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Article

Screening for Microbial Airborne Spores from Unauthorized Urinary Spots on Road Sides in Igwuruta, Rivers State, Nigeria

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Abstract

Indiscriminate urination at unauthorized roadside spots creates unhygienic microhabitats with offensive odor and potential airborne microbial hazards. This study investigated the air microbiome of such sites in Igwuruta, Rivers State, Nigeria, by exposing sterile Petri dishes containing nutrient agar, anaerobic blood agar, and Sabouraud dextrose agar at thirty sampling points for 10-60 seconds during the dry season; the plates were suspended three meters above ground to approximate human breathing height. Microbial loads ranged from 2-18 SFU/s for aerobic bacteria, 0-1 SFU/s for facultative anaerobes, and 0-2 SFU/s for fungi, with statistical analysis showing significant differences (R^2=0.5914, 0.0171 and 0.2286 for aerobic, facultative anaerobic bacteria and fungi, respectively). Twenty-eight isolates were recovered, and three genera were identified: *Pseudomonas* sp., *Bacillus* sp., and *Penicillium* sp. The predominance of aerobic spore-forming bacteria, particularly *Bacillus* sp., and the opportunistic presence of *Pseudomonas* sp. highlight their adaptive survival in airborne environments, while *Penicillium* sp. was the sole fungal genus detected. Importantly, 59% of aerobic spores inhaled by passers-by represent a likely health threat, underscoring the need for preventive measures such as nose masks or handkerchiefs and improved sanitation practices to mitigate environmental and public health risks.

Keywords

Airborne microbial spores, Roadside environments, Unauthorized urinary spots, Time of exposure, Public health risk, Protective measures, Rivers State, Nigeria

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1. Introduction

The importance of passing-out urine cannot be overemphasized as it is important the urine leaves the human system when the urge comes [1]. The urge comes consciously, in situations when urine passage can be embarrassing, hence, it is excreted sometimes on roadside where people frequently pass [2]. Urine, is sometimes disposed in public spaces indiscriminately without any consideration on the effect it might have on road users or passer-by [3]. The indiscriminate urine passage points or spots are thus declared illegal and is fast becoming popular to road users and even passer-by themselves. The urine excreted smells and comes with diverse colors (dark, brown, blue) when it settles after several hours [3]. Fruit pebbles and fruity odor are amongst the commonest odor that evolves from urine deposited points. As the smell vary so also the microbial composition [4]. The quality of the air we breathe is fundamental to the quality of life for the growing millions of people living in the world, hence the atmosphere is a global community that responds to many types of emissions into it, as well as to changes in the surface beneath it [5]. Open urination along the roadside maybe attributed to no toilet facility provided by private or government agencies, hence passer-by most specially the male gender identify special spots where they can urinate without been interrupted [6]. The high volume of urine deposited indiscriminately, in public space is noted to be associated with odor and poor aesthetic. Hence public urination seems to drift to an environmental issue. The pungent smell is mostly inhaled by passer-by or road users particularly, during wind movement [6]. Passer-by or road users en-route of these urinary points breathe in air effluent from the spots in which the air is supposedly composed of particulate matter, dust and droplet. In addition, the air effluent is composed of gases (carbon dioxide, carbon monoxide, nitrogen oxide and other volatile organic compounds) released from vehicles [6,7]. The air components / aerosols and airborne agents are thus transmitted via inhalation to passer- by / roadside users. Although, studies to this effect continue to remain scanty. Microorganisms laden atmosphere may also be breath in, most specifically, fungal spores which are more in the atmosphere than bacteria spore. The microorganism in the atmospheric environment presents a dangerous risk factor to human with respect to aerosolization as expelled from surfaces [8]. The aerosolization of surface deposited organic and inorganic waste is reported to disperse or release microbial spores which are detrimental to human health when humans are exposed [8]. Human exposure to certain inorganic substances via the atmosphere can result to respiratory challenges such a breathing difficulty, chronic bronchitis and many more respiratory issues. These diseases are accompanied with symptoms such as common cold, fever, pain and hotness of the body [9]. With respect to organic exposures, the atmosphere transports certain microorganisms which are ubiquitous and microscopic in size, although cannot be seen with the naked eye but with the aid of a simple microscope. The ubiquitous feature enables the microbe disperse and colonize a wide range of environment causing a hazardous threat to public health [10]. Basically, airborne microorganism originates from different sources such as soil, animals, humans, plants and water. Although, it is reported that microbes present in the air can affect human health, causing mainly respiratory and related diseases transmitted via respiratory route [9]. Consequently, as passer-by or road users regularly move along theses urinary spots, there is need to the investigate the level of atmospheric exposure on road users since these illegal points cannot be totally controlled by law. The air been breath has become necessary for screening following many particulate mater including microorganism that the urinary air quality of that sport may carry which could inflame lungs. This study aims to screen and evaluate microbial airborne spores originating from unauthorized roadside urinary spots in Igwuruta, Rivers State, Nigeria, to quantify aerobic, anaerobic, and fungal spore loads at varying durations of exposure, and to identify the microbial genera present in the air effluent from these sites. In addition, biochemical tests (catalase, indole, methyl red, Voges-Proskauer, citrate, urease, motility, and spore detection) were performed to characterize bacterial isolates. Furthermore, the study seeks to assess potential public health risks associated with inhalation of airborne microorganisms and particulates, and to propose preventive measures to mitigate such risks.

2. Materials and Methods

2.1 Study Area

The area chosen for the study is the Igwuruta metropolis area of Ikwerre Local Government Area of Nigeria. The climatic condition was justified humid and characterized by high temperature. Thus, the hot temperate characteristics disapproved the study for a cold controlled area as this was a major limitation. The area is densely populated with high human and vehicular movement points associated with indiscriminate urination. Thus, the study was limited to points or areas were open urination are observed, thus, along the streets of Igwuruta, Nigeria.

2.2 Materials and Equipments

The following culture media and chemicals were put into use Sabouraud Dextrose Agar, Nutrient Agar, Anaerobic Blood Agar, all manufactured by Oxoid and purchased from Scantrik Medical Supplies, Nigeria. Similarly, distilled water, NaCl reagent, were purchased from Koeman Group of Companies, Nigeria.

Laboratory instruments put into use were 18L autoclave sterilizer machine, 15L digital thermostats incubator, and optical binocular microscope, all of which were manufactured by SH SCIENTIFIC. BACTRON300 anaerobic chamber, an equipment for facultative anaerobic bacteria growth was put into use and was purchased from Sheldon manufacturers. Petri dishes, pippet and other equipment’s were provided/supplied by Scantrik Medical Supplies, Nigeria.

2.3 Experimental Procedure

The design for the study involved taking aerobic bacteria, facultative anaerobic bacteria and fungal counts within 10, 20, 30, 40, 50 and 60 seconds from thirty (30) sampling urinary points. In the forgoing the direction of the wind was considered but the wind speed and direction were not measured. The media plates were suspended three meters above ground level on nearby platforms in nearby urination points, with respect to male stationed character. Furthermore, the design specifically considered the counts of the microbes during the dry season as these offers better investigation unlike raining season wherein the atmosphere is continuously cleaned through-out the rainy season.

2.4 Preparation of Media

The preparation of sabouraud dextrose, a media designed for the isolation of fungi and nutrient media, a general-purpose media designed for the isolation of aerobic bacteria were adopted. For facultative anaerobic bacteria, an anaerobic blood agar was adopted. Hence, the required amount of anaerobic blood agar, nutrient agar and sabouraud agar were dissolved in distilled water, autoclaved and dispensed into petri dishes [11].

2.5 Enumeration of the Air Borne Fungi and Bacteria

Standard microbiological air quality techniques were adopted for the enumeration of fungi and bacteria spores. Following the media preparations, the freshly prepared sabouraud and nutrient agar media plates were placed/exposed to the atmosphere at points along the study area, three (3) meters above ground level to recover fungi and bacteria from the atmosphere. The plates were placed aseptically for a period of 10, 20, 30, 40, 50 and 60 seconds accordingly [12]. Thereafter, the exposed media plates were covered and taken to the laboratory, were they were incubated. For fungal growth, the plates were incubated at room temperature (approximately 27℃) for 72 hours, while for aerobic and facultative anaerobic bacteria growth, the nutrient agar plates were incubated at 37 ℃ for 18-24 hours while blood agar was incubated in an anaerobic chamber devoid of oxygen for 24-48 hours. Thereafter the media plates after incubation were observed for microbial growth and the isolates counted. Counts were recorded of classified as spore forming unit per seconds (SFU/s) [13].

2.6 Fungal Morphology and Identification

The morphogenesis of the isolated fungi was examined and recorded accordingly, as they were sub-cultured. Wet preparations where made from the sub-cultured fungi on a clean greased free slide, covered and examined under the light microscope and identified based on their cultural and microscopic appearance using standard methods [13]. Features observed were compared with fungal sample from a pictorial atlas. Similarly, macroscopic morphology was observed based on colony appearance on the media plates [14].

2.7 Bacteria Morphology and Biochemical Identification

The bacteria isolate on the nutrient media plates were characterized and identified based on their colonial morphological features, with respect to; texture of the colony, color of the colony, shape of the colony, size of the colony and elevation. Following, the morphological characterization, biochemical tests were carried out to identify the isolates. Biochemical test such as: Catalase, indole, methyl red, oxidase, citrate utilization, urease, motility and spore detection test were carried out [15].

Oxidase Test

The test involved the use of a sterile wire loop to pick the bacteria colony and streak onto a filter paper that contain 0.5% tetramethyl-p-phenylene diamine hydrochlorine. A visual observation of the paper after 10 seconds, showed a dark purple color signifies oxidase positive bacteria, while an absence of a change signifies a negative oxidase bacteria colony [15].

Indole Test

The test involved the use of tryptone broth to culture the test bacteria. The tryptone broth is prepared and with the aid of a sterile platinum needle, the test colony is inoculated into the broth. The inoculated broth was then incubated for 24 hours at a temperature of 35℃. after incubation, 5 drops of Kovac reagent was added to the broth and the broth visualized after 10 seconds. A change in pink color to red, indicate a positive urease, while an absence in color change signifies a negative indole [15].

Catalase Test

The test was achieved by placing of Hydrogen Peroxide reagent on a clean slide and there after the bacteria colony is inoculated on the reagent with the use of a sterile wire loop. Bubbles of gases after 5 seconds signified catalase positive bacteria colony while an absence denote a negative colony [15].

Methyl Red Test

The test was done to confirm the production of acid from the test isolate. Hence, the test was done by inoculating the test bacteria into MR-VP broth and the broth incubated for 24 hours at a temperature of 37ºC. a change in the broth from yellow to red indicated a positive methyl red while an absence indicated a negative methyl red bacterium [15].

Citrate Test

The test involved the use of Simmon Citrate agar. The agar was prepared according to the manufacturers specification and the media dispensed on a test tube. Thereafter, with the use of a wire loop, the test bacteria were inoculated into the media and incubated for 24 hours for 37°C. Results were obtained by visual observation of the media after incubation. Blue color appeared as a result of change in the green color of the media. Hence, signifies citrate positive while an absence of the color change, indicate citrate negative bacteria [15].

Urease Test

The test was done to confirm the ability of the test colony to produce enzyme. The test bacteria were inoculated into a freshly prepared urea broth, and with the aid of a wire loop. The broth was then incubated for 24 hours at a temperature of 35°C. A change in red color to pink after incubation indicated a urease producing bacteria while an absence indicated a urease negative bacterium [15].

Motility Test

The test was done to confirm the movement status of the test bacteria colony. The test involved the use of platinum needle to pick and stab a freshly prepared semi-solid nutrient media. Thereafter the semi solid media was then incubated. After incubation, the line of stab was visually observed and diffused growth spread along the line of stab indicated a positive motility while growth only along the stabbed line indicated a non-motile bacterium [15].

Spore Test

The test involved the use of malachite green stain to view the test bacteria under an optical binocular microscope. The text bacteria were heat fixed onto a clean microscopic slide and thereafter the isolate was decolorized and stained with malachite green stain before viewed under a binocular microscope. Un-viewing, the spored bacteria appeared green while the vegetative cell appeared red [15].

2.8 Statistical Test

The fungi and bacteria spore load recovered in relation to the time (10, 20, 30, 40, 50 and 60) of exposure were analyzed using the Pearson's correlation analysis. The Pearson's correlation test was adopted to test the relationship between the time of exposure of the media plates and the microbes recovered on the media plate however, the test for normality was not considered. Data recovered were tabulated and the graphical relationship represented [16].

3. Results

3.1 Enumeration of Aerobic Bacteria Spore Population and Pearson's Correlation Coefficient

Table 1 showed the counts of the aerobic bacteria and the correlation coefficient at several seconds on the media plate exposed. The mean counts of aerobic bacteria obtained ranged from 2 to 18 SFU/s, within an exposure time range of 10 to 60 seconds. Counts showed the least aerobic bacteria count was obtained in 10 seconds while the highest count, 60th seconds.

Table 1. Mean count of aerobic bacteria population.

Seconds (s)	10	20	30	40	50	60
Aerobic Bacteria (SFU)	2	3	3	4	5	18

Keys: SFU=Spore-Forming Unit; s=seconds

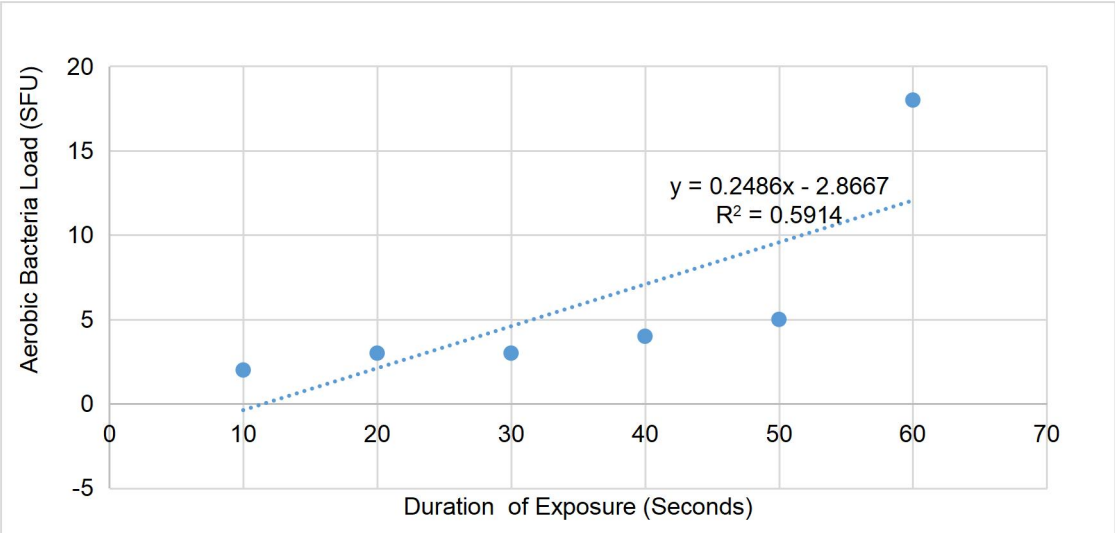

Figure 1. Correlation of the aerobic bacteria to time of exposure to the atmosphere.

The correlation analysis of the aerobic bacteria obtained with respect to duration of media exposure, expressed a R^2 value of 0.5914 as the level of variation (Figure 1). Thus, 59.14% of the variation in the counts of aerobic bacteria obtained were depended on the time of exposure of media plates. This implies that moderate relationship exists between time of exposure and aerobic bacteria load on the media plates. Hence the urinary points support the dispersal of aerobic bacteria.

3.2 Enumeration of Facultative Anaerobic Bacteria Spore Population and Pearson's Correlation Coefficient

Table 2 showed the counts of the anaerobic bacteria population and the correlation coefficient at several seconds on media plate exposure to the urinary points. The mean counts of facultative anaerobic bacteria obtained ranged from 0 to 1 SFU/s, within an exposure time range of 10 to 60 seconds. Counts showed the highest facultative anaerobic bacteria count was obtained in 30 seconds while the least anaerobic count was obtained in 10 and 60 seconds.

The correlation analysis of the facultative anaerobic bacteria obtained with respect to duration of media exposure, expressed a R^2 value of 0.0171 as the level of variation (Figure 2). Thus, 1.71% of the variation in the counts of facultative anaerobic bacteria obtained were depended on the time of exposure of media plates. This implies that a weak relationship exists between time of exposure and facultative anaerobic bacteria load on the media plates. Hence, the urinary points do not support the dispersal of facultative anaerobic bacteria.

Table 2. Mean count of facultative anaerobic bacteria population.

Seconds (s)	10	20	30	40	50	60
Facultative Anaerobic Bacteria (SFU)	0	0	1	0	0	0

Keys: SFU=Spore-Forming Unit; s=seconds

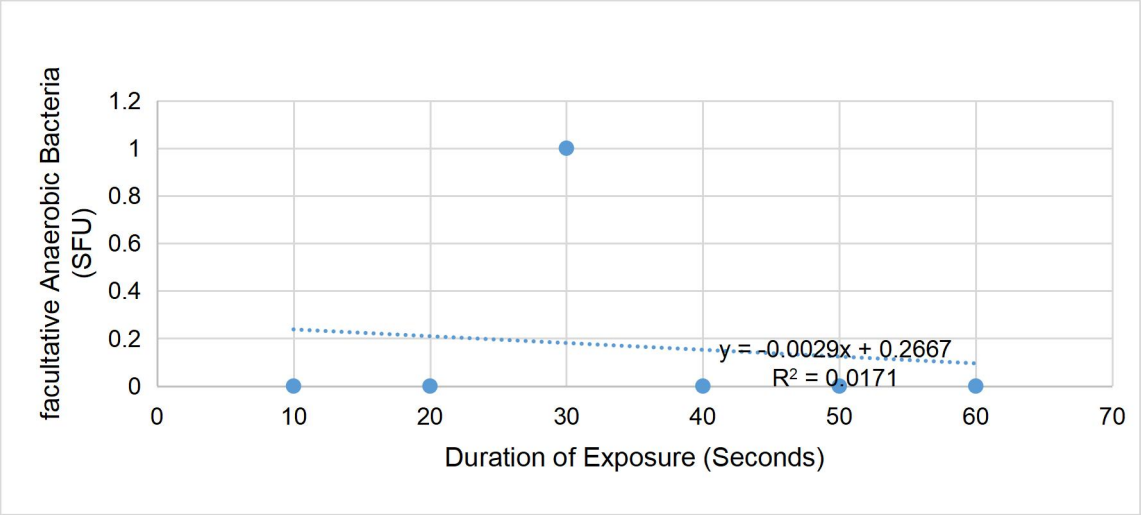

Figure 2. Correlation of the facultative anaerobic bacteria to time of exposure to the atmosphere.

3.3 Enumeration of Fungi Spore Population and Pearson's Correlation Coefficient

Table 3 showed the counts of the fungi and the correlation coefficient at several seconds on the media plate exposure. The mean counts of fungi obtained ranged from 0 to 2 SFU/s, within an exposure time range of 10 to 60 seconds. Counts showed the least fungi counts were obtained in 30 and 40 seconds while the highest fungi counts were obtained in 50 and 60 seconds.

The correlation analysis of fungi obtained with respect to duration of media exposure, expressed a R^2 value of 0.2286 as the level of variation (Figure 3). Thus, 22.86% of the variation in the counts of fungi obtained were depended on the time of exposure of media plates. This implies that a weak to moderate relationship exists between time of exposure and fungal load on the media plates. Hence the urinary points do not support the dispersal of fungi.

Table 3. Mean count of fungal population.

Seconds (s)	10	20	30	40	50	60
Fungi (SFU)	1	1	0	0	2	2

Keys: SFU=Spore-Forming Unit; s=seconds

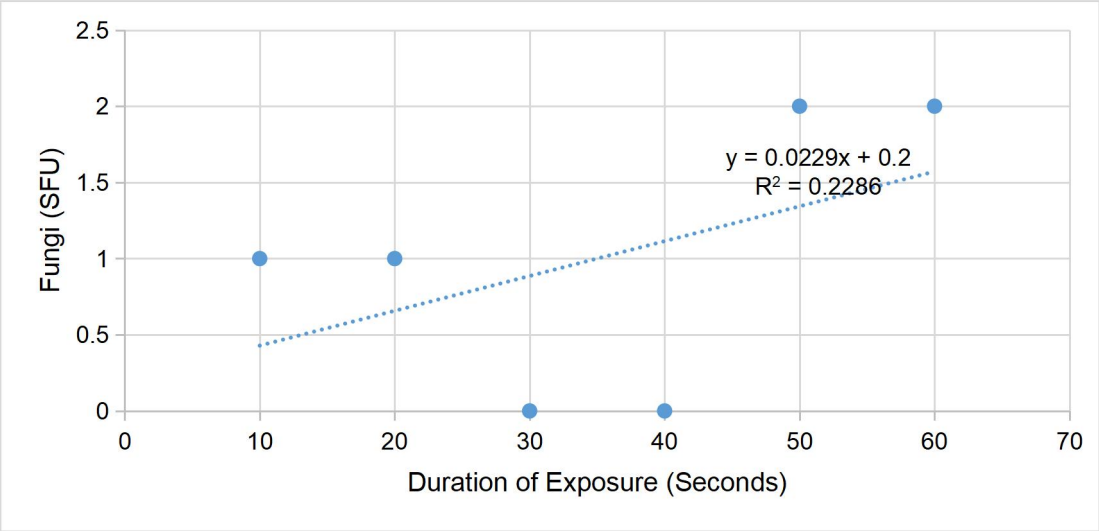

Figure 3. Correlation of the fungi to time of exposure to the atmosphere.

3.4 Morphological Characterization of the Bacteria and Fungal Isolates

3.4.1 Morphological Characterization: Bacteria Isolates

Table 4 showed the isolation and colonial characterization of the bacteria isolates recovered. Two (2) common bacteria genera were recovered from the urinary points. The bacteria *Bacillus* sp. appeared gray white with large size and round shape. Similarly, *Pseudomonas* sp. was noted with a blue green color, irregularly shaped and flat.

Table 4. Colonial characterization of the bacteria isolated.

Edge	Colour	Size	Shape	Elevation	Surface	Probable Bacteria
Curved	Gray white	Large	Round	Convex	Rough	*Bacillus* sp.
Curved	Blue-green	Large	Irregular	Flat	Smooth	*Pseudomonas* sp.

3.4.2 Morphological Characterization: Fungal Isolates

Table 5 showed the structural and physical characteristics of the fungi obtained. Purple like blue color, small in size and a woolly texture was identified for *Penicillium* sp.

Table 5. Macroscopic characterization of the *Penicillium* sp.

Isolates	Structural Description		Growth Rate	Texture	Identification
	Color	Size			
I	Purple	Small	Fast	Woolly	*Penicillium* sp.

3.5 Biochemical Characterization of the Bacteria Isolated

Table 6 reported *Bacillus* sp. and *Pseudomonas* sp. showing varying biochemical results. *Bacillus* spp. was observed with spore and spore absent in *Pseudomonas* sp. *Bacillus* sp. showed positive for citrate, VP and MR reactions but negative for oxidase, motility and urease. However, *Pseudomonas* sp. was positive for catalase, oxidase, motility and urease but negative for citrate, indole MR and VP.

Table 6. Biochemical characterization of the bacteria isolated.

Cal	Oxi	Cit	Ind	Mot	MR	Ure	Spore	Probable Bacteria
-	-	+	-	-	+	-	+	*Bacillus* sp.
+	+	-	-	+	-	+	-	*Pseudomonas* sp.

Keys: CAL-Catalase; **OXI**-Oxidase; **CIT**-Citrate; **IND**-Indole; **MOT**-Motility; **MR**-Methyl Red; **URE**-Urease; **Spore**+=Positive; -=Negative

4. Discussion

The diversity in microbial counts (bacteria and fungi) of the urinary site varied across the time of exposure of the media plates. These basically, is due to the impact of the atmospheric condition of the urinary points on the media. The atmospheric condition initiated large concentration of aerobic bacteria, with no or little facultative anaerobic bacteria and a moderate fungus, all of which may be due to microbial aerosolization from surface points and the ability of the microbes to survive in the atmosphere [17]. Although, aerosol emission from urinary site constitute an important source

of bioaerosols that act as point or diffuse sources [18]. The advent of aerobic bacteria over the facultative anaerobic bacteria, showed clearly, the support of aerobes which grows in an oxygen rich environment whereas toxicity to facultative anaerobic bacteria was observed [19]. Consequently, the ability of the aerobes and the fungal to form spores cannot be overemphasized as the urinary spots, prio to receiving urine, may witness harsh environmental pollutants [20] and in some cases extreme doses of ultra violet radiation , all of which were resisted by the aerobic bacteria and the fungi spored organism [21]. Absence of facultative anaerobes in the urinary sites may be attributed to the fact that the cultivation of facultative anaerobes is challenging. Similarly, the low impact of facultative anaerobes in the atmosphere of the urinary site environment is however attributed to the fact that facultative anaerobes do not grow in the presence of oxygen, as the atmospheric oxygen is toxic to their survival [17]. The diversity of fungal counts in the urinary site varied across the time of exposure on the media plates to a large extent human inhalation. Although, the success of the fungal emission was considered, following metrological factors such as temperature, wind speed and solar radiation, which have severally, been noted to influence the fungal air concentration [22], however not considered in this study. The presence of fungi in the atmosphere may also be attributed to their natural dispersal mechanism. Although, the study noted weak presence in the atmosphere which invariable implies no health threat to both passer-by and road users of such site [23]. The bacteria, *Pseudomonas* sp. and *Bacillus* sp. were observed and identified biochemically, although the identification of *Bacillus* sp. was attributed to its efficient dispersal properties which is believe to originate from dust, soil and other contaminated surfaces. Basically, *Bacillus* sp. ability to form spore, and disperse itself may have resulted to its occurrence in the urinary site [2]. Hence, *Bacillus* sp. survival and dispersal as identified biochemically, is also reported in soil and gastrointestinal tract samples. The flourish of *Bacillus* sp. signifies it a major contaminant in the urinary site environment [4]. Thus, the health genera, *Bacillus* sp. sporulate on exposure to air and produce a heavily in contaminated environmental site [24]. In a similar circumstance, *Pseudomonas* sp. which was identified, was without spore property yet was observed in the air of the urinary sites. *Pseudomonas* sp. is an opportunistic pathogen that thrives in wet contaminated environment. Although, *Pseudomonas* sp. was oxidases positive as reported [15]. Furthermore, its identification can be traced to urinary tract infection which by implication is dispersed by road users, who may be carriers of the infection [1]. Thus, the sporulation ability of *Bacillus sp.* and the metabolic versatility of *Pseudomonas sp.* are considered adaptive features accounting for their airborne presence and their flourished status in the present study. In addition, the study identified a fungal genus*, Penicillium* sp. as the sole fungi identified to have suspended in all sampling points investigated [25,26]. The sole presence of *Penicillium* sp. may be attributed to its ubiquitous saprophytic nature on decaying organic matter [27]. It is reported that *Penicillium* sp. reproduce by releasing high concentration of spores which are easily dispersed by air current [28]. However, the predominant fungi, *Candida* sp. isolated in urine was not recovered, hence does not hold in the present study [29]. The isolation of *Penicillium* sp. and the health implication following inhalation is so diverse, as it is reported to trigger respiratory issues [30,31].

5. Conclusion

The study pointed out likely exposure to aerobic spored bacteria at the study area or urinary sites to passer-by or road users. Consequently, road users' inhalation of the said isolated microbes namely; *Bacillus* sp., *Pseudomonas aeriginosa* and *Penicillium* sp. as recovered and identified in the air microbiome of the urinary spots are dependent on the time of exposure. Thus, the identification of the various microbes showed the urinary sites creates a microhabitat that allows the establishment of pathogenic airborne microbes.

6. Recommendation

Following the indiscriminate public urination on unauthorized sites which results to aerobic bacterial and fungal spore impacted air, it has become necessary for the public to be mindful of this and hereby adopt preventive measures such as; the use of clean handkerchief to cover nose or the use of nose mask when in transit along such spot/points on the road.

Conflict of Interest

The authors declare no conflict of interest with respect to this article, screening for microbial airborne spores from unauthorized urinary spots on road sides in Igwuruta, Rivers State, Nigeria.

Generative AI Statement

The authors declare that no Gen AI was used in the creation of this manuscript.

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