Invitro filtration efficiency for selected face masks to bacteria with a size smaller than SARS-CoV-2 respiratory droplet

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Abstract

Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) can be transmitted between people through respiratory droplets (droplet particles are >5-10 μm in diameter). We conducted an *invitro* experiment to determine the filtration efficiency for selected face masks (cloth and medical face masks and N95 respirators) to bacteria with a size (0.5-1.5 μm in diameter) smaller than SARS-CoV-2 respiratory droplet. Bacteria suspension was prepared using normal saline (NaCl) and bacteria (*Staphylococcus aureus* and *Escherichia coli*) and maintained at a turbidity of 0.5 MacFarland. The suspensions was put in a 100ml plastic spray bottle (with an approximated 250 μl and flow rate of 31.5 ft^3/min per spray) and then a single spray was performed to the test masks. Swabbing was done to unsprayed side of the test mask within 0 and after 4 hrs. The swab was streaked on CLED media then incubated for 48 hours at 37^oC in ambient air. Bacterial filtration efficiency (BFE) was determined as the proportions of colony forming units (CFUs) between the test and control mask. The selected face masks had BFE of 100% and >99% for medical and double layer cotton cloth masks, respectively. This study supports the use of cotton cloth (at least double layer) face coverings in public settings where other social distancing measures are difficult to maintain to prevent the spread of infection from the wearer.

Introduction

Since its identification in December 2019 in Wuhan, Hubei Province, People's Republic of China [1], World Health Organization (WHO) [2], Centre for Disease Prevention and Control (CDC) and other national health authorities have been advocating different measures to curb the spread of Coronavirus disease-2019 (COVID-2019). These measures include, diligent handwashing/use of hand sanitiser, avoiding touching the face with hands, respiratory hygiene and social distancing [2]. Initially, WHO reported lack of enough evidence, that Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) a virus responsible for COVID-19 can be transmitted through respiratory droplets [3]. But, it is now becoming clear that, SARS-CoV-2 can be transmitted between people through respiratory droplets. This usually happens when respiratory droplets from an infected person (released during coughing, sneezing, or talking) makes direct contact with mucous membranes of another person [4].

In this regard, the use of face masks including cloth masks for the protection of the community has received renewed interest following the updated preventive measures by CDC and WHO. Unlike WHO which recommends the use of face masks to health care workers and people with symptoms [5], other countries and organization like the CDC currently recommends mass masking but reserving N95 and medical masks for use by health care workers and symptomatic patients. With the increasing demands for face masks, WHO reported that, since the start of the Covid-19 outbreak, prices have surged, surgical masks have seen a 6-fold increase, N95 respirators have trebled [6]. In Tanzania, Dar es Salaam Regional Commissioner, a region which is hardest hit by the virus in Tanzania, declared that no person is allowed to go to the public without wearing facemasks [7].

In response to this, different types of masks ranging from N95 to locally made cloth masks are now available for community use in Tanzania especially Dar es Salaam region [7]. This adds to the ongoing debates whether locally made cloth masks save the same purpose as medical masks in blocking respiratory droplets from the wearer (source control) [8-12]. Microorganisms such as bacteria and virus have different features which determine their penetration ability on the face mask material, this include shape, size and their surface characteristics [13, 14]. On the other hand, SARS-CoV-2 can be transmitted through respiratory droplets with >5-10 μm size in diameter, transmission through respiratory nuclei (airborne: <5μm in diameter) is possible under certain hospital environment where invasive procedures are performed [4]. Community transmission of SARS-CoV-2 is primarily through respiratory droplets when an individual is in close contact (within 1 m) with an infected person [4]. In this experiment, *S. aureus* and *E.coli* were selected to determine BFE for the selected facemasks. *S. aureus* is a gram positive cocci that is irregular in shape and often in grape like clusters with an average size of about 0.5- 1.0 μm while *E. coli* is a gram negative, rod shaped bacteria with an averages size of 1.1 – 1.5 μm in diameter [13].

Materials And Methods

Study design, period and area

This was laboratory based (in-vitro) study where bacteria *S. aureus* and *E. coli* were employed in testing the filtration efficiency of the selected cotton cloth mask made locally (Dar es Salaam, Tanzania) and surgical masks during Covid-19 pandemic. Dar es Salaam, is the biggest business city in Tanzania with approximately 6 million people. According to the current Covid-19 status in Tanzania, Dar es Salaam region is leading in terms of morbidity and fatality [11]. In this regard, Dar es Salaam Regional Commissioner has declared mass masking [7]. Tanzania, a low income country, hence the majority of its citizens cannot afford buying medical facemasks available in the Dar es Salaam market, therefore most rely on locally made cloth masks which are cheaper and re-usable [15]. Hence, a need to carry out this study.

Study subject

Facemasks were purposively selected from the Dar es Salaam market to include different cloth masks designs made up of 100% cotton and medical facemasks as described below [13]; *Sample 1 (S1)*; Was made from thick wax cotton fabric, commonly known as "*kitenge*"; Swahili local name". It consisted of two layers of the kitenge fabric; an inner and outer surface of the mask. It had no middle filter layer. *S2*; composition for sample 1 plus middle layer/filter made of fabric lining commonly known as canvas material. *S3*; composition for sample 1 plus laminate breathable middle layer or filter made of non-woven fabric lining commonly known as stiff material (Kiarasheba tailoring mart in Dar-es-Salaam, Tanzania). *S4*; N95 respirator (Kimberly Clark Corp, USA). *S5* Surgical mask (BBL, China) *S6:* Locally made surgical mask (PMCL, Tanzania*)*. *S7:* Was made from thick wax cotton fabric, commonly known as "*kiteng*e"; It consisted of two layers of the kitenge fabric; an inner and outer surface of the mask. The kitenge was pleated both sides but had no middle filter layer.

Experimental design

Bacterial filtration efficiency, *in vitro* is widely accepted method for evaluating face masks [13,16], in this case bacteria penetrating the face masks are collected, cultured and counted to determine colony forming units. In this experiment, a 100 ml spray plastic bottle (Aroma, India) was used for spraying bacterial suspension to determine facemask filtration efficiency. A single spray by 100ml plastic spray bottle was equivalent to 250 µl with an approximated flow rate of 31.5 ft^3/min (A. Singl, personal communication, April 28, 2020). The flow rate used in this study was greater than the normal range of human respiration and higher than the flow rate of 1 ft^3/min as recommended American Society for Tropical Medicine [16]. A single spray of bacteria suspension was performed to every test facemask to represent an average number of times a normal person can sneeze within 4 hours [17], a time recommended to change a facemask. During spraying the facemask and spray bottle were kept in contact. This was done to simulate the a person wearing a facemask to prevent or slow the spread of aerosol.

Laboratory procedures

The mask was prepared and labelled according to mask descriptions above (S1-7). Test bacteria were selected to represent gram positive and negative, spherical and rod- shaped, and of sizes smaller than SARS-CoV-2-respiratory droplet; *S. aureus* ATCC 25923 and *E. coli;* ATCC 25922 (American Type Culture Collection Inc., Rockville, Md), respectively. Briefly, each bacteria suspension which consisted of normal saline (NaCl; 0.9%w/v [Aculife Health Care, India]) and the test bacteria were prepared by keeping the turbidity comparable to 0.5 MacFarland [13] using both visual comparison to turbidity standards and a Densichek photometer (bioMérieux). A 0.5 MacFarland standard inoculum was prepared from 48 hours cultures using cystine–lactose– electrolyte-deficient agar (CLED) (Oxoid LTD, England) incubated at 37°C in ambient air. Since cloth masks were locally manufactured sterilization using an hot air oven at 170 °C for 1 hour was performed to avoid possible contamination from the manufacturer. While in contact, each test facemask (S1-7) was sprayed once using 100 ml plastic spray bottle. Then using regular cotton swabs (Medico, China), dipped in normal saline, swabbing was performed to unsprayed side of the mask immediately (at 0 hours) and after 4hrs. The swab was streaked by spreading it over the surface of an agar plate containing CLED followed by 48 hours incubation at 37°C in ambient air [13].

Quality control

A duplicate bacteria culture were performed to represent each design of the facemask (S1-7) for every type of bacteria strain (*S. aureus/ E. coli*). *In vitro* tests uses positive and negative controls to determine the initial number of bacteria. The control was prepared by inoculating 250 µl of 0.5 MacFarland *S. aureus* ATCC 25923 and *E. coli;* ATCC 25922 (volume of single spray by 100 ml plastic spray bottle); this was done in assumption that no innervation was applied. To enable enumeration of colonies a serial dilution to 10^6 by diluting was used as an inoculum by inoculating 1 µl of each bacteria suspension to CLED media followed by 48 hours incubation at 37°C in ambient air.

Interpretations

The piles of bacteria cells observed after an incubation period were termed as a colony forming units (CFUs). For each plate, colonies were counted using the Protocol Bacteria Colony Version 2.05 to obtain the CFUs counts from 0.5 MacFarland *E. coli* and *S. aureus* suspensions for the test (T) and control (C) per single 100 ml plastic spray bottle. The results were expressed as CFUs per 250 µl of 0.5 MacFarland *E. coli* or *S. aureus*. Growth on culture were determined by comparing the colony characteristics, i.e., colony color and texture, any growth out of the streaked line was regarded as contamination. Absence of growth were determined by comparing with a negative control (un-inoculated plate). Two independent readers observed the culture plates to determine the colony growth and number of colonies. Discrepancies were resolved by consulting an experienced microbiologist. Then, bacteria filtration efficiency was mathematically calculated by BFE = 100% (C-T)/C [14].

Data analysis

Data from laboratory sheet data collection was entered in Microsoft excel sheets (Microsoft Corporation, Redmond, WA). BFE was expressed as a percentage of differences in CFUs between the control and the test face mask divided by CFUs of the control.

Ethical consideration

Ethical approval was sought from the Institutional Review Board of the Muhimbili University of Health and Allied Sciences (MUHAS). This was an in-vitro study where no human subject was investigated, the study used standard organisms, *S. aureus*; ATCC 25923 and *E. coli;* ATCC 25922). Additionally, all methods were carried out in accordance with relevant guidelines, regulations and good laboratory practice of MUHAS.

Results

Description of the selected facemasks

Cotton cloth (S1-S3 & S7) and medical masks (S4-S6) were selected to be challenged their filtration efficiencies using *S. aureus* and *E. coli*. The cotton masks were known as "*Kitenge*" where they were made of 100% cotton. All the selected cotton masks were designed by two layers with an additional of filter between the layer (S2 & S3). The medical masks were selected as described by their manufacturers (**Table 1**).

Growth of bacteria for plates

After 48 hours incubation at 37°C in ambient air, growth of colonies were examined for plates inoculated within 0 hr followed with those inoculated after 4hrs. No colony was observed in all the plates inoculated with swabs from medical face masks both within 0 hr and after 4 hrs (S4, S5 and S6). While for cloth masks (S2, S3 and S7), colonies were observed in some of the plates within 0 hr and/ or after 4 hrs (**Table 2**).

Enumeration of bacteria on plates for BFE

The controls were prepared by inoculating 250 µl of 0.5 MacFarland *S. aureus* ATCC 25923 and *E. coli;* ATCC 25922. The total bacteria count for *S. aureus* and *E.coli* were 389x10^3CFUs/ µl (973 x 10^5 CFUs per 250 µl) and 133 x 10^3 CFU/ µl (333 x 10^5 CFUs per 250 µl), respectively. All medical masks and N95 respirator (S4, S5, S6) had zero CFUs both within 0 and after 4 hrs (BFE: 100%) while a single colony (BFE: 99.9%) was observed in some of the cloth face masks within 0 and/ or after 4 hrs (**Table 3**).

Discussion

To the best of authors' understanding, this is the first study from this setting to evaluate the BFE for selected Tanzanian made (cloth masks) and medical masks using *S. aureus* and *E. coli* bacteria. The described purpose of cloth face masks is to prevent the spread of infection from the wearer (source control), whereas medical respirators are used to protect the wearer from others with confrmed or possible respiratory infections [18].

The study found high bacterial filtration efficiency of 100% and >99% for medical masks and cotton cloth masks made up of two layers tested, respectively. The test used bacteria with size smaller than those of SARS-CoV-2 respiratory droplets [4]. Furthermore, there was no difference in filtration efficiency among cloth masks with or without middle filter fabric layer. These findings correlate to those reported from different studies across the global (cross sectional and in vitro studies) conducted to evaluate cotton face masks in preventing the spread of infection from the wearer where the cotton masks were found effective [19]. The differences in BFE between the current studies and those conducted elsewhere can be contributed by several factors such as the size of the selected challenge organisms [8], type material and facemask [13] and experimental design [16].

CDC recommends use of cloth face masks in public areas where other social distancing measures are difficult to maintain (e.g., grocery stores and pharmacies), especially in areas of significant community-based transmission. The use of cloth face masks as one of the interventions in reducing transmission of SARS-COV-2 in the general population has received a lot of attention lately since the virus is highly transmissible with evidence of transmission during asymptomatic phase [4-6]. Furthermore, CDC recommended that, surgical masks or N-95 respirators are critical supplies that must continue to be reserved for health care workers [20]. CDC do not recommend cloth face coverings to young children under age 2, anyone who has trouble breathing, or is unconscious, incapacitated or otherwise unable to remove the mask without assistance. There is little evidence to recommend the use of face masks in hospitals settings, however some researcher have recommended the use of cloth masks in case of adverse effects during the long use of respirators [21,22].

This study was limited to bacterial species *S. aureus* and *E.coli* with average size between 0.5-1.0 µm and 1.1 – 1.5 µm in diameter, respectively. Furthermore, these bacteria were selected to represent gram positive (*S. aureus*) and gram negative (*E.coli*) with cocci and bacillus shape, respectively. It should be noted that although the size of the bacteria used in this study are larger than SARS-COV-2, the size of the bacteria are smaller compared to that of the SARS-CoV-2 respiratory droplet (>5-10 μm).

The current study did not assess the use of face masks as personal protective equipment (PPE) since this requires assessment of both filtration and fit performance. It is well known that fit performance is very crucial for respiratory protection [23]. However, as compared to N95 respirators, medical and cloth face masks are not designed to fit tightly around the face [12,19,24]. Nevertheless, given the better filtration efficiencies observed for selected cloth masks in this study, cloth face masks could provide some respiratory protection if they are properly designed to provide a better face seal. This study did not test the breathability and particulate filtration efficiency from the selected face masks. In this study, only a few types of fabric materials were tested. Some other fabric materials could have different BFE. In addition, the cloth face masks used in the study had not been worn, washed or ironed, which could also affect their BFE.

In conclusion, selected facemasks had the bacterial filtration efficiency of 100% and >99% for medical and double-layer cotton cloth masks respectively. The filtration efficiency was tested against bacteria with size smaller than the reported SARS-CoV-2 respiratory droplet. While observing other preventive measure against Covid-19 such diligent hand washing and social distancing, findings from this study support the use of double-layer cotton cloth face coverings in public settings where other social distancing measures are difficult to maintain to prevent the spread of infections from the wearer.

Declarations

Data Availability

The datasets generated and/or analyzed in this study are provided in the manuscript.

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Author Contributions

MM participated in conception, study design, data collection, analysis and revising the manuscript. HHM participated in study design, data collection, data analysis and revising the manuscript. GMB participated in conception, study design, data collection, analysis and revising the drafting the manuscript. All authors read and approved the final manuscript.

Competing Interests*:* The authors declare that they have no competing interests.

Consent for Publication: Not Applicable.

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Tables

Table 1: Description of facemasks selected for invitro BFE

Sample ID	Common name	Description	Category (medical/ cotton cloth mask)
S1	Kitenge face mask	Double cotton wax layer	Cotton cloth
S2	Kitenge face mask	Double cotton wax layer with canvas filter in between	Cotton cloth
S3	Kitenge face mask	Double cotton wax layer with stiff filter in between	Cotton cloth
S4	N-95	Respirator (Kimberly Clark Corp, USA).	Medical respirator
S5	Surgical mask	Surgical mask (BBL, China)	Medical
S6	Surgical mask	Surgical mask (PMCL, Tanzania).	Medical

S7	Kitenge face mask	Double cotton wax layer with pleats on both sides	Cotton Cloth

Table 2: Observation of bacterial growth on culture plates

Sample ID	**Bacteria sp.**	**Bacterial growth status on culture plates**	
		Within 0 hr	**After 4 hrs**
S1	*S. aureus*	Colony not observed	Colony not observed
	E. coli	Colony not observed	Colony not observed
S2	*S. aureus*	Colony observed	Colony observed
	E. coli	Colony not observed	Colony observed
S3	*S. aureus*	Colony observed	Colony observed
	E. coli	Colony not observed	Colony not observed
S4	*S. aureus*	Colony not observed	Colony not observed
	E. coli	Colony not observed	Colony not observed
S5	*S. aureus*	Colony not observed	Colony not observed
	E. coli	Colony not observed	Colony not observed
S6	*S. aureus*	Colony not observed	Colony not observed
	E. coli	Colony not observed	Colony not observed
S7	*S. aureus*	Colony observed	Colony not observed
	E. coli	Colony not observed	Colony not observed

Table 3: Colony forming units and BFE

Sample ID	**Bacteria sp.**	**Number of CFUs per 250 µl**			
		Within 0 hr		After 4 hrs	
		CFUs	BFE (%)	CFUs	BFE (%)
S1	*S. aureus*	0	100	0	100
	E. coli	0	100	0	100
S2	*S. aureus*	1	99.9	1	99.9
	E. coli	0	100	1	99.9
S3	*S. aureus*	1	99.9	1	99.9
	E. coli	0	100	0	100
S4	*S. aureus*	0	100	0	100
	E. coli	0	100	0	100
S5	*S. aureus*	0	100	0	100
	E. coli	0	100	0	100
S6	*S. aureus*	0	100	0	100
	E. coli	0	100	0	100
S7	*S. aureus*	1	99.9	0	100
	E. coli	0	100	0	100