Original Research Article

Microbiological Assessment of Used Face Masks in Port Harcourt Secondary Schools in COVID-19 Era

ABSTRACT

Aim: This study was aimed to identify bacterial and fungal contaminations in used face masks from different secondary schools in Port Harcourt during COVID-19 Era
Study Design: The study employs statistical analysis of the data and interpretation.
Place and Duration of Study: Five Secondary Schools–Three public schools: Federal Government College Rumuokoro; Rumueme and Rumuokuta Girls' Secondary Schools; Two Private Schools: Solid Steps and Istan Secondary Schools; all located in the city of Port-Harcourt, Nigeria. Sample collection lasted for a week and the analysis lasted for six months.
Methodology: The research study was facilitated through Laboratory analysis and the use of questionnaire to get the age and sex from the school children. A total of 25 used face masks samples were collected from school children between ages of 12-18years and they were examined microbiologically. Sterile swab sticks soaked in sterile nutrient broth were employed to swab the inner surface area of the used face mask of circular diameter 10 cm. The swabbed samples were dipped and shaken in 9ml of sterile saline water for 1-3 minutes to dislodge the organisms; the mixture was then diluted through a ten-fold serial dilution, after which an aliquot of 0.1ml were inoculated unto Nutrient Agar (dilution used 10^{-6}, incubated at 37^0C for 24h), Mac Conkey Agar (dilution used 10^{-3}, incubated at 44 ± 0.2^0C for 24-48h) and Sabouraud Dextrose Agar (dilution used 10^{-3}; incubated at 37^0C for 5-7 days). Frequency evaluation and identification of isolates were carried out using standard microbiological techniques.
Result: The entire face masks sampled were found contaminated with microorganisms. The Microbial load (Log10 CFU/cm^2;) and Percentage (%) occurrence of bacterial isolates from used facemask were; Bacillus spp (6.10±2.13)(30.81) > *Staphylococcus auerus* (3.89±3.01)(19.57%) > *Proteus* spp (2.25±2.45)(11.35) > *Paenibacillus* spp (1.55±2.52)(7.82) > *Escherichia coli* (0.36±0.81)(1.82) while fungal isolates were *Aspergillus* spp (2.20±0.55)(11.09) > *Mucor* spp (2.19±0.96)(11.04) > *Penicillum* spp (1.29±0.61)(6.51). The contaminated used face masks with microorganism were highest in school children of ages 16-18years (72%) and the lowest occurred in children of 12-14years of age (12%).
Conclusion: The presence of potential pathogen such as *Staphylococcus auerus, Bacillus* spp etc. are of public health significance. It is therefore recommended that crowd should be controlled in such environments with high bacterial and fungal load such as schools and COVID-19 protocols duly observed.

Keywords: Face mask, *Staphylococcus aureus*, *Bacillus* sp., *Aspergillus* sp., Secondary School students

INTRODUCTION:

Presence of micro-organisms in the air is ubiquitous but then proportion varies according to the environmental condition and location [1]. Spore forming bacteria and fungi are able to survive in bioaerosols and stay viable for a long time in the air. It is generally known that bacteria aerosols present in the air can affect human health, causing mainly respiratory and related disease transmitted via respiratory route, allergic and toxic reaction [2]. In addition, long-term contact of people with bacterial aerosols can be a source of serious illnesses that can influence a person's mental power and learning ability [3]. Exposure to these aerosols can impose adverse effects on local inhabitants as well as tourists in crowded environments. For the above reasons, it is important to monitor the sanitary state of the air in environments where people spend time every day [4].

Up to now, airborne bacteria and fungi have been predominantly approached in the context of their toxicity, allergic and general medical implication in specific environments such as hospital premises [5], markets, schools, homes and offices [6][7]. There is an emergent need for bacteria and fungi aerosols in the atmosphere to support many applications related to public health and international security. Such studies face significant challenges, including the broad diversity of bacteria that can be carried into the air from soil and plant sources and the tremendous variability (both locally regionally) in microbial load and composition owing to sea effects, local climate, weather pattern, local human activities [8].

Crowd scene investigation and their monitoring is very evolving and an enliven field of learning. Crowd event is of immense concentration in a variety of realistic application [9]. The use of face mask evolved during the great pandemic - Corona virus disease 2019 (COVID-19);.an infection disease caused by sever acute respiratory syndrome Coronavirus 2 (SARS-Cor-2) which was first reported in China, December 2019. It has spread globally and resulted in an ongoing pandemic. Various measures have been taken to contain the pandemic. The measures advocated and implemented differ between countries but in general, social distancing and wearing of face mask are measures adopted in some form by virtually all countries now. This is based on the knowledge that the virus is primarily spread during close contact and by droplets or aerosols produced when people sneeze, cough or talk. In this context, PPE includes gloves, medical/surgical facemasks hereafter referred as “medical masks”, googles, face shield and gowns as well as items for specific procedures – filtering face mask respirators (i.e. N95 or FFP2) or FFP3 standard or equivalent [10]. When you hear about facemask for COVID-19 prevention, it is generally three (3) types; (i) Homemade Cloth Facemasks (ii) Surgical Mask (iii) N95 Respirator

To prevent the transmission of the virus from people with symptom, the Centres of Disease Control and Prevention (CDC) is now recommending that everyone wears cloth face masks; such as homemade facemasks. The recommendation is for when you are in public places where it is difficult to maintain a 6-foot distance from others.Risks of Homemade Facemasks –(i) They may provide a false sense of security while homemade facemasks offer some degree of protection; they offer a lot less protection than surgical masks or respirators (ii)They don’t replace or reduce the need for other protection measures. Proper hygiene practice and physical distancing are still the best methods of keeping yourself safe. Moreover the constant use of face mask which some use for 2 to 5 days before disposing has resulting in microbial contamination.

Then, it is necessary to collect detailed information about airborne bacteria all around the world, both indoor and outdoor environments with typical characteristics to enrich the database. Many studies were carried out about the bacteria community in outdoor environment [5][11], however little are known about the composition concentration and distribution of bacterial and fungal aerosols in Used Face Mask in Port Harcourt. It is indispensable to survey both concentration variation pattern and composition of airborne bacteria and fungi systematically and extensively in crowded environment especially secondary schools.

The main goal of this research work is to determine the prevalence of bacteria and fungi associated with nose masks from different secondary schools in Port Harcourt.

2.0 MATERIAL AND METHODS

2.1 Study area/Sampling sites

Port Harcourt is situated within the Niger Delta region at the southernmost part of Nigeria and it is bound by longitude $6^0$56’ to $7^0$$07^0$E and latitude $4^0$44 to $4^0$52’N of the equator. It has a plane topography and about 5m above sea level.

The sampling sites include five (5) different secondary schools within Port Harcourt metropolis, (One Federal Government School, Two State Government School and Two Private School) namely; Federal Government College Rumuokoro, Rumuokuta Girls’ Secondary School, Rumueme Girls’ Secondary School, Istan Comprehensive School and Solid Step School.

2.3 Sampling Technique

A total of Twenty-Five (25) used facemasks which comprises of homemade face masks and surgical masks were collected aseptically from the school children (exchanged with new ones), the used facemasks was taken and carefully put in a sterile container and transported to the laboratory.

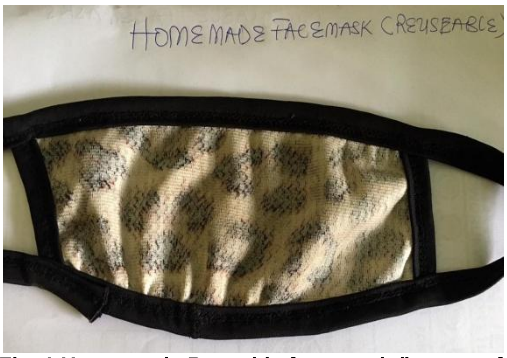

Fig. 1 Home made Reusable face mask (inner surface)

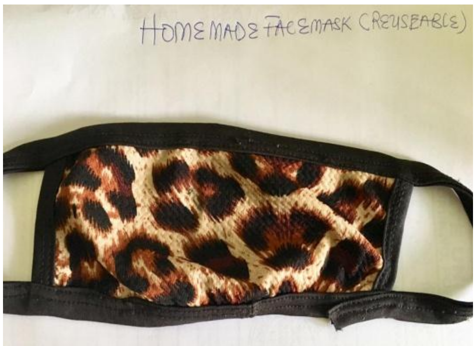

Fig. 2 Home made Reusable face mask (Outer surface)

Fig. 3 Factory made Reusable face mask (inner surface)

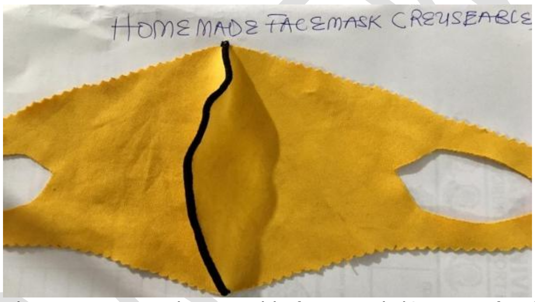

Fig. 4 Factory made Reusable face mask (Outer surface)

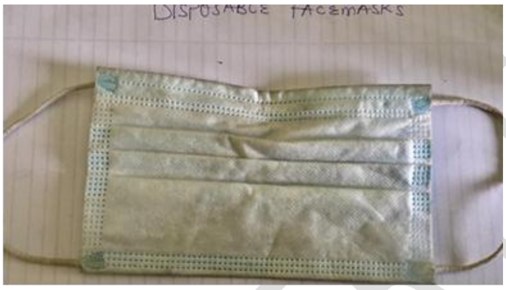

Fig. 5 Factory made Disposable face mask (inner surface)

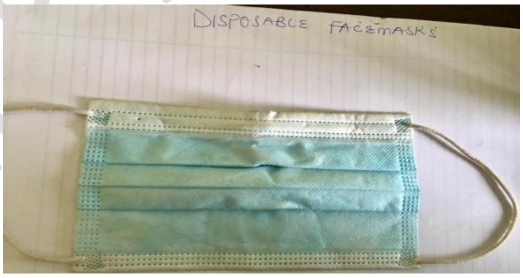

Fig. 6 Factory made Disposable face mask (Outer surface)

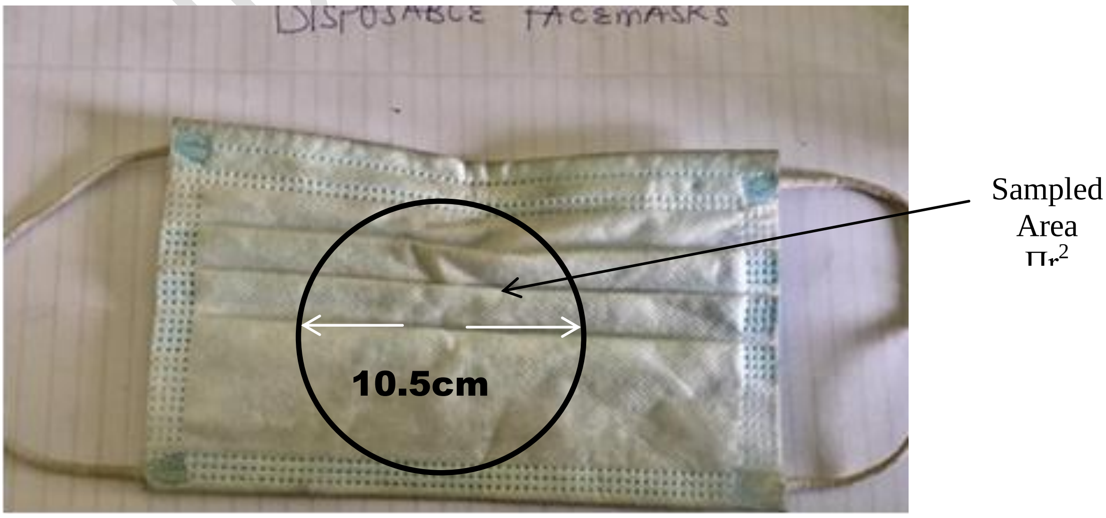

Fig. 7 Picture of Disposable face mask (inner surface) showing diameter of sampled area

B = Sampled Area = (Πr^2)
Diameter = 10.5cm
Radius = 5.25cm
Π = 3.142

2.4 Sample Treatment and Enumeration of microbial population

Sterile swab soaked in nutrient broth prepared and sterilized through autoclaving were used to rub consistently on the inner surface of the used face mask within a circular diameter of 10.5cm. The swabbed samples were dipped and shaken in 9ml of sterile saline water for 1-3 minutes to dislodge the organisms; the mixture was then diluted through a ten-fold serial dilution process, then an aliquot of 0.1ml were inoculated unto Nutrient Agar (dilution used 10^{-6}, incubated at 37^0C for 24h), Mac Conkey Agar (dilution used 10^{-3}, incubated at 44±0.2^0C for 24-48h) and Sabouraud Dextrose Agar (dilution used 10^{-3}; incubated at 37^0C for 5-7 days). Frequency evaluation and identification of isolates were carried out using standard microbiological techniques

2.5 Microbial load of the swabbed Surface Area:

Counts of the colonial isolates on the plates were used to calculate the bacterial and fungal population with the formula.

$$CFU/cm^2 = Ax\frac{B}{C} \quad \ldots equation\ 1$$

Where,

A = *CFU/ml of the Suspension

$$*\,CFU/ml = \frac{No.\ of\ Colonies}{Dilution\ x\ Volume\ Plated} \quad \ldots equation\ 2$$

B = Sampled Surface Area (SSA)

$$SSA = \pi r^2 \quad \ldots equation\ 3$$

C = Volume of Diluent used in Sample Collection

3.0 RESULT AND DISCUSSION

To reveal the adverse effects of airborne bacteria and fungi on human beings, firstly acknowledging the microbial profile of the atmosphere in crowded environments such as schools is essential [4]. Recently, many studies about bacterial and fungal investigation in the air have been carried out in such indoor environments as crowded [3] such as crowded classrooms [4][8][12].

Table 1: Level of significance of bacterial and fungal population (Log10 CFU/cm²) at the various secondary schools in Port Harcourt

					Bacterial Isolates (Log10 CFU/cm²)					*Fungal Isolates (Log10 CFU/cm²)*		
S/N	School	Reusable	Disposable	Colour	*Staphylococcus aureus*	*Bacillus spp*	*Proteus spp*	*Penibacillusspp*	*Escherichia coli*	*Aspergillus spp.*	*Mucorspp*	*Penicilliumspp*
1	RMG		-	Blue	9.188	9.551	9.344	0	0	3.283	0	0
2	RMG		-	White	9.938	0	0	0	0	0	2.983	2.983
3	RMG		-	Blue	0	9.225	0	0	0	3.592	0	0
4	RMG	-		Yellow	0	9.626	0	0	0	3.283	0	2.983
5	RMG		-	Blue	9.928	9.840	0	0	0	3.590	2.983	2.983
6	ISC		-	Blue	9.886	0	0	0	0	0	3.283	0

7	ISC		-	Blue	0	9.682	9.461	0	0	3.591	3.591	3.283
8	ISC		-	White	0	0	9.461	0	0	3.761	0	0
9	ISC		-	Blue	9.897	0	0	0	0	3.283	0	3.283
10	ISC		-	Blue	0	9.489	0	0	0	0	0	3.283
11	FGC	-		Red	0	9.584	0	9.776	0	3.283	3.591	0
12	FGC		-	Blue	0	9.334	0	9.886	0	0	3.283	0
13	FGC		-	Blue	0	9.430	0	0	0	0	3.886	3.283
14	FGC		-	Blue	9.992	0	0	0	0	3.591	3.591	0
15	FGC		-	Blue	0	9.897	0	9.400	0	3.283	3.283	0
16	RMM	-		Red	0	0	0	0	9.104	3.283	3.283	0
17	RMM	-		Black	9.983	9.460	0	0	0	0	0	3.591
18	RMM	-		Brown	9.090	0	0	0	0	0	3.283	0
19	RMM		-	Blue	9.841	9.130	0	0	0	3.761	3.591	0
20	RMM		-	Blue	9.130	0	0	0	0	0	3.279	3.283
21	SSS		-	Blue	0	0	9.430	0	0	3.591	3.591	0
22	SSS		-	Blue	0	9.595	9.364	0	0	3.283	3.591	0
23	SSS		-	Blue	0	9.064	0	0	0	3.283	0	0
24	SSS		-	Blue	0	9.897	9.283	0	0	0	3.591	0
25	SSS		-	Blue	0	9.875	0	9.626	0	3.283	0	3.283

KEY: RMG – Rumuokuta Girls' Secondary School, ISC – Istan Comprehensive School, FGC – Federal Government College, Rumuokoro, RMM – Rumueme Girls' Secondary School, SSS – Solid Step Secondary School.

Table 2: Mean Analysis of Bacterial and fungal load on used face masks

	Bacterial Isolates (Log10 CFU/min-m^2)					***Fungal Isolates (Log10 CFU/min-m^2)***		
School	*Staphylococcus aureus*	*Bacillus* spp	*Proteus* spp	*Penibacillus* spp	*Escherichia coli*	*Aspergillus* spp.	*Mucor* spp	*Penicillium* spp
RMG	5.81±5.31	7.63±4.28	1.87±4.18	0.00±0.00	0.00±0.00	2.75±1.54	1.19±1.63	1.79±1.63
ISC	3.96±5.42	3.83±5.25	3.78±5.18	0.00±0.00	0.00±0.00	2.13±1.95	1.37±1.89	1.97±1.80
FGC	2.00±4.47	7.65±4.28	0.00±0.00	5.81±5.31	0.00±0.00	2.03±1.86	3.53±0.25	0.66±1.47
RMM	7.61±4.27	3.72±5.09	0.00±0.00	0.00±0.00	1.82±4.07	1.41±1.94	2.69±1.51	1.37±1.89
SSS	0.00±0.00	7.69±4.31	5.62±5.13	1.93±4.30	0.00±0.00	2.69±1.51	2.15±1.97	0.66±1.47
Net Mean	3.88±3.01	6.10±2.13	2.25±2.45	1.55±2.52	0.36±0.81	2.20±0.55	2.19±0.96	1.29±0.61

Table 3: Bacterial and fungal percentage occurrences for used nose masks in secondary schools in Port Harcourt

	Bacterial Isolates (%)					***Fungal Isolates (%)***		
School	*Staphylococcus aureus*	*Bacillus* spp	*Proteus* spp	*Penibacillus* spp	*Escherichia coli*	*Aspergillus* spp.	*Mucor*spp	*Penicillium* spp
RMG	29.30	38.58	9.43	0	0	13.87	6.00	9.03
ISC	19.97	19.31	19.06	0	0	10.74	6.91	9.93
FGC	10.09	38.58	0	29.30	0	10.24	17.80	3.33
RMM	38.38	18.76	0	0	9.18	7.11	13.57	6.91
SSS	0	38.78	28.34	31.0%	0	13.57	10.84	3.33
Net Mean %	19.57%	30.81%	11.35%	7.82%	1.82%	11.09%	11.04%	6.51%

KEY: RMG – Rumuokuta Girls' Secondary School, ISC – Istan Comprehensive School, FGC – Federal Government College, Rumuokoro, RMM – Rumueme Girls' Secondary School, SSS – Solid Step Secondary School.

The five (5) genera of bacterial isolates identified, occurred with varied frequencies in all location with *Staphylococcus aureus* having highest value (7.61±4.27 Log10CFU/cm^2; 38.38%) in Rumueme Girls' Secondary School, *Bacillus* spp was highest in Solid Step Secondary School (7.69±4.31 Log10CFU/cm^2; 38.78%), P*roteus* spp had highest in Solid Step Secondary School (5.62±5.13 Log10CFU/cm^2; 28.34%), *Penibacillus* spp was highest in Federal Government College (5.81±5.31 Log10CFU/cm^2; 31.0%), Rumuokoro with (69.0%) and *Escherichia coli* was found only in Rumueme Girls' Secondary School (1.82±4.07 Log10CFU/cm^2; 9.18%) probably due to poor sanitary conditions of the environment (Table 1-3).

Fungal evaluation shows that *Asperigillus* spp occurred highest in Rumuokuta Girls' Secondary Schools (2.75±1.54 Log10CFU/cm^2; 13.87%) *Mucor* spp occurred highest in Federal Government College (3.53±0.25 Log10CFU/cm^2; 17.80%) while *Penicillum* spp had its highest value in Istan Comprehensive School (1.97±1.80 Log10CFU/cm^2; 9.93%) (Table 1-3).

Mean Microbial load (Log10 CFU/cm^2;) and mean Percentage (%) occurrence of bacterial isolates from used facemask were; Bacillus spp (6.10±2.13)(30.81) > *Staphylococcus auerus* (3.89±3.01)(19.57%) > *Proteus* spp (2.25±2.45)(11.35) > *Paenibacillus* spp (1.55±2.52)(7.82) > *Escherichia coli* (0.36±0.81)(1.82) while fungal isolates were *Aspergillus* spp (2.20±0.55)(11.09) > *Mucor* spp (2.19±0.96)(11.04) > *Penicillum* spp (1.29±0.61)(6.51) (Table 2-3).

The contaminated used face masks with microorganism were highest in school children of ages 16-18years (72%) and the lowest occurred in children of 12-14years of age (12%).(Table 4) while Table 5 shows that contamination were higher among the female school children than the males.

Table 4: Occurrences of microorganisms amongst secondary school children in different age groups

Age Group	No. of Examined Face Masks	No. of contaminated Face Masks	Percentage (%)
12-14	3	3	12
14-16	4	4	16
16-18	18	18	72
Total	**25**	**25**	**100**

Table 5 Contamination of used face masks among gender in secondary school children

Organism	No. of School Children	Male	Female
Staphylococcus aureus	13	1	12
Penibacillus	3	0	3
Proteus spp	3	1	1
Escherichia coli	1	1	1
Penicillum spp	1	1	0
Aspergillum spp	3	3	1
Mucor spp	1	1	0
Total	**25**	**7**	**18**

There are many important factors that influence biological pollution in indoor environments. According to several studies, the moisture content of building material, relative humidity and temperature, [8][13][14], human activities [11][15] significantly affect the levels of indoor bioaerosols. In addition, housing condition, the activities and life style of occupants can contribute to the varying concentration. The survival and quantity of microorganism in air depends on microbiological condition such as relative humidity, temperature, uv-radiation and wind velocity. The degree to which these factors influence the survival of microorganism in aerosols depends strongly on the type of microorganism and the time it has to spend on the atmosphere.

Table 6 Percentage of occurrence of bacterial and fungal genera

	GENERA	PERCENTAGE (%)	GENERA	PERCENTAGE (%)
1.	*Staphylococcus* spp	19.57	*Aspergillus* spp	11.09
2.	*Bacillus* spp	30.81	*Mucor* spp	11.04
3.	*Proteus* spp	11.35	*Penicillum* spp	6.51
4.	*Penibacillus* spp	7.82		
5.	*Escherichia coli*	1.82		

Table 6 shows that *Bacillus* spp (30.81%) was the highest occurring bacterial genera, followed by *Staphylococcus aureus* (19.57%), *Proteus* spp (11.35%), *Penibacillus* spp (7.82%) and lowest being ,*Escherichia coli*(1.82%). With respect to fungal isolates; *Aspergillus spp*(11.09%) occurred highest, followed by *Mucor spp*(11.04%) and the least occurring was *penicillium* spp (6.51%). Nrior and Adiele [8] reported percentage frequency occurrence of bacterial isolates of aerosol from school environment; university cafeteria: *Enterobacter* spp. (46.20%) > *Bacillus* spp. (26.72%) > *Micrococcus* spp.(16.10%) > *Escherichia* coli (5.78%); Student toilet: *Staphylococcus aureus* (33.10%) > *Micrococcus* spp. (31.78%) > *Bacillus* spp. (16.44%) > *Escherichia* coli (12.00%) > *Enterobacter* spp. (6.66%); Main laboratory: *Micrococcus* spp. (23.74%) > *Enterobacter* spp. (23.50%) > *Bacillus* spp. (22.34%) > *Escherichia coli* (11.80%) > *Staphylococcus aureus* (1.00%); Laboratory preparatory room: *Micrococcus* spp. (40.72%) > *Escherichia coli* (25.00%) > *Bacillus* spp. (17.84%) > *Staphylococcus aureus* (5.22%). *Staphylococcus aureus* are major pathogenic microorganisms which can create a lot of health problem to humans associated with air, *Staphylococcus aureus* are frequently found in human respiratory tract and minor skin infection, it is a common cause of leg, boil, respiratory disease, food-poisoning and scaled skin syndrome.
Microbial load evaluated from used face mask collected from students in the different secondary schools in Port Harcourt were very high probably due to overcrowding, old buildings and limited /lack of space which makes the environment crowded. Three groups of viable bacteria were identified, gram positive *cocci*, gram positive rods, gram negative rods. Gram positive rods were most abundant. Children's activity is normally high and thus, *Staphylococcus aureus* can be transmitted to the air from their bodies and respiratory tracts. *Staphylococcus aureus* are indicators of the severity of air pollution and their presence may indicate the further presence of pathogenic bacteria. Similar results were also obtained by Kim *et al*., [16] in South Korea, they found that 84% of identified bacteria were gram positive and *Staphylococcus aureus* were the most abundant.

CONCLUSION AND RECOMMENDATIONS

In conclusion, this study was carried out in a number of Secondary Schools in Port Harcourt, the study revealed the high bacterial and fungal contamination on used face masks collected from different Secondary Schools students. Staphylococcal species and heterotrophic bacteria are ubiquitous and quite dominant in the outdoor environment, as a result, to reduce the load of bacterial and fungal contamination on the used masks, the school environments, especially microbial air quality should be improved by preventing overcrowding and reduce the risk of exposure to these organisms.

It is recommended that crowd should be controlled in environments with high level of bacterial and fungal load such as school. This can be achieved by reducing the number of students admitted each session, consciously spacing the students in the classrooms and COVID 19 protocols duly observed.

Having enough windows in rooms to reduce poor ventilation will help to reduce bacterial and fungal load especially in schools, homes and church. Dust control can be a preventive measure in reducing pathogens sources.

General sanitation should be carried out at least twice every month to reduce bacterial and fungal load in crowded environments.

Lastly, regular cleaning, inspection, good personal hygiene should be practiced. It is recommended that hand sanitizer should be provided at the entrance of places like schools, church etc. to reduce the transmission of airborne diseases in the environment.

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