



BACTERIAL CONTAMINATIONS OF USED FACE MASKS COLLECTED FROM DIFFERENT CLINICAL SECTIONS IN A UNIVERSITY TEACHING HOSPITAL DURING COVID -19 PANDEMIC CRISES IN NIGERIA

*¹Enaigbe, A.A. , ¹*Akpoka, O.A. , ²Irodi, C.C.

Address (es):

¹Department of Biological Sciences, College of Natural and Applied Sciences, Igbinedion University, Okada, Nigeria.²Department of Nursing, School of Clinical Medicine, College of Health Sciences, Igbinedion University, Okada, Nigeria.*Corresponding author: ausbones@gmail.com, ausbones@iuokada.edu.ng<https://doi.org/10.36547/be.2021.4.1.8-10>

ABSTRACT

This study was carried out to identify and characterize the fungi present in used face masks, obtained from some clinical sections of University Teaching Hospital in Nigeria. The phenotypic; microbiological and biochemical identifications of the microorganisms were determined by the pour plate techniques using, nutrient agar according to established standard protocols. The results recorded a mean total bacterial count that ranged from $1.75 \pm 0.12 \log_{10}$ CFU/ml to $4.36 \pm 0.28 \log_{10}$ CFU/ml. However, the samples collected from the Anatomy section recorded the lowest bacterial count ($1.75 \pm 0.12 \log_{10}$ CFU/ml) while samples obtained from the Nursing section had the highest bacterial load ($4.36 \pm 0.28 \log_{10}$ CFU/ml). The overall screening test showed the presence of three bacterial isolates: *Peptococcus*, *Pseudomonas* and *Staphylococcus*; Consequently, from the total 64 counted bacterial colonies, the frequency of occurrence was predominated by the *Pseudomonas* 40 (62.5 %) while, mild counts were respectively reported for *Staphylococcus* and *Peptococcus* 18 (28.1 %) and 06 (9.40 %). Succinctly, this work has highlighted that, the underlying illnesses such as lung abscesses, asthma, otitis, pneumonia and sinusitis associated with the isolated organisms in this experiment are also directly linked or similar to the symptoms displayed in mild and severe cases of COVID-19 patients.

Keywords: Contamination, COVID-19, face mask, hospital environment and microorganisms

INTRODUCTION

The outbreak of the dreaded COVID-19 has spread to all parts across the world with infection cases manifesting in cold, fever, chest pain, dry cough, sore or itching throat, difficulty in breathing and loss of speech and movement. It takes an average of 5 to 8 days from where symptoms start to manifest in infected persons; however, it can take up to 14 days (CDC., 2020).

The virus envelop is covered by a characteristic ring of little (crowns) bulbous structures and its morphology is produced by viral spikes; peplomers which are proteins that surround the surface of the virus and determine host tropism and specificity. However, both cellular and viral proteins in the host cell are required for replication and transcription. When the Corona virus locates some viral proteins in the nucleus of the host cell, it triggers host alteration in the transcription and translation patterns, resulting in inflammation, immune and stress responses (Calaneri, 2020; Ji *et al.*, 2020).

The World Health Organization (2020) stated that, in dire need for respite to the intimidating negative impact on humanity, outlined some regulations to include; stay at home orders, avoidance of crowded places, physical distancing, constant hand washing with detergents, use of alcohol-based sanitizers and wearing of face masks. The mask is intended to be worn by health personnel during health-care clinical services and in public, when someone is with persons that are coughing, talking and sneezing. It is made to stop infections in patients and the treating health professionals, by preventing organisms released in respiratory liquid droplets and aerosols from wearers' mouth and nose.

People touch their eyes 15 to 20 times per hour on average, due to itchy, sweating or poorly fitted mask, indicating that people touch their eyes, mouth and nose almost every time. This eventually will result with the risk that, your hands become contaminated and thereafter, distribute the virus to other surfaces, door handles, railings, tables and machines. More so, wearing a face mask allows the exhaled air move into the eye, generates an impulse or feeling to touch the eye, thereby infecting your hands (Jannesson, 2007; WHO., 2020).

The masks containing a humid habitat where corona viruses and other organisms persist and proliferate due to the water vapour regularly released by the breathing and received by the mask fabric, can stimulate a rise in viral counts resulting in defective innate immunity and increased infections (Desai and Aronoff, 2020).

Recent study suggested that, face masks are effective at reducing the spread of these minute particles, stressing that droplets fell out of the air within 1.5 meters of the person who was wearing mask, relatively to 5 meters for those not wearing a mask. Therefore, masks were 100 % effective in preventing seasonal viruses in droplets secreted during breathing, coughing and sneezing. Furthermore, masks create humidity, thereby stopping virus-containing droplets from turning into droplet nuclei. This permits the fabric of the mask to block the droplets and considered the mask good for source control (Desai and Aronoff, 2020; Hunter *et al.*, 2020).

Apparently, when determining a safety precaution that is worth enunciating at scale, it is pertinent to stabilize the positive aspects against potential hazards. Consequently, masks may serve as extra transmission pathway or enhance other tendencies that can transmit the virus such as regular touching of face. However, to avoid the practice of turning mask into alternate transmission route, mask need to be safely placed on and taken off properly.

The National and International public health authorities have recommended that; people should endeavour to use face masks in places where it is inevitable to observe good hand and environmental hygienic practices and social distancing (WHO, 2020).

MATERIALS AND METHODS

Samples Collections

At the commencement of samples collections, hand hygiene and use of appropriate personal protection equipment (PPE) were worn as stipulated. Two used face masks were each collected from 4 clinical sections of the teaching hospital; Anatomy, Microbiology, Nursing and Pharmacy. Samples were collected from each used face mask with separate swab sticks, premoistened with sterile peptone water, rubbed the area (about 5 cm diameter) around the middle of the face mask with the soft end of the swab and left for 10 s to facilitate absorption of microorganisms. The cap of the swab collection tube was carefully removed, then placed the soft end into sterilized sealed containers in sealable leak-proof plastic bags and immediately transported to Microbiology laboratory of the Institution for analyses.

MICROBIOLOGICAL ANALYSIS

Isolation and Enumeration of bacterial isolates

Isolation of bacteria from used face masks was performed by standard methods of pour plating using nutrient agar and minimum salt agar (MSA) (Barrow and Feltham, 2003). The plates were incubated at 28 ± 2 °C for 48 hr. and distinct bacterial colonies in the nutrient agar plates were used to respectively deduce the heterotrophic bacteria counts (HBC).

Identification and characterization of bacteria

Three bacterial colonies were picked based on their different colonial morphologies and each of them was phenotypically characterized with prescribed standard methods (Barrow and Feltham, 2003). The colonies were repeatedly streaked onto nutrient agar slants until pure cultures were obtained and identified. The purified cultures were stored at 4 °C.

Gram staining

This was performed to differentiate the bacterial isolates into Gram positive and negative bacteria.

Biochemical tests

The following biochemical tests were conducted according to stipulated guidelines to characterize and further identify the organisms: Catalase, oxidase, methyl red, Voges Proskauer, lactose and citrate (Ostenfied *et al.*, 2001).

RESULTS

The total heterotrophic bacterial counts (HBC) of the face mask samples are presented in Table 1. The bacterial colony counts were as followed; Anatomy: $1.75 \pm 0.12 \log_{10}\text{CFU/ml}$, Microbiology: $3.75 \pm 0.15 \log_{10}\text{CFU/ml}$, Nursing: $4.36 \pm 0.28 \log_{10}\text{CFU/ml}$ and Pharmacy: $2.37 \pm 0.08 \log_{10}\text{CFU/ml}$ for the samples.

Table 1 Counts of Heterotrophic Bacterial Counts in The Face Mask Samples

Sample	Anatomy		Microbiology		Nursing		Pharmacy	
	Count X=1	Mean \pm SE Count X=4	Count X=1	Mean \pm SE Count X=4	Count X=1	Mean \pm SE Count X=4	Count X=1	Mean \pm SE Count X=4
	\log_{10} cfu/ml	\log_{10} cfu/ml	\log_{10} cfu/ml	\log_{10} cfu/ml	\log_{10} cfu/ml	\log_{10} cfu/ml	\log_{10} cfu/ml	\log_{10} cfu/ml
1	1.62		3.63		4.13		2.51	
2	1.68	1.75 ± 0.12	3.40	3.75 ± 0.15	3.90	4.36 ± 0.28	2.32	2.37 ± 0.08
3	1.90		4.12		5.31		2.40	
4	1.79		3.85		4.09		2.23	

Key: X: total number of samples examined; SE: Standard error of mean and cfu/ml: coliform forming unit per milliliter

Table 2 Results of The Phenotypic Characterization of The Bacterial Isolates

Representative Isolates	Colony and Morphological characteristics	Biochemical characteristics							Identified Species
	Colony characterization	Gram Staining	Ca	Ox	Mr	Vp	La	Ci	
1	Black colouration, convex, shining and smooth appearance.	+	+	-	+	+	+	-	<i>Peptococcus</i>
2	Greenish pigmented colony, rod shape with entire margin.	-	+	+	+	-	-	+	<i>Pseudomonas</i>
3	Yellow pigmented cocci, circular with entire margin.	+	+	-	+	+	+	+	<i>Staphylococcus</i>

Key: Ca: catalase, Ox: oxidase, Mr: methyl red, Vp: Voges Proskauer, La: lactose and Ci: citrate

The phenotypic characterization of bacterial colonies isolated from the contaminated face mask samples is presented Table 2 above. The identified bacterial isolates were; *Peptococcus*, *Pseudomonas* and *Staphylococcus* species.

Table 3 The relative occurrence and percentage frequency of bacterial isolates.

Identified Species	Frequency of occurrence (F)	Representative percentage of occurrence (%)
<i>Peptococcus</i>	06	9.40
<i>Pseudomonas</i>	40	62.5
<i>Staphylococcus</i>	18	28.1
Total counts	64	100

Table 3 represented the occurrence and percentage frequency of bacterial isolates, determined by the following formula: Relative occurrence = total occurrence of a particular fungal isolate divided by the total number of all isolates, multiplied by 100 and expressed in percentage.

DISCUSSION

This study isolated and characterized some bacteria and fungi that can be found in used face masks in the present-day COVID-19 pandemic. The bacteria phenotypically identified were; *Peptococcus*, *Pseudomonas* and *Staphylococcus* species. The mean bacterial counts ranged from $1.75 \pm 0.12 \log_{10}\text{CFU/ml}$ to $4.36 \pm 0.28 \log_{10}\text{CFU/ml}$ in the 4 units of the hospital. The lowest count was recorded from face masks collected from the Anatomy section of the teaching hospital ($1.75 \pm 0.12 \log_{10}\text{CFU/ml}$) and the highest count was reported from the Nursing section ($4.36 \pm 0.28 \log_{10}\text{CFU/ml}$) (Table1). This could be attributed to the regular interaction resulting from coughing, talking and sneezing of health workers with large number of patients and out-patients, as well as constant handling of hospital equipment as compared to the level of interface with persons in the anatomy (morgue) unit.

The phenotypic characterization results confirmed the presence of the following bacterial isolates; *Peptococcus*, *Pseudomonas* and *Staphylococcus* (Table 2). The screening outcome shown that *Pseudomonas* predominated, with relative

frequency of 40 (62.5%) *Staphylococcus* and *Peptococcus* recorded 18 (28.1%) and 06 (9.4%) respectively from all samples surveyed (Table 3). Previous study had implicated the occurrence of *Pseudomonas* species as one of the commonest organisms found in hospital environment, being regarded as an opportunistic (nosocomial) pathogen (Ji *et al.*, 2020).

The *Pseudomonas* is very difficult to eradicate due to its high intrinsic resistance to a variety of antibiotics including β lactams, aminoglycosides and fluoroquinolones. More so, it has been identified as a causal agent of septicaemia, bacteraemia and ear infection. The *Staphylococcus* has been reported to be highly resistant to many antibiotics and involved in toxic shock and scalded skin syndromes, pneumonia and endocarditis, while the *Peptococcus*, though mildly recorded in this analysis is a normal flora of the mouth, has been linked with respiratory diseases, lung abscesses, sinusitis and otitis (Piftet, 1999).

The primary risk of infection for the health service providers are the transmission from patients caring devices and the hospital environment. The aerosol particles of biological aetiologies such as viruses, bacteria and fungal spores have been linked with respiratory tract infections, asthma, bronchitis tuberculosis and aspergillosis which are proximal to known symptoms of COVID-19. This however confirmed that those with underlying diseases and whose immunities have been suppressed or compromised are vulnerable and easily prone to die from COVID-19 attack.

Ji *et al.* (2020) using animal models, investigated that, the Reovirus stimulated infections caused by *Staphylococcus aureus* infections and the Cytomegalovirus facilitated the infection caused by *Pseudomonas aeruginosa*. The established Koch's postulate of disease manifestation can be facilitated by viruses to increase the ability of bacterial pathogens to effect infection. This mechanism is being highlighted to reveal the concomitant association between the bacteria and viruses, in relation to infections of the respiratory tracts, that is a common feature of COVID-19 severe cases.

However, a study has indicated that, the use of face mask has juxtaposed its productive impacts due to improper handling, dirty, damaged, wetted, worn out face masks, indiscriminate disposal, littering and accumulation (Anthonio, 2020; WHO., 2020). These have contributed to a rise in the spread of Corona virus disease; hence, more research is needed to isolate other associated bacteria that

could enhance or be a causal agent to the debilitating public health threats of Corona virus and its related diseases.

CONCLUSION

This study has proved that, the inherent pathological properties expressed by the isolated organisms, are similitudes of the mild and severe clinical manifestations exhibited by COVID-19 patients. Therefore, there is need to embark on personal hygiene practices, as outlined by the World Health Organization (WHO) to stop the spread of COVID-19 devastating effects on mankind.

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