

Sterility Assessment of Some Face Masks for Public Use During COVID-19 Pandemic in Lagos, Nigeria

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To cite this article:

Toyosi Yekeen Raheem, Kazeem Osulale, Samuel Kayode Akindele, Oluranti Ojerinola, Samuel Amoo, Ahmed Ismaila Ochacha. Sterility Assessment of Some Face Masks for Public Use During COVID-19 Pandemic in Lagos, Nigeria. *Biomedical Sciences*. Vol. 7, No. 4, 2021, pp. 114-119. doi: 10.11648/j.bs.20210704.14

Received: October 24, 2021; **Accepted:** November 18, 2021; **Published:** December 24, 2021

Abstract: Globally, the use of face masks is one of the non-pharmaceutical interventions recommended as a method of preventing spread of SARS-CoV-2. Lagos State was an epicenter of COVID-19 and as such, the State Government made the use of facemasks mandatory while in the public and social gatherings. Due to the alarming rate of spread of COVID-19 pandemic, shortage of masks and respirators has been observed and reported globally. This has led to production and use of different types of facemasks including locally made facemasks of different fabrics. A total of 400 consisting of 200 made of local fabrics, 100 imported face/surgical masks and 100 N95 respirators were selected from different sources in Lagos State between May 2020 and November 2020. Samples were immersed aseptically into conical flask containing 100ml Nutrient broth and incubated 18-24hrs. The broth culture was sub-cultured onto Sabouroid Dextrose Agar in duplicates. One of the SDA was incubated at room temperature and the other at 37°C. Blood, Chocolate and MacConkey agar plates were also inoculated and incubated at 37°C for 18-24 hrs. Isolates were identified using phenotypic identification methods. Of the total 400 samples, 346 (86.5%) had no bacterial or fungal growth while 44 (11.0%) had one bacterial isolate and 10 (2.5%) had mixed growth of bacterial isolates. Of the 200 locally-made face masks, 39 (19.5%) had one bacterial isolate and 9 (4.5%) had two bacterial isolates and 5 (2.5%) had fungal isolates while out of the 100 imported surgical masks, only 4 (4.0%) had one bacterial isolate and one (1.0%) had mixed growth of bacterial isolates. One (1.0%) of the imported N95 respirator had only one bacterial isolate (*Lactobacilli spp*). Generally, the isolated bacteria were *Staphylococcus aureus*, *Coagulase negative Staphylococcus*, *Escherichia coli*, *Klebsiella species* and *Lactobacilli species* while fungal isolates were *Candida albicans* and *Aspergillus fumigatus*. The locally-made face masks were contaminated with both bacterial and fungal isolates. Face masks with no bacterial or fungal growth had a p-value of 0.02 and it is statistically significant in terms of the face masks tested in this study. Face masks with one bacterial isolate had a p value of 0.35 and those mixed growth of two different bacterial and fungal isolates had a p value of 0.36 which indicated a non-statistically significant results of the face mask tested in these categories. Locally made face masks were more contaminated with single or mixed bacterial and fungal agents. Cautionary use of the masks is recommended.

Keywords: Face Masks, Respirators, Sterility, SARS CoV-2, COVID-9, Lagos, Nigeria

1. Introduction

There are different types of masks used in infection control measures- face masks and respirators. The main difference between the two are the intended use. While face masks are to

protect the spread of infections from the wearer to others, and also prevent body fluid splashes or sprays to others, respirators on the other hand, are used to protect its users from infections from others with respiratory pathogens [1]. The pandemic nature of the COVID-19 disease which occurred in December

2019 is a major global health concern. Since the first index case of the disease (COVID-19), was reported in Nigeria on February 27, 2020, there had been a steady increase in the number of reported positive cases of COVID-19, especially through community transmission of SARS-CoV-2 virus. Nigeria Centre for Disease Control since then recommended the use of face masks by Health Professionals, Health Workers and the public. Lagos State Government and few other states in Nigeria, even criminalized the appearance in public without the use of face masks. This, in addition to other measures such as coughing or sneezing etiquette, hand hygiene and physical or social distancing were the major interventions promoted by the World Health Organization for the control of the Pandemic [1].

SARS-CoV-2 which is the cause of coronavirus disease 2019 (COVID-19), is a transmissible virus that infects the upper and lower respiratory tract [2] leading to presence of a high viral quantity in saliva and respiratory secretions [3]. Rate of spread of SARS-CoV-2 and number of relevant deaths continue to elevate as the time elapses since its first outbreak [3]. Transmission of corona virus occurs through respiratory droplets, close personal contact as well as touching stuff or surfaces polluted by the viral particles. However, several studies have already proven the effectiveness of using various face masks, according to the circumstances, in preventing the dissemination of COVID-19. A key public health control strategy for mitigating SARS-CoV-2 transmission is use of masks or face coverings by the public [3, 4]. Practicing personal protection measures remain the key strategic and effective way to stay protected from COVID-19 attack. WHO and Centre for Disease Control and Prevention (CDC) [5, 6] also advised those who are directly exposed to the care of any confirmed or suspected COVID-19 patient, including the health care workers and caregivers to wear proper face masks or respirators. Masks serve a dual purpose to protect the wearer and others. These analyses were designed to quantify the protection that masks offer to the wearer when exposed to others who may be infected. There are different types of face masks and these are (i) Medical mask (also known as surgical mask and made of non-woven polypropylene material). World Health Organisation (WHO) recommends that medical masks and respirators should be dispensed to and used by the health care workers only (ii) N-95 respirators and equivalent. This for Health Care Workers to prevent inhalation of minute infectious aerosols. It is recommended in circumstances where there is likelihood of excessive risk of aerosolization, such as in the laboratory procedures, bronchoscopy and intubation. (iii) Non-medical mask. These are made up of different woven cotton fabrics or materials with a larger pore size than the medical masks [3]. Masks that completely cover the nose and mouth are effective at reducing seasonal coronavirus and influenza transmission when worn by infected persons [5, 6] and noninfected persons who may come into contact with infected individuals [7, 8] This is supported by emerging epidemiologic data that indicate that community-wide use of masks can effectively contribute to the prevention of SARS-CoV-2 transmission [9].

In almost all countries, the use of masks in public with several other non-pharmaceutical interventions have been an important health measures during the ongoing COVID-19 pandemic. The use of face masks during the COVID-19 pandemic becomes common non-pharmaceutical intervention. There has been a rapid expansion in the public use of commercial, homemade, and improvised masks that vary considerably in design, material, and construction [10]. Many studies have been done on Facemasks, e.g. fitness and filtration efficiencies (FTEs) of facemasks [3], Use, Reuse, Disinfection and Disposal of Masks and Filtering Facepiece Respirators [10]. The perception is that if masks protect in high transmission settings, they should also protect in crowded public spaces, including workplaces, buses, trains, planes and other closed settings [11, 12].

This study intends to assess quality of face masks, guide cautious use of face masks to promote healthy living and prevent people from being infected with other bacterial or fungal pathogens through use of face masks, in their efforts to seek protection against SARS-CoV-2 infections.

1.1. Goal of the Study

The study aims to assess the sterility of imported and /or the locally made face masks to ensure that they are free from transmissible bacterial and fungal pathogens.

1.2. Specific Objectives

- 1) To determine the proportion of the face masks that were contaminated by bacteria and or fungal agents in imported and locally made facemasks.
- 2) To investigate the type of pathogens if present, in the face masks.
- 3) To compare the difference between bacterial and fungal contaminants of imported and locally made face masks.

1.3. Rationale for the Study

Following the occurrence and spread of COVID-19 pandemic globally, many different types and fashion of face masks (medical and non-medical) are recommended for use [1] Previous studies to assess quality of face masks had been conducted [1, 2] locally made and imported face masks are on the increase. Face masks should be able to prevent pollutants and pathogens from the environment or from individual to individuals. Face masks must also not contain pollutants and pathogens from source of production/through manufacturing processes [3]. There is need for quality control of face masks used in-country to prevent further respiratory health challenge. Even with the advent of emergency use of vaccines against SARS CoV-2, non-pharmaceutical interventions are still strongly recommended [4]. Despite the fact that locally-made face masks of different fabrics are used in the community, there is little or no mention of locally made face masks in the infection control guidance policy in Lagos and generally in Nigeria. This probably may be due to inadequate data on such face masks in-country. This has led to emergence and use of many varieties of face masks (both locally-made and imported)

of which sterility were not assessed. Bacterial or fungal contamination of facemasks could constitute source of bacterial infection of the upper respiratory track and health hazards.

2. Methods

2.1. Sample Collection

Samples were collected between May and November, 2020. Samples collected were from open market such as Mushin, Oshodi and Yaba Markets, NIMR (local and imported brands), Oyingbo, Ojuelegba, private sewing entrepreneurs and selected importers of face masks. Naked (exposed) ones were aseptically collected in sterile paper bag and were taken to the laboratory for processing.

2.2. Culture and Identification of Bacterial and Fungal Isolates

400 different samples were randomly and aseptically selected from 20 different sources into sterilized paper bag. The selected face masks were aseptically immersed in two separate sterile flasks containing 100ml nutrient broth and physiological saline each. The flasks were incubated at 37°C and at room temperature for 18-24 hrs. The cultures were sub-cultured into blood agar, chocolate, MacConkey Agar and Sabouraud 4% dextrose agar (SDA) for isolation of bacterial and fungal agents respectively. The cultures were incubated at 37°C for 18-24 hours to isolate bacterial isolates. SDA were incubated at room temperature and at 37°C for up to 3 weeks for isolation of fungal pathogens. Positive and negative controls (suspension of standard *Escherichia coli* and sterile normal saline respectively) were also cultured to check sterility and ability of the culture media to support growth. Pure culture of bacterial isolates from each sample was performed and identified based on their colonial morphologies and phenotypic biochemical tests such as gram staining reaction, catalase, coagulase, oxidase, indole, vogues proskor and citrate utilization tests (Table 2). Fungal isolates were identified based on phenotypic characteristics such as colonial morphologies, germ tube test, microscopic appearance using lactophenol cotton blue mounts and sugar assimilation test. Data were analysed using descriptive and inferential statistics.

2.3. Ethical Considerations

The protocol was approved by the Institutional Review Board of Nigerian Institute of Medical Research with IRB number IRB/20/045. Confidentiality of the producer of the sampled face masks was maintained by using unique identifiers for the sampled face masks and not the trade names.

3. Results

A total of 400 consisting of 200 made of local fabrics, 100 imported face/surgical masks and 100 N95 respirators were selected from different sources in Lagos State between May 2020 and November, 2020 (Table 1). Different methods of sterilization of the face masks claimed by the producers were indicated in Table 3 which showed 150 out of the 200 (75.0%) locally-made face masks did not indicate method of sterilization of the masks after production. Of the total 400 samples of the face masks tested, 346 (86.5%) had no bacterial or fungal growth while 44 (11.0%) had one bacterial isolate and 10 (2.5%) had mixed growth of bacterial isolates (Tables 1 and 4). Of the 200 locally-made face masks, 39 (19.5%) had one bacterial isolate and 9 (4.5%) had two bacterial isolates and 5 (2.5%) had fungal isolates while out of the 100 imported surgical masks, 4 (4.0%) had one bacterial isolate and one (1.0%) had mixed growth bacterial isolates (Tables 1 and 4). One (1.0%) of the 100 imported N95 respirators had one bacterial isolate (*Lactobacilli spp*). Generally, the isolated bacteria were *Staphylococcus aureus*, *Coagulase negative Staphylococcus*, *Escherichia coli*, *Klebsiella species* and *Lactobacilli species* while fungal isolates were *Candida albicans* and *Aspergillus fumigatus*. All imported face masks indicated that ultraviolet radiation was used for sterilization of the face masks; however, 150/200 (75.0%) of locally-made masks did not indicate method of sterilization of their products (Table 3). The locally-made face masks were contaminated with both bacterial and fungal isolates (Table 1). Inferential statistics showed face masks with no bacterial or fungal growth had a p value of P=0.02, those with one bacterial isolate had P value=0.35 and those mixed growth of two different bacterial isolates had a p value=0.36 (Table 4).

Table 1. Bacterial and fungal isolates in the different types of facemasks.

Brand of facemasks	N (face masks tested)	N (without growth)	N (%) Face masks with one or mixed bacterial growth	N (%) (Face masks with fungal growth)
Made from local fabrics	200	152	43 (21.5)	5 (2.5)
Imported Face/Surgical masks	100	95	5 (5.0)	0 (0)
Imported N95 respirator	100	99	1 (1.0)	0 (0)
Total	400	346	49 (12.3)	5 (1.25)

N=number

Table 2. Biochemical reactions of bacterial isolates.

Isolates	<i>Staphylococcus aureus</i>	<i>Coagulase Negative Staphylococcus</i>	<i>Escherichia coli</i>	<i>Klebsiella spp</i>	<i>Lactobacilli Spp</i>
Gram staining	+ Cocci	+cocci	Negative bacilli	Negative bacilli	Positive bacilli
Catalase test	+	+	NA	NA	-
Coagulase test	+	-	NA	NA	NA

Isolates	<i>Staphylococcus aureus</i>	Coagulase Negative <i>Staphylococcus</i>	<i>Escherichia coli</i>	<i>Klebsiella</i> spp	<i>Lactobacilli</i> Spp
Gram staining	+ Cocci	+cocci	Negative bacilli	Negative bacilli	Positive bacilli
Oxidase test	-	-	-	-	-
Indole test	NA	NA	+	-	-
Urea	NA	NA	-	+	-
VP	NA	NA	-	+	-
Citrate	NA	NA	-	+	-
Glucose	NA	NA	+	+	+
Mannitol	+	+	+	+	+
Sucrose	NA	NA	+	+	+
Lactose	+	+	+	+	+
Motility	NA	NA	+	-	-
KIA					
Glucose fermentation			+	+	+
H ₂ S	NA	NA	-	-	-
Gas production	NA	NA	+	+	+

+ = reaction, - = no reaction, NA = not applicable

Fungal isolates identified by phenotypic characteristics such as colonial morphologies, gram staining reaction, germ tube test, microscopic appearance using lactophenol cotton blue mounts and sugar assimilation test.

Table 3. Types of face masks and indicated methods of Sterilization.

Types of face masks	Indicated Method of Sterilization	
	Radiation	Unknown
Locally made face masks	50	150
Imported face masks	100	0
Imported N95 Respirator	100	0
Total	250	150

Table 4. Types of face masks and bacterial/fungal isolates.

Brand of face masks	Number tested	Number without bacterail/fungal growth	Number with only one bacterial isolate	Number with mixed growth (two or more bacteria or fungal isolates)	Bacteria or fungi isolated
Made of local fabrics	200	152 (76.0%)	39 (19.5%)	9 (4.5%)	<i>Staphylococcus aureus</i> , <i>Coagulase negative staphylococcus</i> , <i>Escherichia coli</i> , <i>Klebsiella</i> spp, <i>Candida albicans</i> , <i>Aspergillus fumigatus</i>
Imported surgical face masks	100	95 (95.0%)	4 (4.0%)	1 (1.0%)	<i>Lactobacilli</i> spp
Imported N95 Respirator	100	99 (99.0%)	1 (1.0%)	0 (0%)	<i>Lactobacilli</i> spp
Total	400	346 (86.5%) P=0.02	44 (11.0%) P=0.35	10 (2.5%) P=0.36	

4. Discussion

Locally-made face masks were the most contaminated in this study. This finding could be so due to lack of standardized method of sterilization because all imported face masks indicated that ultraviolet radiation were used for sterilization of the face masks unlike the locally-made masks which the method of sterilization were unknown. guideline in some countries as to the manufacturing and sales of facemask especially, where the use of personal protective equipment for coronavirus disease (COVID-19) has been controversial, with differing guidelines issued by different agencies [11]. The fact that some bacterial and fungal contaminants were isolated from locally made face masks in this study further implied that newly procured locally-made face masks should be used with caution. This is line with the report that cloth masks can be used for an extended period as long as they are not wet or

soiled, but that they can be reused after they are washed with soap and water or other appropriate methods [8]. Findings in this study stressed the need to wash and iron locally-made face masks before use. Simple modifications such as washing, ironing and proper packaging to improve the sterility of locally made mask is suggested. Our study found that some of the face masks had no significant bacterial and fungal contamination. This implied that the face masks can offer some level of protection without constituting vehicle of infection. This further supports the claim in previous studies that community-wide use of masks can be beneficial and effectively contribute to the prevention of SARS-CoV-2 transmission [10, 11]. This study therefore contributed to helping the public to feel confident and safe in their ability to acquire and wear both locally-made and imported facemasks consistently and appropriately. More so that, SARS-CoV-2 virus being a highly infectious pathogen, one must avoid spreading it in to the environment and that the wearing of a

face masks is better than not wearing a mask at all in the public [12]. However, this study showed that some locally made facemasks were contaminated with one or more bacterial pathogens. The contamination of face masks with bacterial and some fungal (*Candida albicans* and *Aspergillus fumigatus*) organisms could constitute source of upper respiratory infections especially if such contaminated face masks were used by immunocompromised individuals. A similar report conducted in Vietnam where 2-layered masks compared to medical masks showed a lower rate of infection in the medical mask group, and a 13 times higher risk of infection in the cloth mask and suggested that cloth masks may increase the risk of infection [13]. This study showed that imported face masks and N95 respirator were less contaminated. This finding was similar to previous report that performance of locally-made cloth masks is inferior to those of medical masks and respirators [8]. This may probably be due to the method of production and sterilization (exposure to radiation). Method of sterilization of locally-made face masks after production, were unknown unlike the imported ones that indicated sterilization by radiation. It has been previously reported that locally-made face masks can be reused after being decontaminated by various techniques, especially, washing in hot water with soap [8], it is likely that the locally-made face masks will be cheaper and readily available to the public than the imported ones. The contamination of some of the locally-made masks by bacterial and some fungal pathogens can be mitigated by the material, design, adequacy of washing, drying and ironing without any damage or alteration to the shape of locally made face masks as previously reported [3, 12-14].

5. Conclusion

Bacterial and fungal agents were detected in the face masks used by the public. Locally made face masks showed presence of significant pathogenic bacteria and fungi contamination compared with the imported ones. This may be due to methods of sterilization after production. In public settings and communities, locally-made cloth masks can be used to prevent community spread of SARS-CoV-2 infections if they are properly washed and sterilized.

6. Recommendations

More research on locally made cloth face masks is needed to update their use as an alternative to imported medical or surgical masks and or N95 respirators especially, in case of shortage or high-demand situations in possible future pandemic(s). Further analysis of the *Lactobacilli spp* isolated from local and imported face masks is required. Simple modifications such as washing, ironing and proper packaging of locally-made face masks to improve their sterility is suggested in order to avoid bacterial infections of upper respiratory tract due to use of contaminated face masks (especially in the immuno compromised individuals) while trying to prevent SARS-CoV-2 infection.

Conflict of Interest

The authors declare that they have no competing interests.

Acknowledgements

Instigators acknowledge the Federal Government of Nigeria for providing funding for this study through Emergency COVID-19 Research Grant.

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