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Bacterial Profile of Multiple-Used Covid 19 Compliant N-95 Non-Surgical Face Mask

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Abstract:-A face mask is a protective device that covers the user's nose and mouth. It may be used once or multiple times. If for multiple use, it may be laundered or cleaned depending on its texture. The bacterial profile of Covid-19 compliant N-95 face masks were examined during this study. A pack of the N95 Face Mask was bought and given to twenty nine (29) informed and consenting young adult volunteers to use for different number of days. When retrieved, it was immersed in warm water for five (5) minutes. A 10-fold serial dilution was carried out using the Miles and Misra's method on Nutrient agar Petri dishes and incubated for 24 hours at 35^0C using the pour plate method. Bacteria colonies were counted and subsequently identified using standard procedures. The result obtained showed that the average colony forming unit per mask (cfu/mask) ranged from $2.5x10^7$ - $1.2x10^{10}$. Bacteria isolated from this study were Bacillus polymyxa, Proteus vulgaris, Bacillus subtilis, Klebsiella pneumonia, Staphylococcus aureus, Citrobacter freundii, Bacillus megaterium, Moraxella catarrhalis, Staphylococcus epidermidis. Although some of these organisms may not be out rightly pathogenic, their presence indicates the need for proper handling and hygienic use of all face mask. Single and multiple users of face mask are advised to maintain adequate hygienic condition that will guarantee good keeping quality and healthy use in-order to reduce the microorganisms load as well as improve the quality of air taken in.

Keyword:- *Bacteria-profile, Facemask, bacteria, respiratory infection, personal hygiene.*

I. INTRODUCTION

Face mask is a protective device that shields the nose and mouth, filtering the air entering through the nasal or buccal cavity (Hayavadana & Vanitha, 2009). It covers the user's nose and mouth and it may be used once or multiple times (Smith *et al*., 2016). Face mask for multiple uses are usually laundered or cleaned (Chellamani & Thiruppathi, 2009).

SARS-CoV-2 (severe acute respiratory syndrome corona virus 2) is spread primarily via respiratory and close contact with symptomatic individuals (Jefferson *et al*., 2008). However human-to-human transmission can occur from unknown infected persons (e.g. asymptomatic carriers or individuals with mild symptoms), as well as individuals with virus shedding during the pre-incubation period before symptoms develop (Cai *et al*., 2020).

Respiratory diseases are caused by a variety of microorganisms (Huang *et al*., 2020). They pose significant risks to life and health of individuals (Simmerman *et al*., 2011). The use of facemasks has been recommended as one of the preventive and control measures to limit the spread respiratory infections (Van-Doremalen, *et al*., 2020). Thus, doctors and other healthcare workers usually use facemask during clinical examinations or in operation room to reduce the risk of contaminations from droplets from mouth and nose (Ong *et al*., 2020).

The spread of COVID-19 occurs primarily through droplets routes and contacts as evidence suggests that the virus can remain viable and infectious in aerosols for hours (Worden *et al*., 2020). Therefore, the use of facemasks remains the appropriate paraphernalia for preventing the spread of COVID-19 and respiratory infections (Konda *et al*., 2020).

The N95 non-medical facemasks are made mostly from non-woven fabric and are available in the two-layer and three-layer form (Ong *et al*., 2020). The layers are ultrasonically welded for efficient bacterial filtration and are used as a protective barrier to prevent cross-contamination among patients and individuals (Smith *et al*., 2016). The micropores of N95 facemask is about 8 microns in diameter and can effectively prevent the penetration of virions (Johnson *et al*., 2009). The micropores of the mask blocks dust particles or pathogens that are larger than the size of micropores (Ong *et al*., 2020). Hence, it is recommended for prevention of diseases transmitted through droplets and respiratory aerosols including the pandemic Covid-19 (Xiao *et al*., 2020).

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However, in-view of the increasing concern raised on compulsory use of facemask to reduces one's risk of being exposed to respiratory infections, in this phase of the COVID-19 pandemic, some have argued that facemasks if not properly used, can become a rich source of the respiratory infections it was originally intended to prevent (Ong *et al*., 2020). Therefore, this study was conducted to investigate the bacteriological profile of COVID-19 compliant N95 face mask worn over different days.

II. MATERIAL AND METHOD

A. Culture media preparation

All the culture used for this study were prepared according to the manufacturer's instructions and sterilized in an autoclave at 15ibs pressure (121^{o}C) for 15 minutes and were allowed to cool to about 45^{o}C according to the method described by Agholor *et al*., (2020)

B. Collection of samples

A packet of N95 non-medical facemask was purchased from a store in Auchi Edo state. The N95 Facemask was given to twenty (29) informed and consenting young adult volunteers to use 8 hours for different number of days, retrieved, tied in different sterile polyethylene bags and transported to Microbiology Laboratory in Auchi Polytechnic for microbial analysis.

C. Bacteriological screening of the samples

Each of the retrieved Nose mask was immersed in sterile warm water for five (5) minutes with continuous agitation to dislodge the bacteria (Abubakar *et al*., 2018). Thereafter, a 10-fold serial dilution was carried out using the Miles and Misra's method and inoculated into Nutrient agar Plates using the pour plate method as described by Agholor *et al*., (2020). The inoculated plates were then incubated for 24hours at 37^{0}C under aerobic and anaerobic condition as described by Abubakar *et al*., (2018) and the bacteria colonies were counted and subsequently identified using standard procedures.

D. Examination and identification of bacteria isolates

The isolated bacteria were identified using the standard conventional method described by Agholor *et al*., (2020) observation of the cultural morphology, Gram staining and biochemical characterization.

E. Statistical Analysis

The experimental data obtained was analyzed using analysis of variance (ANOVA). The days and number of persons associated with organisms was tested using Least Significant Difference (LSD). The analysis was done electronically using Statistical Package for the Social Sciences (SPSS) version 20.0 and the results were presented in tables and interpreted.

III. RESULTS AND DISCUSSION

Dependent Variable: Bacterial organism

Source	Sum of Squares	df	Mean Square	F	Sig.
Days	9.492E19	4	2.373E19	2.886	.050
Number of persons	4.521E19	6	7.535E18	.916	.506
Error	1.480E20	18	8.223E18		
Total	2.882E20	28			

Table 1: Tests of Between-Subjects Effects showing the ANOVA results of the experimental sample data

a. R Squared = .486 (Adjusted R Squared = .201)

Investigation, enumeration and identification of bacterial profile of used covid-19 compliant non-medical N95 face mask was carried out in the laboratory and the data obtained were analyzed using analysis of variance (ANOVA). The results of the ANOVA (Table 1), shows that that there is no significant difference in the number of persons associated with the bacterial count as the probability value associated with F-stat (0.506) is greater than 0.05 at 5% level. This implies that the bacterial count obtained were irrespective of the persons using face mask within the limit

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of the study. However, the result also revealed that there is significant difference in the mean number of days and number bacterial isolated from the used N95 face mask. The days of the experiment shows significant difference as probability value associated with F-stat (0.050) is equal to 0.05 at 5% level. The experimental model value of days based on sample of persons associated with organisms is positively correlated at 0.486 indicating 48.6%.

- Post Hoc Tests Days of experiment Multiple Comparisons

	(I) Days of experiment	(J) Days of experiment	Mean Difference (I-J)	Std. Error	Sig.	Decision Sig (p <0.05)	Conclusion
LSD	Day 4	Day 1	4.3019E9*	1.67913E9	.020	.020<0.05*	Sig.
		Day 2	4.3204E9*	1.67913E9	.019	.019<0.05*	Sig.
		Day 3	3.8611E9*	1.67913E9	.034	.034<0.05*	Sig.
		Day 5	3.7067E8	2.09424E9	.861	.861>0.05	Not Sig.

Table 2: Dependent Variable: Bacterial organism

Based on observed means.
The error term is Mean Square (Error) = 8223434047619040000.000.
*. The mean difference is significant at the 0.05 level.

In testing the significance of days of the experiment of N95 face mask, shown in Table 2, the Least Significant Difference (LSD) of the post hoc test of multiple comparison statistic result indicates that day 4 of sampled experiment are significantly different from day 1, day 2 and day 3. However, day 4 is not significantly difference from day 5.

Bacteria isolates	Gram rxn	Sugar Fermentation Test			Catalyst Test	Indole Test
		Glucose	Maltose	Fructose		
Bacillus polymyxa	+ Rods	AG	A	AG	+ Ve	+ Ve
Proteus vulgaris	- Rods	AG	A	AG	-Ve	-Ve
Bacillus subtilis	+ Rods	AG	A	AG	+ Ve	+ Ve
Klebsiella pneumonia	- Rods	AG	A	A	+ Ve	-Ve
Stahylococcus aureus	+ Cocci	AG	A	AG	+ Ve	+Ve
Citrobacter freundii	- Rods	AG	AG	AG	+ Ve	-Ve
Bacillus megaterium	+ Rods	AG	A	AG	+ Ve	+Ve
Moraxella catarrhalis	-Cocci	AG	A	AG	+ Ve	-Ve
Staphylococcus epidermidis	+ Cocci	AG	AG	AG	+ Ve	+ Ve

Table: 3 Bacteria isolates, Gram reactions and some biochemical test results

Key: AG= acid with gas, A= acid, +ve = positive. - ve = negative, rxn = reaction

Number of days	Number Of Person/ Organisms Associated With Each Person						
	1(cfu/ml)	2(cfu/ml)	3(cfu/ml)	4(cfu/ml)	5(cfu/ml)	6(cfu/ml)	Control
Day 1	*Proteus vulgaris, Bacillus subtilis, Staphylococcus aureus, Bacillus megaterium* $1.7x10^9$	*Bacillus megaterium, Staphylococcus epidermidis* $8.0x10^8$	*Bacillus megaterium, Moraxella catarrhalis* $1.4x10^9$	*Bacillus megaterium, Bacillus subtilis* $2.5x10^9$	*Bacillus subtilis, Moraxella catarrhalis, Bacillus megaterium* $2.5x10^8$	*Bacillus subtilis* $2.5x10^7$	
Day 2	*Bacillus megaterium* $5.0x10^7$	*Bacillus megaterium, Bacillus polymyxa.* $3.2x10^8$	*Bacillus megaterium, Moraxella catarrhalis* $5.0x10^8$	*Bacillus megaterium, Moraxella catarrhalis* $1.3x10^8$	*Bacillus subtilis* $2.5x10^7$	*Moraxella catarrhalis* $5.7x10^8$	

Day 3	*Citrobacterfreundii, Bacillus megaterium, Moraxella rrhalis* 2.6x10^{8}	*Bacillus megaterium* 6.0x10^{8}	*Bacillus megaterium, Moraxella catarrhalis* 1.0x10^{9}	*Bacillus megaterium, Moraxella catarrhalis, Staphylococcus epidermidis* 6.2x10^{8}	*Bacillus megaterium, Bacillus subtilis* 1.1x10^{9}	*Bacillus megaterium, Moraxella catarrhalis* 1.0x10^{8}	
Day 4	*Proteous vulgaris, Bacillus megaterium* 7.5x10^{9}	*Bacillus megaterium, Moraxella catarrhalis* 8.1x10^{9}	*Bacillus megaterium, Moraxella catarrhalis* 1.8x10^{9}	*Bacillus megaterium,* 8.7x10^{9}			
Day 5	*Bacillus megaterium,* 2.3x10^{9}	*Bacillus megaterium,* 1.2x10^{10}					

Table: 4 Occurrence of Bacteria Isolated from each Person from day1 to day 5

IV. DISCUSSION

The mandatory use of facemasks has been recommended as one of the non-pharmaceutical preventive and control measures to limit or prevent the spread of COVID-19 and other respiratory infections (Van-Doremalen, *et al*., 2020). However, less attention has been given to microorganisms that could be transferred to the mask while wearing it and the possibilities of the organisms causing infection for the user. Investigation, enumeration and identification of bacterial profile of used Covid-19 compliant non-medical N95 face mask was carried out in the laboratory and in this study, twenty (29) informed, consenting young adult volunteers were involved and the results obtained showed that all the mask were contaminated with different bacteria. The average colony forming unit per mask ranged from 2.5 x 10^{7} cfu/mask to 1.2 x 10^{10} cfu/mask. This agreed with the work of Lize *et al.*, (2021) that shows that cotton and surgical face mask of 13 healthy volunteers after 4 h of wearing were contaminated with different bacteria species with the colony forming unit ranging from an average of 1.46 x 10^{5} cfu/mask and 1.32 x 10^{4} cfu/mask for cotton and surgical face mask respectively. The bacteria isolated from this study includes Bacillus *polymyxa, Proteus vulgaris, Bacillus subtilis, Klebsiella pneumonia, Staphylococcus aureus, Citrobacter freundii, Bacillus megaterium, Moraxella catarrhalis,* and *Staphylococcus epidermidis* (Table 3). The presence of these organisms may have resulted from the user's saliva, exhaled breath or inhaled air from the environment. Microorganisms from the external air may be trapped in the mask when breathing (Johnson *et al*., 2009), while those in the hand may also be a source of contamination during the process of wearing (Abubakar *et al*., 2018). However, since microorganisms have the ability to multiply on moist and warm surfaces (Agholor *et al*., 2020), the increase in the population of these organisms, could increase the number of bacteria being inhaled which could distort the balance between the normal nasal microbiota and increase the risk of infection (Ong *et al*., 2020). *Staphylococcus aureus* and *Staphylococcus epidermidis* are commonly found in the body surface of human as commensal, helping to maintain healthy skin but these bacteria have also been associated with respiratory tract infection and inflammatory skin diseases (Lize *et al*., 2021).

The Analysis of Variance result (Table 1 and 2) showed that there is significant difference in the mean number of days the face marks were used and the associated microorganisms as the probability value associated with F-stat (0.506) is less than 0.05 at 5% level. This was also confirmed by the Duncan Multiple Range Test as the mean of day 4 recorded the highest number of persons associated with microorganisms followed by day 5. While Day 1 and 2 recorded least mean of number of persons associated with organisms (Table 4). In other words, the longer the usage, the more the microbial load and higher the chances of being infected.

V. CONCLUSION

In conclusion, N95 non-medical face mask are very important as they are recommended for use against diseases transmitted through droplets and respirators from respiratory aerosols. However these face masks if not properly used, can become a rich source of the respiratory infections it was originally intended to prevent. This study has showed that Face mask could become a potential fomite and there is need for biosafety concern even though some of the organisms identified in this study may not out rightly be pathogenic but their presence indicates the need for proper handling and hygienic use of face masks.

CONFLICT OF INTERESTS

The authors declared that there are no conflicts of interest regarding this manuscript.

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