. [2-tailed]
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference		Mean	Std. Deviation	Std. Error Mean
		Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper
Pair 1	*Candida* sp – *Gardnerella vaginalis*	11.500	9.950	4.975	-4.332	27.332	2.312	3	.104
Pair 2	*Candida* sp – *Trichomonas vaginalis*	7.750	8.221	4.110	-5.331	20.831	1.885	3	.156
Pair 3	*Candida* sp – *Neisseria gonrrrhea*	12.000	10.893	5.447	-5.334	29.334	2.203	3	.115
Pair 4	*Gardnerella vaginalis* – *Trichomonas vaginalis*	-3.750	2.062	1.031	-7.030	-.470	-3.638	3	.036
Pair 5	*Gardnerella vaginalis* – *Neisseria gonrrrhea*	.500	2.082	1.041	-2.812	3.812	.480	3	.664

COMPETING INTEREST

Collectively speaking, we appreciate this opportunity which encourages freedom of expression of feelings of interest. One of the challenges we scientists face in this part of the world is funding. Researchers are rarely funded and making use of the state-of –the-art equipments becomes unaffordable. Scientific research is more expensive in a country like ours where power supply is epileptic and more money has to be spent to obtain and maintain reliable scientific results which is paramount to us as scientists/researchers. We are, however, optimistic that government and concerned institutions take proactive actions to combat the menace.

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