Original Article

# Household Air Pollution and Respiratory Symptoms of Women and Children in a Suburban Community in Nigeria

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Abstract **OBJECTIVE:** Globally, the morbidity and mortality caused by exposure to household air pollution from the use of solid fuels remain a significant public health burden. This study assessed the levels of PM<sub>2.5</sub> in households using clean and unclean fuels and their associations with the respiratory health of women and children.

**MATERIAL AND METHODS:** Daytime PM<sub>2.5</sub> sampling was done in 62 households (31 in each group) in Isiohor, a suburban community in southern Nigeria using Casella CEL-712 Microdust Pro Real-time Dust Monitor. Validated American Thoracic Society questionnaire was used to assess respiratory symptoms.

**RESULTS:**  $PM_{2.5}$  levels exceeded World Health Organization-recommended limits in most households. The median (range) concentration of  $PM_{2.5}$  was lower in households using clean fuels (26 (14 to 358) µg/m<sup>3</sup>) than those using unclean fuels (29 (14 to 650) µg/m<sup>3</sup>). This difference was not statistically significant (P = .272). At least 1 respiratory symptom was reported by women (25.8% vs. 22.6%) and children (64.5% vs. 77.4%) in household using clean and unclean fuels, respectively. The most commonly reported respiratory symptoms were being woken up by an attack of cough (41.9% vs. 51.6%) and cough first thing in the morning (16.1% vs 38.7%) for clean and unclean fuels, respectively (P = .046). More children in household using unclean fuel missed school for up to a week because of respiratory illness when compared to those in households using clean fuel, 61.3% vs. 29.0% (P = .011). In the sample as a whole, burning of candles in the house (22.6%) was associated with respiratory symptoms (Adjusted Odds Ratio = 14.81, 95% CI 1.79 to 122.51) among the women.

**CONCLUSION:** The use of unclean fuel was associated with higher levels of PM<sub>2.5</sub>. The household air pollution resulting from the use of unclean fuels and activities like burning of candles in the home may compromise the respiratory health of women and children.

KEYWORDS: Household air pollution, household cooking fuels, respiratory symptoms, women and childrenReceived: February 14, 2021Accepted: June 13, 2021

# INTRODUCTION

The morbidity and mortality caused by exposure to household air pollution (HAP) from the use of solid fuels remain a significant public health burden in many developing countries of the world. It is estimated that globally, about 3 billion people mostly in low-income countries use solid fuels for domestic cooking and heating with close to 646 million of these people living in sub-Saharan Africa, and this number is expected to continue to rise over the years.<sup>1</sup>

The use of solid (biomass) fuels and kerosene exposes household members to health-damaging pollutants such as fine particulate matter ( $PM_{2.5}$  and  $PM_{10}$ ), carbon monoxide (CO), and polycyclic aromatic hydrocarbons.<sup>2</sup> Each year, close to 4 million people die prematurely from illness attributable to HAP from inefficient cooking practices using polluting stoves paired with solid fuels and kerosene.<sup>3</sup> A 10-µg/m<sup>3</sup> increase in indoor  $PM_{10}$  has been shown to increase cardiovascular and respiratory mortality by 0.36% and 0.42%, respectively. Similarly, a 10-µg/m<sup>3</sup> increase in indoor  $PM_{2.5}$  increases cardiovascular mortality by 0.63% and respiratory mortality by 0.75%. In the long term, every 10 µg/m<sup>3</sup> increase of household  $PM_{10}$  increases the risk of mortality by 23% to 67%.<sup>4</sup>

Exposures to HAP and its detrimental consequences affect all ages of life from preconception to old age, including all the systems in the body, and its effects resonate over an entire lifetime with the respiratory system bearing the most burden.<sup>5</sup> Women and children are mostly affected because the mothers are primarily responsible for cooking and their children especially the under-fives are usually with them.<sup>6-8</sup> A study by Rumchev et al<sup>9</sup> recorded high levels of indoor air pollutants in rural kitchens in Zimbabwe and a high prevalence of respiratory symptoms in both women and children. The levels of respirable particles and CO were higher than accepted guidelines and may be contributing to the high prevalence of respiratory symptoms.<sup>9</sup>

In Nigeria, three-quarters of all households use solid fuels for cooking in poorly ventilated dwellings. The use of solid fuel is moderate in urban areas (45.0%) but very high in rural areas (90.0%).<sup>10</sup> About 40% of households cook inside their

houses, while one-quarter (25%) cook outdoors. The percentage of households that cook in their dwelling is higher in urban areas (43%) than in rural areas (38%).<sup>11,12</sup> These conditions are likely to result in constant and continuous exposures to pollutants during cooking and for a very long time after cooking. The use of biomass fuels and kerosene indoors in poorly ventilated kitchens leads to high concentrations of air pollutants including respirable particles and CO. Unfortunately, data on HAP and the resultant health effects especially among women and children are lacking in Nigeria.

Given the fact that the use of unclean fuels will continue to rise in many households in Nigeria without corresponding measures put in place by local authorities to provide clean fuels for the people and improve ventilation in houses, this study assessed the levels of PM<sub>2.5</sub> produced in households using clean (electricity and liquefied petroleum gas) and unclean (firewood and kerosene) fuels and their associations with respiratory health of women and children in a suburban community in Nigeria.

#### MATERIALS AND METHODS

#### **Study Site**

This community-based study was carried out in Isiohor Community, a suburban community located in Edo State, Nigeria. Edo State is located in the heart of the rain forest, and it lies in longitudes 5°E and 6° 42″E and latitudes 5° 45″N and 7° 35″N of the equator.<sup>13</sup> The inhabitants of Edo State Isiohor community are mainly junior and middle cadre civil servants, artisans, and farmers. Passage houses (a building with a long middle walkway and single rooms on both sides) were the predominant housing type in the community.

#### Study Population and Sample Size

The household environmental monitoring was done in households using clean and unclean fuels in the community. Women and their children under age of 5 years in the selected household participated in the questionnaire survey.

The sample size for this study was calculated using the OpenEpi statistical package for sample size calculation. In order to detect a 30% difference in the levels of PM in the household using clean fuel (mean  $PM_{2.5}$  of 100 µg/m<sup>3</sup> and standard deviation of 50 µg/m<sup>3</sup>) and those using unclean fuel (mean  $PM_{2.5}$  of 150 µg/m<sup>3</sup>) and standard deviation of 75 µg/m<sup>3</sup>), at a power of 80% and two-sided alpha of 0.05, the calculated sample size was 60. This was split into 30 each for the 2 groups.

#### MAIN POINTS

- The PM<sub>2.5</sub> levels in the Nigerian suburban community exceeded the World Health Organization-recommended limits in most households.
- The use of unclean fuels (biomass and kerosene) for cooking was associated with higher levels of PM<sub>2.5</sub> in households when compared to households using clean fuels (electricity and liquefied petroleum gas).
- The prevalence of respiratory symptoms was higher among children in households using unclean fuels.

#### Sampling Technique

The households that participated in this study were selected using a systematic sampling technique. There were a total of 250 households in the community with 100 and 150 using clean and unclean fuels, respectively, and this constituted the sampling frame. Based on the calculated sample size of 30 in each group, we arrived at the sampling interval of 3 and 5 for households using clean and unclean fuels, respectively, by dividing the sampling frame with the calculated sample size. The starting point was determined using a simple random sampling technique among households within the sampling interval, and subsequent households were systematically selected by adding the sampling interval to the first household selected. If there are 2 women in a selected household, the one with the youngest underfive child was recruited for the study. Similarly, if a selected women has more than 1 underfive children, the youngest underfive child was recruited for the study.

# **Data Collection**

Data collection was carried out using 2 methods – household environmental monitoring and a questionnaire survey.

#### Household Environmental Monitoring

PM<sub>25</sub> sampling was carried out using Casella CEL-712 Microdust Pro Real-time Dust Monitor (Casella, Bedford, United Kingdom) between the hours of 8:00 AM and 6:00 PM each day of survey. During sampling, the PM monitor was fitted on a tripod stand and held firmly in a stationary position, at a height range of 1.5 to 2.0 m above the ground. Total suspended particulate matter (TSP) was detected and quantified automatically. In each household, the concentration values obtained were recorded at 5-minute intervals over a period of 30 minutes to 1 hour. For PM25 monitoring, a polyurethane foam filter was positioned inside the adaptor attached to the probe to reduce the passage of particles above the PM<sub>2.5</sub> size category, before monitoring was performed. The obtained values were also recorded at 5-minute intervals. The PM monitor was pre-calibrated before use to ensure accurate results were achieved.

#### Questionnaire Survey

The respiratory symptoms of women and underfive children were assessed using a questionnaire adapted from the validated American Thoracic Society (ATS) questionnaire for respiratory diseases. The first part of the questionnaire contained the socio-demographic characteristics of the women and their corresponding children. The second part contained questions on the presence of respiratory symptoms of wheeze, chest tightness, cough, phlegm, shortness of breath, difficulty in breathing, and asthma in both women and the children. The final part sought information on the housing characteristics which included type of fuel used in cooking, the place of cooking (whether indoor or outdoor), the ventilation of the cooking area, burning of candles or oil lamps in the house, and the use of gasoline generator in the house.

#### Measurements

Blood pressure (BP) was measured using the *ACCOSON* sphygmomanometer. The measurement was done in the sitting position with an appropriate-sized cuff encircling the left arm held at the level of the heart. Hypertension was defined

as systolic BP >140 mmHg and/or diastolic BP >90 mmHg according to the Joint National Committee (JNC 7) on Hypertension classification.<sup>14</sup> Weight and height were measured using a standardized stadiometer. The body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. The World Health Organization (WHO) BMI classification was adopted as follows: underweight (BMI < 18.5), normal weight (BMI 18.5 to 24.9), overweight (BMI 25.0 to 29.9), and obese (BMI > 30).<sup>15</sup>

#### **Statistical Analysis**

Data analysis was done using The Statistical Package for Social Sciences version 21.0 software (IBM Corp.; Armonk, NY, USA). An initial univariate analysis was performed to determine the distribution of the variables in a stratified analysis on the basis of the type of fuel (clean vs. unclean fuel). The primary outcome was the household level of PM2 5, while the secondary outcomes were the respiratory symptoms in both women and underfive children. The covariates include place of cooking in the home, burning of candles, frying of food in the house, use of generator set, and neighbor's use of generator set. Chi-square test was used to test the association between socio-demographic characteristics and the fuel type used by respondents. Mann-Whitney U-test was used to test the difference in the PM<sub>2.5</sub> levels in households using clean and unclean fuels. Logistic regression was modeled for the socio-demographic characteristics (type of fuel, age, and level of education) and all the covariates to identify factors associated with the presence of respiratory symptoms among the women. The level of significance adopted for each statistical test was P < .05.

# RESULTS

Thirty-one households each using clean (electricity and liquefied petroleum gas) and unclean (firewood and kerosene) fuels, respectively, were sampled. Table 1 shows the sociodemographic characteristics of the women and children. Almost all the socio-demographic characteristics of the women and children studied were basically similar by fuel type. However, a higher proportion of mothers with only primary level of education used unclean fuel (P = .009).

The atmospheric, housing, and anthropometric characteristics of the respondents are shown in Table 2. In the households using clean fuel, we recorded lower median (range) levels of PM<sub>2.5</sub> of 26 (14.0 to 358.0)  $\mu$ g/m<sup>3</sup> compared to 29 (14.0 to 650.0)  $\mu$ g/m<sup>3</sup> in those using unclean fuel. For TSP, the median (range) was 64 (3.0 to 414.0)  $\mu$ g/m<sup>3</sup> in households using clean fuel and 70.0 (37.4 to 4772.8)  $\mu$ g/m<sup>3</sup> in households using unclean fuel. Over two-thirds (22/31) of the households using clean fuel cook indoors, while only about a third (10/31) of the households using unclean fuel cook indoors (P = .002). There was no statistically significant difference by fuel type in other housing characteristics such as frying of food in the house, burning of candles, and use of gasoline-powered generators. For BP, more than half (54.8%) of those in unclean fuel households had normal BP when compared to a third (32.3%) of those using clean fuel (P = .182).

**Table 1.** Socio-Demographic Characteristics by Fuel TypeUsed by Respondents

	Type of Fuel		
Characteristics	Clean Fuel, n = 31	Unclean Fuel, n = 31	Р
Mother			
Age group (years)*			
20 to 29	10 (32.3)	13 (41.9)	.582
30 to 39	15 (48.4)	11 (35.5)	
40 to 49	6 (19.4)	7 (22.6)	
Level of education			
Primary	3 (9.7)	9 (29.0)	.009
Secondary	19 (61.3)	21 (67.7)	
Tertiary	9 (29.0)	1 (3.2)	
Marital status			
Married	29 (93.5)	29 (93.5)	.999
Single	2 (6.5)	2 (6.5)	
Children			
Age group of child (months)			
0 to 11	7 (22.6)	5 (16.1)	.931
12 to 23	5 (16.1)	7 (22.6)	
24 to 35	7 (22.6)	6 (19.4)	
36 to 47	4 (12.9)	5 (16.1)	
48 to 59	8 (25.8)	8 (25.8)	
Sex of child			
Male	11 (35.6)	17 (54.8)	.126
Female	20 (64.5)	14 (45.2)	
Birth order of child			
First	6 (19.4)	6 (19.4)	.830
Second	10 (32.3)	8 (25.8)	
Third	9 (29.0)	8 (25.8)	
Fourth and above	6 (19.4)	9 (29.0)	

past 12 months preceding the study was generally low and similar in nature between women in the 2 groups. However, for the children, the prevalence of these respiratory symptoms was higher among those in households using unclean fuel. A greater proportion (12 or 38.7%) of children in the households that use unclean fuel usually cough first thing in the morning when compared to children (5 or 16.1%) in the households that use clean fuel (P = .046). Similarly, more than half (19, 61.3%) of the children in the households that use unclean fuel (P = .046). Similarly, more than half (19, 61.3%) of the children in the households that use unclean fuel were kept away from school by respiratory illnesses for 1 week in the past 1 year compared to children (9, 29.0%) in the households that made use of clean fuel (P = .011) (Table 3).

Table 4 shows the logistic regression model for possible factors associated with respiratory symptoms among the

The prevalence of respiratory symptoms of cough, wheezing, shortness of breath, and difficulty in breathing for the

	Type of F		
Variables	Clean Fuel, n = 31	Unclean Fuel, n = 31	Р
PM <sub>2.5</sub> (Median (range)) <sup>a</sup>	26.0 (14.0 to 358.0)	29.0 (14.0 to 650.0)	.272
Total suspended particulate matter <sup>a</sup>	64.0 (3.0 to 414.0)	70.0 (37.4 to 4772.8)	.063
Humidity, % (mean (SD))	67.0 (7.4)	58.8 (8.7)	<.001
Temperature, °C (mean (SD))	34.0 (2.0)	35.2 (3.0)	.076
Social and housing factors, n (%)			
Place of cooking at home			
Indoors	22 (71.0)	10 (32.3)	.002
Outdoors	9 (29.0)	21 (67.7)	
Burn candles, oil lamp in the house	8 (25.8)	6 (19.4)	.544
Burn/fry food in the house	28 (90.3)	26 (83.9)	.707
Use generator set at home	21 (67.7)	16 (51.6)	.196
Use of generator set by neighbor	20 (64.5)	22 (71.0)	.587
Anthropometry, n (%)			
Body mass index category			
Underweight	1 (3.2)	0 (0.0)	.365
Normal	11 (35.5)	17 (54.8)	
Overweight	12 (38.7)	10 (32.3)	
Obese	7 (22.6)	4 (12.9)	
Blood pressure category*			
Normal	10 (32.3)	17 (54.8)	.182
Pre-hypertension	19 (61.3)	11 (35.5)	
Stage 1	1 (3.2)	2 (6.5)	
Stage 2	1 (3.2)	1 (3.2)	

**Table 2.** Atmospheric, Housing, and AnthropometricCharacteristics of Respondents by Type of Fuel Used

<sup>a</sup>Mann–Whitney *U*-test; \*JNC classification of blood pressure. JNC, Joint National Committee; SD, standard deviation.

women. Selected variables were fitted into the model to identify independent factors associated with the women having had at least 1 respiratory symptom in the past 1 year preceding the study. Only the burning of candles in the home was found to be a significantly associated respiratory symptom among the women (AOR = 14.81, 95% CI 1.79 to 122.51, P = .028).

# DISCUSSION

The use of solid fuel and the attendant HAP has been found to constitute serious environmental and health challenges, resulting in health-damaging effects and changes in the biosphere/atmosphere. PM<sub>2.5</sub> features prominently among the health-damaging pollutants, and prolonged exposures to high levels of it, above the WHO-recommended air quality guideline values (25  $\mu$ g/m<sup>3</sup>), causes harm to all the body systems. In this study, we found very worrisome high levels of PM<sub>2.5</sub> in all of the households studied, although the levels were higher in those using unclean fuels. This finding may have resulted from the ambient PM concentration in the study area because of the closeness of the houses to each other and the use of biomass fuels for cooking.

A higher proportion of women using clean fuel had tertiary education when compared to those using unclean fuel. This finding was not surprising because the differential use of solid fuel has been found to be associated with household educational level and wealth.<sup>11</sup> Indoor cooking was very prevalent in all the households especially among those using clean fuel. This finding is in keeping with the Multiple Indicator Cluster Survey carried out by the National Bureau of Statistics in Nigeria which found out that a greater proportion of households in Nigeria use solid fuel and most of them cook indoors.<sup>11</sup>

The comparatively higher values of PM2.5 found in the households in this study pose a serious public health concern to families and communities in Nigeria. These high values were similar to the high levels of TSP, inhalable and respirable particles reported in previous studies carried out in some cities in Southern Nigeria.<sup>16,17</sup> The State of Global Air 2019 showed that globally, Western Sub-Saharan Africa is the region with the second highest PM<sub>2.5</sub> exposures and Nigeria as one of the countries having the highest exposures, with 100% of the population living in areas with PM<sub>25</sub> concentrations exceeding the WHO Air Quality Guideline.<sup>18</sup> This is a clear indication that Nigeria is not on course toward achieving the sustainable development goal (SDG) 11 which aims to "make cities and human settlements inclusive, safe, resilient, and sustainable." Women and children mostly bear the brunt of HAP. Household air pollution has been documented to give rise to acute lower respiratory tract infections in children below 5 years of age.<sup>3,12,19</sup> This is corroborated with the finding of more of the children in households that made use of unclean fuel in this study, who cough first thing in the morning and have been kept away from their usual activities by respiratory illness for 1 week.

Although the prevalence of respiratory symptoms was relatively low and similar among the women in both households, the respiratory symptoms were significantly higher in children in households using unclean fuel. The level of immunity of the children could probably explain why the children manifested more respiratory symptoms than the women. Studies in South West Nigeria have shown that exposure to HAP from biomass fuel (cooking with firewood) is associated with pulmonary dysfunction, reduced antioxidant defense, and inflammation of the airways, thus compromising lung health.<sup>20,21</sup> In addition, studies and surveys have also revealed that cooking with kerosene stoves in poorly ventilated structures increases air pollution.<sup>4,8,22</sup> These findings underscore the need for the government authorities in Nigeria to prioritize the implementation of measures that will replace the use

### Table 3. Prevalence of Respiratory Symptoms in the Women and Children

	<b>Type of Fuel</b> ( <i>n</i> (%))		
Respiratory Symptoms	Clean Fuel	<b>Unclean Fuel</b>	Р
Mother			
Had wheezing or whistling in the past 12 months	4 (12.9)	2 (6.5)	.671
Had shortness of breath during the day at rest in the past 12 months	2 (6.5)	3 (9.7)	.641
Had shortness of breath following strenuous activity in last 12 months	1 (3.2)	3 (9.7)	.612
Woken by an attack of shortness of breath at anytime in last 12 months	2 (6.5)	2 (6.5)	.694
Woken by an attack of coughing at anytime in last 12 months	5 (16.1)	4 (12.9)	.500
Usually coughed first thing in the morning	5 (16.1)	1 (3.2)	.195
Ever had trouble with breathing	2 (6.5)	1 (3.2)	.500
Troubled by shortness of breath when hurrying on level ground or walking up a slight hill	0 (0.0)	2 (6.5)	.492
Ever had asthma	2 (6.5)	0 (0.0)	.492
Kept away from work by any respiratory illness for 1 week in the past 1 year	1 (3.2)	0 (0.0)	.500
Child			
Had wheezing or whistling in the past 12 months	0 (0.0)	1 (3.2)	.313
Had shortness of breath during the day at rest in the past 12 months	1 (3.2)	1 (3.2)	.754
Had shortness of breath following strenuous activity in last 12 months	0 (0.0)	1 (3.2)	.313
Woken by an attack of shortness of breath at any time in last 12 months	3 (9.7)	3 (9.7)	.664
Woken by an attack of coughing at any time in last 12 months	13 (41.9)	16 (51.6)	.445
Usually coughed first thing in the morning	5 (16.1)	12 (38.7)	.046
Ever had trouble with breathing	1 (3.2)	2 (6.5)	.500
Troubled by shortness of breath when hurrying on level ground or walking up a slight hill	1 (3.2)	1 (3.2)	.754
Kept away from school by any respiratory illness for 1 week in the past 1 year	9 (29.0)	19 (61.3)	.011

of biomass fuels for cooking with cleaner energy source so that people can breathe in clean air in their households. A study in South West Nigeria showed that introduction of low-emission stoves was effective at improving indoor air quality and reducing exposure-related symptoms.<sup>21</sup>

Another important finding in this study is the presence of other factors that could lead to HAP in the households. These include burning of candles and oil lamp in the house, frying of foods in the house, and the use of gasoline-powered generators which emits carbon monoxide. The regression analysis showed that indoor cooking and burning of candles in the households were the most important significant predictors of respiratory symptoms. Women who cook indoors were 5 times more likely to have respiratory symptoms, while those who burn candles in their houses were 15 times more likely to have respiratory symptoms. These findings are supported by findings from the study by Gordon et al<sup>23</sup> which showed that burning of candles and various other forms of fuels apart from the conventional ones such as kerosene, animal dung, and charcoal have a way of increasing the carbon and particulate emissions and the exposure of the affected

individuals to higher levels with subsequent health-damaging consequences.<sup>23</sup>

This study has the following limitations: First, environmental monitoring was carried out during daytime hours, and the measurement period was limited to a maximum of 1 hour. Apart from the fact that cooking is mostly done in houses during the day in which case it is expected that the levels of HAP will be higher during this period, this study has generated baseline quantitative data of HAP in the study area, which will be useful for future studies. Secondly, although none of the women interviewed ever smoked cigarette or current smokers, we did not explore the possibility of exposure to secondhand smoke if any member of the household smokes inside the house. Thirdly, the small sample size used in this study may have affected the outcome of the multivariate analysis.

In conclusion, the use of unclean fuel was associated with higher levels of  $PM_{2.5}$ . The HAP resulting from the use of unclean fuels and activities like burning of candles in the house may compromise the respiratory health of women and children. The Nigerian government should institute measures to provide clean fuels for household use and improve ventilation

Respiratory symptoms in the women								
Covariates	Crude OR (95% CI)	Р	Adjusted OR (95% C1)	Р				
Type of fuel								
Clean fuel	1.19 (0.37 to 3.82)	0.313	0.25 (0.04 to 1.66)	.374				
Unclean fuel	1		1					
Place of cooking at home								
Indoors	1.57 (0.48 to 5.10)	0.897	5.09 (0.66 to 39.23)	.455				
Outdoors	1							
Burn candles in the house	5.00 (1.37 to 18.23)	0.062	14.81 (1.79 to 122.51)	.028				
Use generator set at home	0.50 (0.15 to 1.61)	0.407	0.83 (0.09 to 8.09)	.365				
Neighbor use of generator set	1.42 (0.39 to 5.18)	0.144	1.18 (0.19 to 7.22)	.755				
Age group (years)								
20 to 29	2.93 (0.52 to 16.6)	0.422	6.08 (0.54 to 68.18)	.590				
30 to 39	1.31 (0.22 to 7.88)		3.25 (0.20 to 52.29)					
40 to 49	1		1					
Level of education								
Primary	0.80 (0.09 to 7.00)	0.756	0.45 (0.01 to 16.23)	.832				
Secondary	0.63 (1.52 to 8.29)		1.82 (0.14 to 23.24)					
Tertiary	1		1					
OR, odds ratio.								

**Table 4.** Variables Associated With Presence of AnyRespiratory Symptoms in the Women

in houses to reduce frequent and continuous exposures to pollutants. This is imperative to the achievement of SDG 7 which is "ensure access to affordable, reliable, sustainable, and modern energy for all."

**Ethics Committee Approval:** The study was approved by the Ethics and Research committee of the University of Benin Teaching Hospital (Protocol Number: ADM/E22/A/VOL. VII/1364).

**Informed Consent:** Written informed consent was obtained from all the participants included in the study.

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