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Evaluating Congregational Strength in a Bioaerosol Communicable Disease Situation in Some Worship Centers

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Abstract: The study aimed at evaluating bioaerosol agents in some congregational worship centers across Port Harcourt City, Nigeria. The sample size population/congregation sizes of 100 - 600 persons were investigated using the settled/exposure plate technique. The analysis screened the atmosphere for the recovery of bioaerosols and the determination of the relationship that exist between congregants and the bioaerosols expelled in the worship centers. Linear regression analysis of the data recovered showed that the bioaerosols expelled where dependent on the congregant's population. This implies that a positive linear regression of the congregants exists with a R^2 of 0.9841 for bacteria bioaerosols, ranged from less than 100 congregants for 80 CFU/30 minutes to less than 600 congregants for 312 CFU/30 minutes. Similarly, a R^2 of 0.981 for fungal bioaerosols was obtained, which ranged from 100 congregants for 8 CFU/minutes to 600 congregants for 106 CFU/30 minutes. The morphological and biochemical characterization of the recovered bioaerosols showed diverse bacteria genera namely, *Staphylococcus* spp., *Bacillus* sp. and diverse fungi genera namely; *Penicillium* sp., *Aspergillus* sp. and *Rhizopus* sp. The results clearly, indicated that a strong correlation exist between the congregation size and bioaerosol load. Inhaling either of the bioaerosols isolated can be detrimental to an immune-compromised congregant. The study therefore recommends total responsiveness of congregants towards signs of associated disease and calls for studies on congregational population greater than 700 persons.
Keywords: Communicable diseases, Congregants, Colony forming unit (CFU), Bioaerosols, Linear regression, Worship centers.

Introduction

Unhealthy congregants come to worship centers with the believe that they could get healed, so also some healthy congregants come, to receive knowledge and blessings from God. Worship centers are referred to as dedicated places where people come together to connect with their God (Peter & Musiliudeen, 2021). The centers include mosque, church, shrine/temple, chapel etc. and the congregants carry out worship activities such as talking, clapping, singing, dancing and other operations as required (Zaluchu, 2021). These activities expel bioaerosols to the atmosphere, which in-turn could be inhaled (Fu & Zhu, 2024). Bioaerosols are very small airborne living particles comprised of microorganisms (bacteria, fungi, virus, protozoa etc). They are pathogenic and dispersed from one environment to another due to their light size and weight (Kim et al., 2017). It is important to note that bioaerosols are causative agents to communicable diseases. Diseases such as whooping cough, tuberculosis, and flu are transmissible from person to person (Fu & Zhu, 2024) and therefore should be observed. In addition, high contamination of fungal bioaerosols have been isolated from an indoor environment (Marcu et al., 2021). Indoor bioaerosols have been noted heavy in some public facility such as schools, residential areas (Adekunle et al., 2025) and hospitals (Upula et al.,2023), however, records of bioaerosols isolation in worship centers, specifically churches in Nigeria have not been reported. Bioaerosols when confined in busy indoor spaces can reach concentrations that affect breath (Agi et al., 2024). Bioaerosols inhalation activates allergic reaction which in-turn inflames the lungs (Roy et al., 2004). Thus, worship centers enclosed in

environment with regular congregation gatherings, and varying ventilation systems could serve as potential hotspots for the accumulation, dispersal, and transmission of bioaerosols which ultimately may result to the emergence of communicable diseases (Heo et al., 2017; Balasubramanian et al., 2012). Moreso, bioaerosols transmission of communicable diseases have not been adequately reported excluding viral airborne, which made headlines in the 20s (Tang, 2021). The corona virus bioaerosols threatened worship centers specifically during spiritual rituals/ceremony such as sprinkling of holy water, self-flagellation to blood brotherhood, communion service amongst many others (Aira et al., 2007). These rituals or activities of the church could transfer corona viruses (Gajurel & Deresinki, 2021). Consequently, some congregants acquired infections from poor church facilities such as limited spaces in situations church facility cannot carry the huge congregation, gives opportunity for disease transmission (Cool, 2021). Evaluating congregation strength and bioaerosol transmission in worship center are important in safeguarding the public health. Sequel to the likelihood of communicable diseases, the study seeks to justify congregation strength and bacterial and fungal bioaerosols expelled.

Materials and Methods

The study area

The study area for the study is the Church in the City of Port Harcourt, Nigeria. The City is home to so many Christian denomination and serves as a communion point for Christians. It is orderly reserved for believers of God Almighty. It is more or less a serene vicinity, and offers congregants safety, security and confidence. Congregants both healthy and unhealthy converge to worship and hear the word of God Almighty. As a result of converging, congregants engage in several activities such as singing, shouting, and praying aloud, which definitely expel bioaerosols from their mouth.

Experimental design

The study adopted a stratified sampling technique for the recovery of heterotrophic bacteria and fungi bioaerosols, in a 30 minutes air exposure of nutrient and sabouraud agar media respectively, in an indoor congregation. Twenty air sampling plates were provided each for the congregation strength of less than 100, 200, 300, 400, 500 and 600 persons, all totaling 120 air sampled plates. The suspension of the plates was made one meter above the ground level to prevent contaminants from the floor into the plates. The process was repeated three (3) different times and results obtained were recorded accordingly.

Nutrient agar and sabouraud dextrose agar media preparation

Nutrient agar media and sabouraud (SDA) agar medium were adopted for the culture of bacteria and fungi spores present in the Church atmosphere. For preparation of the media, four grams (4g) and three grams (3g) of SDA and nutrient agar respectively, were weighed and suspended in 100ml of distilled water accordingly, stirred to obtain a uniform mixture and autoclaved at 121^{o}C for 15 minutes. The nutrient medium was allowed to cool and then dispensed into petri dishes. Prior to the dispensing of the SDA media, 500mg antibiotics was added to the media aimed at inhibiting any bacterial contaminant. Thus, both media were prepared according to the manufacturer's specification, weighed, autoclaved and dispensed accordingly in a petri dish, before exposure to the atmosphere (Cheesbrough, 2000).

Bacterial and fungal bioaerosols (samples) collection

The bioaerosol collection involved taking the sterile prepared media plates to the worship centers and their subsequent exposure to air. Bacterial and fungal bioaerosols in the air where determined using the settled/exposure plate technique, were the freshly prepared nutrient and

sabouraud agar media where air-exposed to the Church congregation strength of less than 100, 200, 300, 400, 500 and 600 attendees for 30 minutes. Thereafter the plates where transferred to the Microbiology Laboratory of Ignatius Ajuru University of Education, Port Harcourt, Nigeria. In the laboratory, the nutrient agar media plated samples where then incubated at 37 degrees centigrade for 20 hours while the sabouraud agar media plates were incubated at a room temperature for 96 – 110 hours (Cheesbrough, 2000).

Determination of the bacteria bioaerosol load

After the nutrient media plates were incubated at a temperature of 37 degrees for 20 hours. Colonies that developed were counted and documented as colony forming unit per 30 minutes (Chakraborty & Nishith, 2008).

Determination of fungi bioaerosol load

After the sabouraud media plates were incubated at a room temperature for 96 - 110 hours. Colonies that developed were counted and documented as colony forming unit per 30 minutes (Chakraborty & Nishith, 2008).

Characterization of the recovered isolates (bacteria and fungi)

Characterization of the bacteria and fungi isolates recovered involved description of the isolate's appearance on the media plates and under the microscope and finally their reaction to certain important chemical reagents. All processes adopted were geared towards identifying the spores recovered from the atmosphere. The biochemical analysis did not involve the fungal spores' isolates (Cheesbrough, 2000).

Colonial morphology/characterization of the bacteria isolates

Colonial morphology considered the physical appearance of the isolated bacteria/fungi colony in terms of shape, color, shape, margin, surface, size, elevation, and opaque as may be required as carried out by Hafezi and Khamar (2024).

Microscopic examination /characterization of the bacteria isolates

In the microscopic examination of the isolates in terms of shape, color, margin, size, and elevation. The isolates were subjected to screening with the aid of a microscope, prior to the use of microscope the isolates were stained. Gram stain was adopted for staining of the bacteria while lactophenol cotton blue stain, was adopted for the staining of the fungi. For Gram staining, it was carried out to differentiates the bacteria recovered into Gram-positive and Gram-negative bacteria based on the differences in the nature of their cell wall. The Gram staining process firstly, involved the heat fix of the bacteria colony unto the microscopic glass slide. Followed by application of the crystal violet stain which lasted for 60 seconds. Thereafter, the stain was washed-off under running water for 2 seconds. Iodine was then applied for 60 seconds and washed-off with water for 2 seconds. Ethanol was applied for 10-30 seconds and washed-off with water again for 2 seconds. Finally, the application of safranin for 30-60 seconds and washed off again with water for 2 seconds. Following all these steps, the slide was allowed to air-dry before viewed under a light microscope. On viewing, Gram positive bacteria showed purple blue coloured background appearance while Gram negative bacteria showed red/pink colour (Teeseling et al., 2017).

Motility test

The motility test was carried out to determine the ability of the isolate to move. Motility as carried out was determined using the hanging drop method. The process as described by Chakraborty and Nishith (2008) involved introducing the isolate into a sterile microscopic

cover slip, thereafter a petroleum jelly was introduced around the slide well and then placed in an inverted position (hanging down). The slide was then viewed under a light microscope with an objective lens of 40x. Cells that move were identified tumbling hence positive result, while cells stationed were declared negative (Hafezi & Khamar, 2024).

Fungi microscopic morphology/characterization

For microscopic examination, lactophenol cotton blue stain was adopted for the staining of the fungi. Firstly, the fungi were impregnated on the microscopic slide before the application of the stain. Thereafter, the slide was covered and the microscope used to view the slide as stated by Olumuyiwa at el. (2025).

Biochemical characterization of bacterial isolates

Biochemical tests were carried out to identify the bacteria reactions to some biochemical agents. Biochemical agents such catalase, oxidase, coagulase, motility, indole, methyl-red, vogue proskauer, citrate utilization and sugar fermentation test were adopted for the test on the bacteria isolates as carried out by Hafezi and Khamar (2024).

Catalase test

In carrying out the catalase test, a colony of the test bacteria was introduced into a clean microscopic slide, thereafter a drop of hydrogen peroxide was added onto the colony, and with the aid of a wooden applicator stick, the components were smeared and observed for gas bubbles. Catalase negative bacteria produced no bubbles while catalase positive bacterial produced bubbles of oxygen (Hafezi & Khamar, 2024).

Heat resistant (spore) test

The heat resistant test was done to confirm the ability of the bacteria to produce spore. In carrying out the test, a colony of the test bacteria was introduced into a nutrient broth with the aid of a wire loop. Thereafter, the broth was heated to a temperature of 80 degrees for 10 minutes. following the heating the broth culture was incubated at 37 degrees for 24 hours. An absence of a growth indicated no spore while an appearance of a growth signified spore formation (Hafezi & Khamar, 2024).

Indole production test

In carrying out the indole production test, a colony of the test bacteria was introduced into a freshly prepared indole medium contained in a test tube with the aid of a sterile wire loop. Thereafter the test bacteria were inoculated onto the medium and the components incubated at 37°C for 48 hours. After incubation, 10 drops of Kovac's reagent was added to the component, then shock, allowed to stand for 5 minutes. Negative result showed no red colour at the surface of the medium/component, while positive result showed indicated a red colony (Hafezi & Khamar, 2024).

Methyl red test

In carrying out the methyl red test, a colony of the test bacteria was introduced into a freshly prepared sterile Methyl Red/ Voges Proskauer broth with the aid of a sterile wire loop r. Thereafter, the broth culture was incubated for 48 hours at a temperature of 37 degrees. After incubation, 2-5 drops of methyl red indicator were added to the broth culture. The medium was well swirled and allowed to stand for 15 minutes. Negative result was noted with a yellow colored appearance while positive results was indicated with a red colored appearance (Hafezi & Khamar, 2024).

Voges Proskauer (vp)test

In carrying out the voges proskauer test, a colony of the test bacteria was introduced into a freshly prepared sterile Methyl Red/ Voges Proskaurer broth with the aid of a sterile wire loop. Thereafter, the broth culture was incubated for 24 hours at a temperature of 37 degrees. After incubation, about 10 drops each of a-naphthal and potassium hydroxide were introduced into the broth culture. The medium was well swirled and allowed to stand for 15 minutes. Negative results indicated no colour change on the surface of the medium while a positive result indicated a pink or red colour at the surface of the medium (Hafezi & Khamar, 2024).

Citrate utilization test

In carrying out the citrate utilization test, a colony of the test bacteria was introduced into a freshly prepared sterile Simmons citrate agar that was prepared according to the manufacturer's instructions with the aid of a sterile wire loop. The tube contained Simmons citrate medium was then slanted and allowed to cool and solidify. The slanted media component was then incubated at 35^0C for 18 to 24 hours. The emergence of a blue color indicated a positive result while a negative result was indicated with no color change in the medium (Hafezi & Khamar, 2024).

Glucose test

In carrying out the glucose test, a colony of the test bacteria was introduced into a freshly prepared sterile broth composed of sugar, phenol red indicator was compounded and an insertion of a Durham tubes, with the aid of a sterile wire loop. The broth culture was then incubated at 35 to 37^0C for 24 hours. Tubes were observed for colour change. Red colored medium was interpreted positive/ reaction with remains of bubbles in the Durham tubes while lack of bubbles indicated no gas production and therefore declared negative / reaction (Hafezi & Khamar, 2024).

Urease test

In carrying out the urease test, a colony of the test bacteria was introduced into a freshly prepared sterile urea agar medium with the aid of a sterile wire loop. The culture media was then incubated at 35°C for 24 hours. Thereafter the culture media was examined for development of pink colour. Pink coloured media indicated a positive reaction while a reverse indicated a negative result (Hafezi & Khamar, 2024).

Coagulase test

In carrying out the coagulase test, a colony of the test bacteria was introduced into a clean microscopic slide contain a drop of normal sale all of which aided by a sterile wire loop, thereafter a drop of human plasma was added onto the colony, and with the aid of sterile wire loop again, the components were smeared and observed for clumping within 10 seconds. Coagulase negative bacteria produced no clump while coagulase positive bacterial produced clump (Hafezi & Khamar, 2024).

Macroscopic morphology of the fungi isolate

The Fungi isolated was again characterized based on macroscopic features as seen on the sabourouad culture media plate. Macroscopic features involved the visual observation of the fungi texture, colour, shape, size etc. as carried out by Cheesbrough (2000).

Statistical analysis

Linear regression analysis of the congregation strength and recovered microbial load was carried out using the simple linear regression analysis to show the relationship between

congregation strength (X) and the microbes expelled (Y). The output expected were the R^2 value and an equation of a line graph. An R^2 at a very high percentage signified a correlation exist in the relationship, that is: the line fits the data. The congregation is said to determine the bacteria expelled, while a low percentage of R^2 signifies no correlation exist. The line therefore does not fit the data and the microbes generated is not dependent on the congregation strength/ population (Montgomery et al., 2021).

Results

Bacteria bioaerosol load recovered from the congregants

Figure 1, showed the regression analysis result of the bacteria bioaerosol load in the air as expelled by a congregation strength of less than 100, 200, 300, 400, 500 and 600. Result showed a R^2 value of 0.9841, which implies a strong correlation between congregation size and bioaerosol load. Hence, the congregation size determines the bacteria load expelled.

The Table 1(b) also showed the mean bacteria bioaerosols generated with respect to the congregation strength accordingly. The mean result ranged from less than 100 congregants for 80 CFU/30 minutes to less than 600 congregants for 312 CFU/30 minutes.

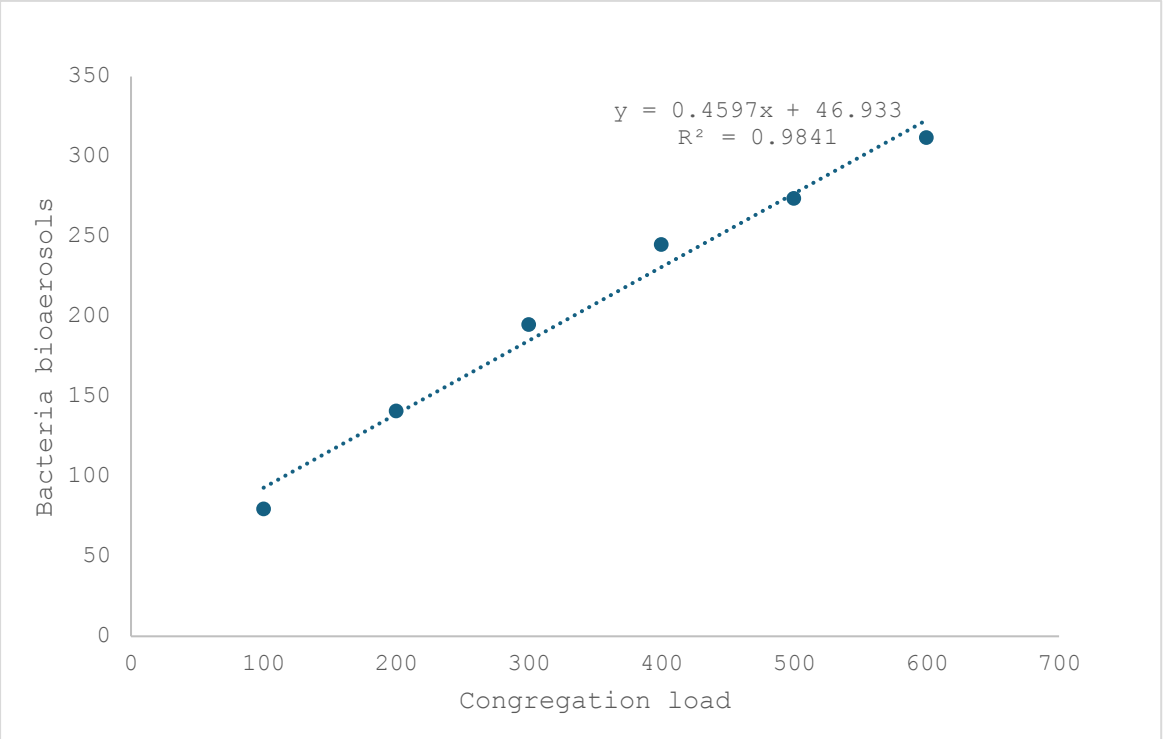

Figure 1: Bacteria bioaerosols generated with respect to congregation strength

Table 1(a) Bacteria bioaerosol load recovered from the congregants

Congregation population	Bacteria (bioaerosols) CFU/30minutes
<100	80
<200	141
<300	195
<400	245
<500	274
<600	312

Fungi bioaerosol load recovered from the congregants

Figure 2 showed the regression analysis result of the fungi bioaerosol load in the air as expelled by a congregation strength of less than 100, 200, 300, 400, 500 and 600. Result showed a R^2 value of 0.981, which implies a strong correlation between congregation size and bioaerosol load. Hence, the congregation size determines the bacteria load expelled.

The Table 2(a) also showed the mean fungal bioaerosols generated with respect to the congregation strength accordingly. The mean result ranged from less than 100 congregants for 8 CFU/30 minutes to less than 600 congregants for 106 CFU/30 minutes.

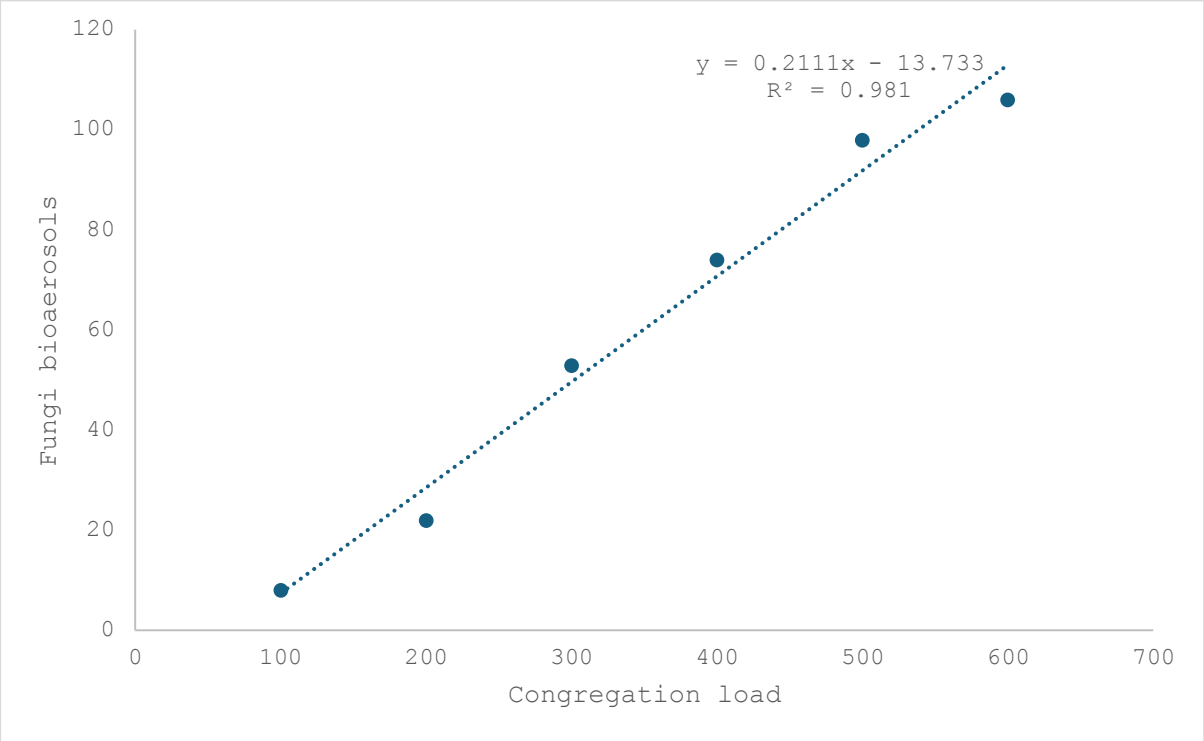

Figure 2: Fungi bioaerosols generated with respect to congregation strength

Table 2(a) Fungi bioaerosol load recovered from the congregants

Congregation population	Fungi (bioaerosols) CFU/30minutes
<100	8
<200	22
<300	53
<400	74
<500	98
<600	106

Colonial characterization of bacteria isolate

Table 1 showed the colonial morphology of the bacteria isolates revealing probable bacteria such as *Staphylococcus* sp. and *Bacillus* sp. with striking/unique appearance of light yellow and off-white colour respectively.

Table 1: Colonial characterization of bacteria isolate

Edge	Colour	Size	Shape	Elevation	Surface	Probable bacteria
Curved	Light Yellow	Small	Circular	Low	Smooth	*Staphylococcus* sp.
Curved	Off- white	Large	Round	Convex	Rough	*Bacillus* sp.

Biochemical characterization of the bacteria isolates

Table 2 showed the biochemical characterization of the bacteria isolates confirming bacteria: *Staphylococcus* sp. and *Bacillus* sp. with a confirmatory formation of spore by *Bacillus* sp. and clumping feature by *Staphylococcus aureus.*

Table 2: Biochemical characterization of the bacteria isolates

CAL	SPOR	CIT	IND	MOT	MR	VP	GLU	URE	COA	Gram Stain	Bacteria
+	-	+	+	+	-	-	+	+	+	+	*Staphylococcus aureus*
+	+	+	-	+	-	+	-	-	-	+	*Bacillus* sp.

Keys: CAL-Catalase; **SPOR**-spore; **CIT**-Citrate; **IND**-Indole; **MOT**-Motility; **MR**-Methyl Red **VP**-Vogues Proskauer; **GLU**-Glucose; **URE**-Urease; **COA** = Coagulase, **Gram Stain**; + = Positive, - =Negative.

Macroscopic and microscopic morphological description of the fungal isolate

Table 3 showed the macroscopic and microscopic morphological description of the fungal isolates. Macroscopic feature showed green, blue green and white gray appearance for *Aspergillus* sp. *Penicillium* sp. and *Rhizopus* sp. respectively, while microscopic showed conidiophores, hyphae, and profuse hyphae for *Aspergillus* sp. *Penicillium* sp. and *Rhizopus* sp. respectively.

Table 3: Macroscopic and microscopic morphological description of the fungal isolates

Isolates	Macroscopic morphology	Microscopic morphology	Fungi
A	Lemon green, powdery growth	Green conidiophores with a septate hypha	*Aspergillus* sp.
B	Blue-green powdery	Hyphae spread evenly	*Penicillium* sp.
C	White-gray	Profuse hyphae	*Rhizopus* sp.

Discussions

The bioaerosols in the air, as expelled by congregants showed a correlation exist. This implies the bioaerosols expelled had significant effect on the congregants, as so reported (Gallego-Cartagena et al., 2024). Basically, Gallego-Cartagena et al. (2024) pointed out the role of environmental and human related factors in shaping bioaerosols distribution in tropical urban context cannot be over emphasized. The significant effect of the expelled bacterial and fungal bioaerosols in relation to the congregation strength showed that the worship centers are likely to be in a bad state (Gajurel & Deresinki, 2021). Gajurel and Deresinki (2021) noted that 38% congregants in church developed Covid 19, hence partially in line with study carried out by Cool (2021). Cool (2021) noted that bioaerosol infections have been attributed to the numbers of congregation. Basically, viral bioaerosols are of various sizes and the viruses remain the highest in recoveries, above bacteria and fungi bioaerosols from hospitals, schools and public transportation system (Kim, 2007). Furthermore, the positive correlation that exist between the bioaerosols and the congregation population in this study is in line with Kim (2007), who noted

that the prevalence of bioaerosols can be associated with certain human diseases. This implies that even if a congregant has a communicable disease, transmission could still be possible. *Staphylococcus aureus, Bacillus* sp., *Penicillium* sp., *Aspergillus* sp., and *Rhizopus* sp. in this study as identified through macroscopically and biochemically assay are well distinguished communicable disease agent of airborne origin (Agi et al., 2024). The isolated bioaerosols (bacteria and fungi) have notably, been recovered, so also communicable diseases of viral origin that have previously been repot by Milton (2020). Milton (2020) reported some communicable diseases of viral origin such as measles, influenza, mumps and hepatitis "A". However, Gautret and Steffen (2016) previously reported meningococcal diseases caused by bacteria and till date no report of fungi airborne in church congregations exist. Basically, the report of fungal bioaerosols is a signal for congregation awareness. Again, Gajurel and Deresinki (2021) pointed out anthrax, brucellosis and cholera diseases, which are bacteria associated disease within religious rituals. Thus, the health implication of inhaling either of the bioaerosols, *Staphylococcus aureus, Bacillus* sp., *Penicillium* sp., *Aspergillus* sp., and *Rhizopus* sp. can result to blister, sore, boils and inflammatory conditions, specifically in an immuno-compromised congregant (Yusof et al., 2025: Al-Shaarani & Lorenzo, 2024).

Conclusions

The study concluded that following the varied strength of worship centers congregation, there is a significant effect in the expelled bacterial and fungal spore in relation to the congregation strength, which showed that the worship centers have likely return to the era of consciousness. *Staphylococcus aureus, Bacillus* sp., *Penicillium* sp. spores, *Aspergillus* sp. and *Rhizopus* sp. were identified and therefore regarded as communicable disease agents.

Recommendations

The study recommends total responsiveness of the congregations towards signs of communicable disease and a call for further study on an exposure limit of 60,120, 200 minutes and an increased congregational strength of 600 persons upward.

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Conflict of interest

We, Amadi-Ikpa, C. N. and Enaregha, E.B, the authors of this work hereby, declare that no conflict of interest exist with respect to this work.

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