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Microbiological Analysis of Selected Washed and Unwashed Nose Masks and its Implication for Public Health

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ABSTRACT

The microbial loads of washed and unwashed medical and fabric nose masks were investigated to determine the efficacy of washing as a means of decontaminating nose masks and also to determine the microbial load of the supposedly new medical and fabric nose mask. Six nose masks were sampled from different sources and were subjected to microbial isolation and identification procedures using the serial dilution and pour plate method, and microbes found were identified using the colonial, microscopic, cultural, morphological and biochemical characteristics.The results obtained revealed that the new medical nose mask had no microbial load while the used unwashed medical nose mask had microbial loads too numerous to count (TNTC) and the used washed medical nose mask had microbial load of 89 cfu/ml. The new and unwashed fabric masks had microbial loads too numerous to count (TNTC) while the washed fabric nose mask had 40cfu/ml. Nine organisms were identified including; Aspergillus *spp* and yeast (Fungi), *Staphylococcus aureus, Salmonella typhi, Proteus bulgaris, Escherichia coli, Proteus mirabilis, Enterobacter cloacae, Micrococcus spp, Streptococcus canis* and *Pseudomonas aeruginosa.* The bacteria isolates were subjected to antibiotic susceptibility test using the disk diffusion method. They were tested against standard gram +ve and gram -ve antibiotics. Antibiotics resistance profile of the isolates to standard antibiotics are as follows; S*taphylococcus aureus* was resistant to 86% of the antibiotic used, *P. bulgaris* was 64%, *S. typhii* was 86%, *P. Mirabilis* has a resistance of 71% While *P. aeruginosa* was 64%. The result of the study showed that the microbial load of new medical nose masks is significantly negligible while microbial load of the used nose masks was high, Furthermore, it reveals that washing reduces the microbial load but does not completely eliminate the presence of microorganisms. This study reveals that there is a need to develop efficient decontamination and antimicrobial protocols that can be universally accessible and easy to use for nose masks that can be reused like the fabric nose mask. For safety purposes, this study recommends that medical nose masks should only be used once and then properly discarded.

KEYWORDS: Medical, Nose mask, Fabric, Washed, Unwashed, Decontamination, Antimicrobial

1.0. Introduction

The aerosolized form of microbial pathogen (such as viruses, bacteria, mycobacterium etc.) and pollutants from the environment has posed an existential threat to healthy living globally with high impact on developing countries recording high morbidity and mortality due to air pollution (WHO, 2012). The transmission of airborne infectious respiratory diseases from infected individuals to susceptible individuals involves the discharge of microorganism-containing aerosols and droplets during various expiratory activities (e.g., breathing, talking, coughing, and sneezing) (Jones and Brosseau, 2015; Tellier *et al*., 2019).

Over the years, infections resulting from airborne pathogens have been mitigated through the use of disposable nose masks (Brook *et al*., 2017). It has been established by previous researches that wearing of nose masks can offer a noteworthy protection to the wearer, although, improper fitting of mask and lack of proper hygiene might make realizing the benefit of protection unrealizable (Bowen, 2010; Rengasamy *et al*., 2010; Chu *et al*., 2020; Leung *et al*., 2020).

The outbreak of the new strain of severe acute respiratory syndrome coronavirus (SARS-CoV-2) from China in the year 2019 resulted in an alarming global pandemic. Many residents in the industrialized world have been using disposable nose masks in an attempt to protect their health from high particulate matter (PM) concentrations so far. Although the disposable nose masks were primarily made for the protection of health-care professionals, who are skilled on how to use and dispose it, to prevent occupational hazards, untrained professionals also use these nose masks during the outbreak of SARS in 2003 and COVID-19 in 2019 (Yang *et al*., 2011; Elachola *et al*., 2020). In the case of the present COVID-19 pandemic, researchers and scientists have urged on the use of nose masks until the mode of transmission of Covid-19 is fully understood, a move which was adopted from the SARS pandemic scenario (Schuchat *et al*., 2011). It has been argued that the use of nose masks offer assistance in decreasing the number of times an individual touch the face/mouth/nose with unwashed hands, which also significantly diminish the chance of contamination. At this time, not only medical masks but also non-medical masks are being produced, from different materials including cotton, silks, e.t c which are uncertified by WHO (Aragaw, 2020). Unfortunately, used fabrics easily present unpleasant odor if they are not washed properly after being used, because there will have been substrates for microorganism's growth under appropriate conditions such as moisture and temperature (Sritoomma and Chantarapanont, 2015).

Whilst nose mask is an important equipment basically used to trap respiratory secretions (bacteria and viruses in air), one cannot compromise its quality and protective effect. The protective quality of a nose mask is dependent on its hydrophobic properties and dryness of its outer layer, also called the protective layer. If the protective layer is not resistive to microorganisms, it may cause health risk to the user. In day-to-day environment, microorganisms are abundantly present and can easily multiply and grow in presence of moisture, temperature and nutrients. The growth and continuous presence of microorganisms on the face, has not only adverse effect on textiles itself but also on the wearer (Hiragond *et al*., 2018).

This study aims to compare the microbial load of various categories of nose masks commonly used as protective barrier against airborne pathogens.

2.0. Materials and Methods

2.1. Sample collection

Six different nose masks were used for this study. Fabric nose masks (new, washed and unwashed) and medical nose masks (new, washed and unwashed) were obtained from different sources into sterile Ziploc bags and taken to the laboratory for further analysis.

The materials and reagents used in this study include: Ziploc bags, conical flasks, test tubes, weighing balance, disposable petri dishes, pipette, cotton wool, spatula, sterile distilled water, sterile nutrient broth, nutrient agar, Potato dextrose agar, eosin methylene blue (EMB) agar and MacConkey agar.

2.2. Serial dilution and isolation of microorganism

Each nose mask was collected into a sterile Ziploc bag. A 10 by 10 cm length was cut out from the middle part of each nose mask and retained in each Ziploc bag. Fifty millilitres (50 ml) of sterile nutrient broth was added to each bag aseptically and swirled around the cut out of each nose mask. The nose masks were allowed to soak in the nutrient broth for 2 hours. One millilitre (1 ml) of the nutrient broth was pipetted out and serially diluted into test tubes containing 9 ml of sterile distilled water. Dilutions 10^{-3} and 10^{-5} was inoculated into nutrient agar, PDA, EMB and MacConkey agar through the pour plate method. The plates were incubated at 37^{o}C for 24 hours. The PDA plates were incubated at room temperature for 5 days.

After 24 hours, the plates were retrieved and the number of colonies were counted. The differential media plates were also observed for growth. The colour, size, shape and elevation of the colonies were observed. The PDA plates were retrieved after 5 days and were observed for presence of growth.

2.3. Identification and characterization of isolated bacteria: The various bacteria colonies were identified based on their colonial, morphological and biochemical characteristics.

2.4. Identification and characterization of isolated fungi: the fungi isolated were identified based on their cultural, microscopic and morphological characteristics.

2.5. Antibiogram of isolated bacteria: The isolates were subjected to antibiotic susceptibility test using the disk diffusion method of Kirby-Bauer *et al*., (2006). The M100 of the CLSI (2019) standard was used to interpret the result of the antibiotic susceptibility test. Isolated bacteria were subjected to antibiotics susceptibility test using the disk diffusion methods of Kirby-Bauer *et al.,* (1996). Standard antibiotic disk including; septrin (30µg), sparfloxacin (30µg), gentamicin (30µg), Augmentin (30µg), chloranphenicol (30µg), ciprofloxacin (30µg), amoxilin (30µg), pefloxacin (30µg), tarivid (30µg), streptomycin (30µg), ampiclox (30µg), zinnacef (30µg), erythromycin (30µg), amoxacilin (30µg), rocophin (30µg) were used.

2.6. Determination of Multiple antibiotic resistance index (MARi). The MAR index for the resistant bacteria isolates was determined according to the procedure described by Krumperman (1983). This is essentially to determine the degree of bacterial resistance to antibiotics. The indices were determined by dividing the number of antibiotics to which the organism were resistant to (a) by the number of the antibiotics tested (b), Resistance to three or more antibiotics is taken as MAR and MAR

greater than 0.2 indicates a high risk source of contamination.

3.0. Results and Discussion

3.1. Results

1. Microbial load of nose masks

After incubation for 24 hours and 5 days. The results obtained is presented in table 1. The new medical masks showed no microbial load while the washed medical mask had 201 cfu/ml. This is an evidence that the medical masks were produced under strict hygienic procedure. However, the used mask shows a high load of microorganisms. Disposable masks were originally developed to filter droplets containing microorganisms expelled from the mouth and nose, and probably to protect the human respiratory system from fine air-borne particles that are known to be associated with various respiratory diseases (Huang 1998). The high microbial load on the used medical mask is not unexpected. Several authors have recorded high microbial load on used nose masks in hospital personnel (Luksamijarulkul, *et al*., 2014). (Gund *et al*., 2021. Zhiqing, *et al.,* 2018) in their work discovered high level of contamination in used surgical nose mask.

Moreover, the nose mask is expected to be able to trap pathogens and produce marked reduction in the bacterial contamination of the respiratory passage. The result presented on the fabric nose mask shows a high level of contamination. Fabrics are known to be a breeding ground for viruses, bacteria and other microbes (Cohen, 2020) (Sharma, *et al*., 2020) in their work opined that fabric nose mask show minimum efficacy in the control of aerosol transmitted infection as compared to surgical mask.

Although, the result from the washed nose mask showed that the microbial load reduced considerably, there were still some residual organisms present. This shows that washing with ordinary soap and water may not completely rid the nose masks of all microbes present. However, several suggestions emerged on the guide to cleaning and disinfection of used mask. WHO (2020a) suggested that boiling and steaming fabric masks can be adopted. It also advised a single use strategy for both surgical and fabric masks. Other guidelines include non-sharing of mask, soaking in 0.1% chlorine for 1 min and rinsing with room temperature water.

Table 1: Microbial Load of each Nose mask on Nutrient agar

Mask type	Medical New	Medical Unwashed	Medical Washed	Fabric New	Fabric Unwashed	Fabric Washed
CFU	0	TNTC	89	TNTC	TNTC	40

Legend: TNTC= Too numerous to count

2. Cultivation and cultural presentation of isolates.

Colony Morphology on Nutrient agar

Figures 1-4 shows the colonial presentation of the isolates. The colonies observed in 1 and 2 were grown on EMB agar and they appear creamy in colour, raised elevation,

some with smooth and some with serrated edges.

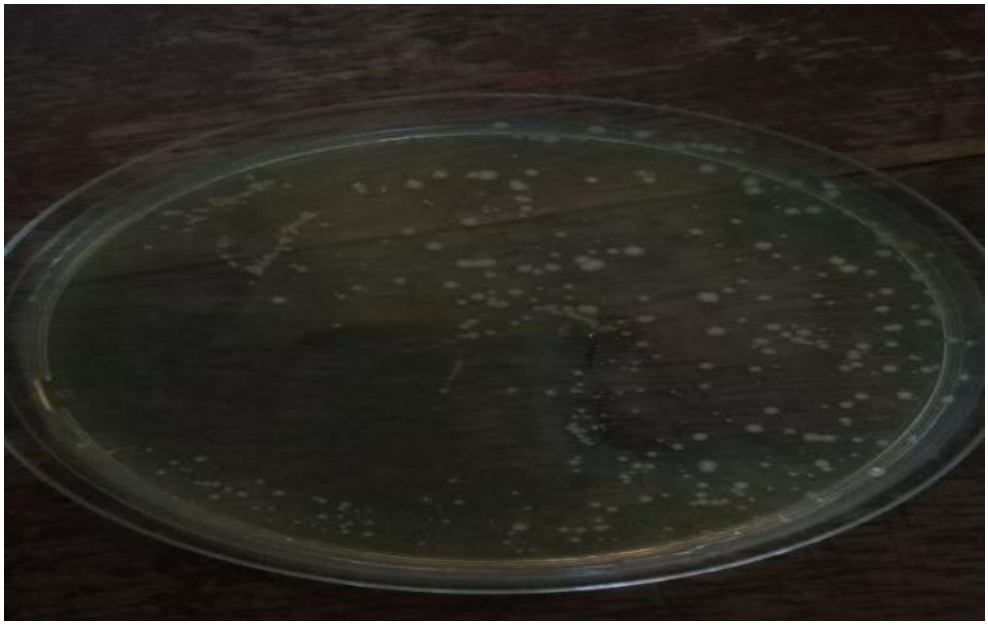

Fig 1. Colony presentation of unwashed

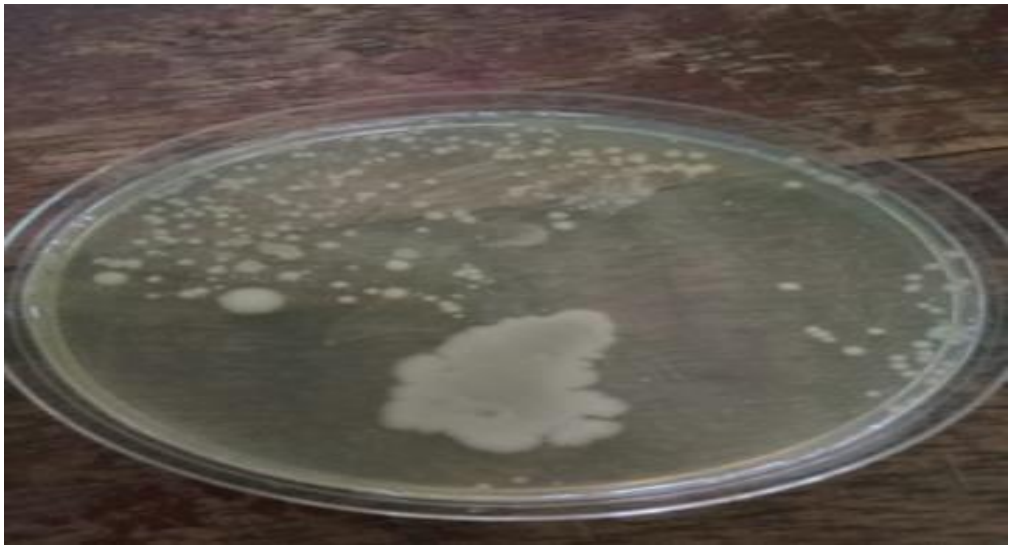

Fig 2. Colonial presentation of unwashed fabric nose mask surgical mask

The colonies on plates 3 were grown on MacConkey agar and they show pigmented isolates while the colonies on plate 4 ware grown on PDA showing fungal growth. Table 2 shows the growth pattern of the isolates on the different media used for their cultivation.

Table 2: Growth on EMB, MacConkey and PDA

Mask type	Medical New	Medical Unwashed	Medical washed	Fabric New	Fabric Unwashed	Fabric Washed
EMB	-	+	+	-	-	-
MacConkey	-	+	+	+	+	+
PDA	-	+	+	+	+	+

Legend: (-): No Growth
(+): Growth

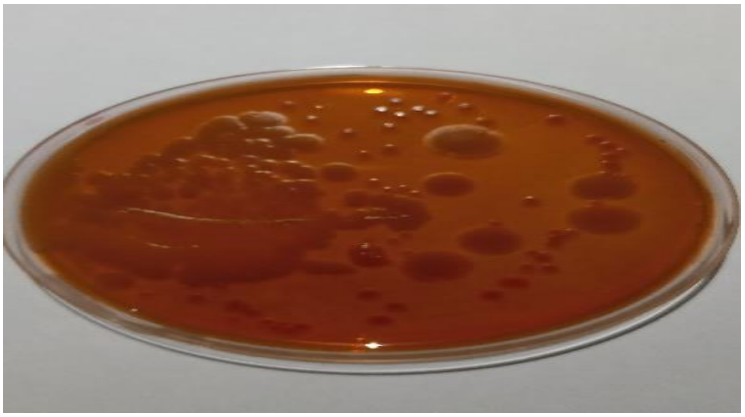

Fig 3: Growth on MacConkey agar, from unwashed fabric nose mask.

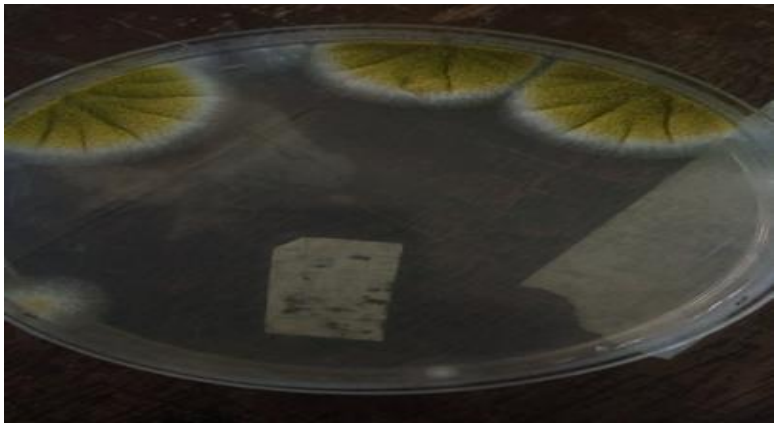

Fig 4: Growth on a PDA plate from unwashed medical nose mask.

3. Identification of isolates.

After subjecting to morphological, cultural and biochemical tests (including Gram staining, sugar fermentation, catalase test, Vogeus Proskeur test, motility test and sugar hydrolysis test). The isolates were characterized to species level using the Bergey's manual. The following isolates were identified; *Staphylococcus aureus, Salmonella typhi, Proteus vulgaris, Escherichia coli, Proteus mirabilis, Enterobacter cloacae, Micrococcus spp, Streptococcus canis* and *Pseudomonas aeruginosa.* The fungi were identified using the microscope and the hanging drop method. *Aspergillus spp* and yeast were confirmed.

4. Antibiotic profile of test isolates:

Table 3 presents the antibiotic profile of the test isolates using standard antibiotics. Zones of inhibition with values lower than 10mm are designated resistant while values ≥10 are designated as sensitive. *S. aureus* was resistant to all the antibiotics used except pefloxacin and streptomycin with a percentage resistance of 86. *S. typhi* was resistant to all antibiotics used except septrin, and erythromycin with a resistant factor of 86%. *P. bulgaris* was resistant to nine of the fourteen antibiotics used which include chloramphenicol, sparfloxacin, ciprofloxacin, augmentin, amoxacillin, ampliclox, Zinnacef, rocophin and erythromycin with a resistance factor of 64%. However, *E.coli* was sensitive to six of the fourteen antibiotics used including septrin, chloramphenicol, ciprofloxacin, gentamycin, pefloxacin, and streptomycin with a percentage resistance of 57%. Similarly, *Proteus mirabilis* was sensitive to chloramphenicol, gentamycin, rocophin and erythromycin with percentage resistance of 71. *Enterobacter cloacae* was also sensitive to ciprofloxacin, pefloxacin, tarivid, amoxacillin, zinnacef and erythromycin with a resistance factor of 57%. On the other hand *Micrococcus spp.* was resistant to sparfloxacin, pefloxacin, tarivid, ampiclox, zinnacef and erythromycin with 43% resistance factor. *Streptococcus canis* was sensitive to chloramphenicol, augmentrin, pefloxacin, and streptomycin with 71% resistance factor and *Pseudomonas aeruginosa* was sensitive to ciproflaxin, augmentrin, tarivid, amxacilin, and zinnacef with a percentage resistance of 64.

Table 3. Antibiotic resistance profile of test organisms to standard antibiotic

Diameters zone of inhibition in mm																		
Ab/ Test organism	*S.aureus*		*S.typhii*		P. *bulgaria*		*E coli*		*P. mirabilis*		*Enterobacter cloaca*		*Micrococcus spp*		*Streptococcus canis*		*P. aeruginosa*	
Septrin	-	R	10	S	10	S	10	S	5	R	7	R	10	S	-	R	6	R
Chloramphenicol	8	R	5	R	-	R	10	S	10	S	5	R	10	S	10	S	-	R
Sparfloxacin	-	R	-	R	5	R	5	R	-	R	6	R	-	R	5	R	5	R
Ciprofloxacin	-	R	-	R	5	R	10	S	8	R	10	S	10	S	7	R	10	S
Augtmentrin	-	R	-	R	5	R	-	R	5	R	-	R	10	S	10	S	10	S
Gentamycin	5	R	-	R	10	S	10	S	10	S	9	R	10	S	6	R	5	R
Pefloxacin	12	S	-	R	10	S	10	S	-	R	10	S	5	R	10	S	-	R
Tarivid	-	R	-	R	10	S	-	R	6	R	10	S	-	R	5	R	10	S
Streptomycin	10	S	-	R	10	S	15	S	55	R	-	R	15	S	10	S	6	R
Amoxacilin	-	R	-	R	6	R	5	R	8	R	10	S	10	S	5	R	10	S
Ampiclox	-	R	-	R	-	R	-	R	-	R	5	R	9	R	5	R	-	R
Zinnacef	-	R	-	R	-	R	-	R	8	R	10	S	-	R	7	R	10	S
Rocophin	-	R	5	R	-	R	5	R	10	S	5	R	6	S	6	R	5	R
Erythromycin	-	R	10	S	7	R	8	R	10	S	10	S	-	R	5	R	-	R

Legend;

R—Resistant

S—Susceptible

- --- No activity

3.2. Discussion

The result of the antibiotic susceptibility profile shows that all the isolates have a high degree of antimicrobial resistance to the antibiotics used. Antibiotic resistance has been a major menace to the human race. The result of this work showed that all the bacterial isolates were multidrug resistant, this fact underscores the danger faced by the use of contaminated (unwashed) nose mask as such can constitute a medium for the transfer of antibiotic resistant pathogens into the respiratory tract of the wearer thereby constituting health risk and hazards. Solomon *et al*, (2017) discovered that air sampled in a hospital environment revealed that all the isolates from the samples were all multidrug resistant, thereby contributing to nosocomial multidrug resistant infections. It is also quite possible that the resistant pathogens were exhaled by the users of the nose mask. Kennedy *et al*., (2018) discovered resistance genes in exhaled aerosol of patients and healthy volunteers in a hospital setting. This opinion underlines the importance of non- sharing of nose masks amongst the populace. Furthermore, contaminated nose masks can also become a source of fomite transmission if not properly disposed or sterilized (WHO, 2020b).

Aerosolisation of antibiotics resistance genes from individuals may contribute to the burden of antibiotics resistance circulating in the environment, and the airborne route has been identified as a potential reservoir of antibiotics resistance elements.

Microbial survival and re-growth on conventional nose masks after usage, improper storage or uncleanliness can also lead to secondary infections in humans (Pasanen *et al*., 1993; Brosseau, *et al*., 1997).

4.0. Conclusion.

The result of this work emphasizes the single use regimen for the use of nose mask. Suffice to say that the use of antimicrobial nose mask is an area that can be explored in the present and no distant future. Antimicrobial coated nose mask can address some of the concerns associated with single-use nose masks by providing in situ real-time antimicrobial protection.

The microbial load of the new, washed and unwashed fabric nose masks were compared. The new and unwashed fabric nose mask had significant growth on all the agar plates. The microbial load of the washed fabric nose mask was lower to the other two categories of fabrics. This is similar to the result obtained by Yilmaz *et al*., 2020 that microbial load of masks increases with time of usage.

Aerosolisation of antibiotics resistance genes from individuals may contribute to the burden of antibiotics resistance circulating in the environment, and the airborne route has been identified as a potential reservoir of antibiotics resistance elements.

For safety purposes, this study recommends that medical nose masks should only be used once and then properly discarded. Nose masks from fabrics should be thoroughly washed with antiseptic soaps, not just toilet soap. Other simple decontamination techniques like swabbing with alcohol, proper sun drying and ironing can be carried out on the nose masks, taking into consideration the materials they are made from.

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