ACTA MICROBIOLOGICA BULGARICA Volume 39 / 4 (2023)

Microbiological Analysis of Consecutively used Face Masks during the Covid-19 Pandemic

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Abstract

It is unanimously approved that face mask greatly supports reducing COVID-19 transmission. And it is still an important tool for dealing with the COVID-19 pandemic. However the utilization and handling of face masks are not so easy. Under this study, we set up an innovative way to investigate the microbial quality of the consecutively used face mask. People in Bangladesh use the same face mask several times, even for days to months. So the possibility of contaminating these masks with different microbes is so high. The frequency of using the same masks multiple times is higher in cloth masks than in surgical masks. The microbial load was also significantly (*P*-value 0.0369) higher in cloth face masks than in surgical masks. The mean TVC of surgical masks was 9.92×10^3 CFU/inch^2 and TCC was 5.38×10^3 CFU/inch^2, while the mean TVC and TCC in cloth masks were 1.76×10^4 and 9.82×10^3 CFU/inch^2, respectively. *E. coli*, *Pseudomonas* spp., and *Proteus* spp. bacterial isolates were assured by standard biochemical tests. Moreoever, enteropathogenic *Escherichia* coli (EPEC) was confirmed by PCR targeting the bfpA gene (300bps). Bacterial isolates were resistant to different commercial antibiotics; *E. coli* resistant to Vancomycin (80%), Ampicillin (80%); *Pseudomonas* spp. resistant to Ampicillin (100%), Chloramphenicol (86.67%), and Vancomycin (80%); and *Proteus* spp. resistant to Miconazole (93.3%), Vancomycin (66.67%), respectively. Face masks protect against coronavirus, but pathogenic microbes contaminated masks may cause health hazards. So we should use face masks properly, keeping them sterile by using disinfectant or washing them properly for consecutive use.

Keywords: COVID-19, face masks, contamination, biochemical test, PCR, antibiotics

Резюме

Единодушно е прието, че маската за лице значително спомага за намаляване предаването на COVID-19. Тя все още е важен инструмент за справяне с пандемията от COVID-19. Но използването и боравенето с маски за лице не е толкова лесно. В рамките на това проучване ние създадохме иновативен начин за изследване на качество на последователно използваната маска за лице по отношение на микробиологичната чистота. Хората в Бангладеш използват една и съща маска за лице няколко пъти, дори от дни до месеци. Така че възможността за замърсяване на тези маски с различни микроби е много голяма. Честотата на използване на едни и същи маски многократно е по-висока при маските от плат, отколкото при хирургическите маски. Микробното натоварване също се оказа значително (P-стойност 0.0369) по-високо в платнените маски за лице, отколкото в хирургическите маски. Средният TVC на хирургическите маски е 9.92×10^3 CFU/inch^2, а TCC е 5.38×10^3 CFU/inch^2, докато средните TVC и TCC в платнените маски са съответно 1.76×10^4 и 9.82×10^3 CFU/inch^2. Бактериалните изолати на *Escherichia coli*, *Pseudomonas* spp. и *Proteus* spp. са получени чрез стандартни биохимични тестове. Освен това, наличието на ентеропатогенна *E. coli* (EPEC) е потвърдено чрез PCR, насочен към bfpA гена (300 bps). Бактериалните изолати са резистентни към различни търговски антибиотици; E. *coli* е резистентна към ванкомицин (80%) и ампицилин (80%); *Pseudomonas* spp. е устойчив на ампицилин (100%), хлорамфеникол (86.67%) и ванкомицин (80%); а *Proteus* е резистентен съответно на миконазол (93.3%) и ванкомицин (66.67%). Маските за лице предпазват от коронавирус, но замърсени с патогенни микроби те могат да бъдат опасност за здравето.

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Acta Microbiol. Bulg. 2023;39(04). https://doi.org/10.59393/amb23390413

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Introduction

COVID-19 is a flu-like and highly contagious disease that evolved from a novel coronavirus in Wuhan, China. The International Committee on Taxonomy of Viruses (ICTV) has declared the disease as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (Gorbalenya *et al*., 2020; Wu *et al*., 2020). It has been believed that the wild animal available for sale in the general market of Wuhan is responsible for the transmission of SARS-CoV-2 (Konda *et al*., 2020). The origin and transmission of this virus is still unknown. Still, many scientists believe that bats may be the natural carrier of SARS-CoV-2, and pangolins may have been suggested as the probable intermediate host (Yuan *et al.*, 2020). The disease rapidly spread globally, affecting millions of people, and more than 5 million people have died due to SARS-CoV-2 (till the end of 2021) (Edwards *et al*., 2022). Thus, this virus created a miserable pandemic with a significant impact on survival and human activities. The world is still going along with this pandemic situation, and proper hygiene and medical care are crucial to reducing the mortality rate and maintaining economic growth (Alhassan *et al*., 2021).

As a highly infectious disease, the expansion of SARS-CoV-2 occurs worldwide within the shortest possible time. To reduce affect and mortality rates, governments of different countries took different steps, including international and domestic travel restrictions, closing schools and nonessential businesses, strictly limiting public gatherings, and so on (Aravindakshan *et al*., 2020; Mendez-Brito *et al*., 2021)governments introduced strict Non-Pharmaceutical Interventions (NPI. Furthermore, using face masks, maintaining social distance, self-isolation while symptomatic, handwashing with disinfectant, and disinfecting surfaces will reduce the transmission of SARS-CoV-2 (Honein *et al.*, 2020)2020 (1.

As Covid 19 is an air-transmitted pathogen, the World Health Organization (WHO) has recommended using face masks as the first line of defense against the coronavirus. Still, it is believed that face masks are a primary way of reducing air-transmitted disease (Akber Abbasi *et al*., 2020; Bazant and Bush, 2021)specifically the Six-Foot Rule, a guideline that offers little protection from pathogen-bearing aerosol droplets sufficiently small to be continuously mixed through an indoor space. The importance of airborne transmission of COVID-19 is now widely recognized. While tools for risk assessment have recently been developed, no safety guideline has been proposed to protect against it. We here build on models of airborne disease transmission in order to derive an indoor safety guideline that would impose an upper bound on the "cumulative exposure time," the product of the number of occupants and their time in an enclosed space. We demonstrate how this bound depends on the rates of ventilation and air filtration, dimensions of the room, breathing rate, respiratory activity and face mask use of its occupants, and infectiousness of the respiratory aerosols. By synthesizing available data from the best-characterized indoor spreading events with respiratory drop size distributions, we estimate an infectious dose on the order of 10 aerosol-borne virions. The new virus (severe acute respiratory syndrome coronavirus 2 [SARS-CoV-2]. During the initial stage of the pandemic, some countries, not only developing countries but also developed countries like the USA, have seen an enormous demand for face masks. But with time, the supply of masks is increasing, and people are trying to maintain face masks as personal protection (Panda *et al.*, 2020). Bangladesh is highly populated, and infectious diseases like corona can be transmitted easily. It isn't easy to ensure social distancing and personal hygiene (Siraj *et al*., 2020). But surprisingly, the coronavirus pandemic is not as devastating as other developed countries like the USA, UK, or other European countries. All classes of people are trying to maintain face masks even though in a developing country, most people are middle class based on economic status.

Bangladeshi people use different kinds of face masks. Most of them use surgical and cloth face masks. They run their family on a lower budget and use the same face masks daily. Sometimes they wash their masks with soap and water and use them for several weeks to months. After use, they store their mask in their pocket or handbag. At the same time, they maintain very little sterile condition. So there is a great chance of bacterial and fungal contamination and virus transmission (Akber Abbasi *et al*., 2020)we highlight the concerns relating to extensive use of face masks in this region, particularly in the context of (micro-.

So we performed an innovative experiment to investigate the role of face masks among different classes of Bangladeshi people during the coro-

navirus pandemic and the microbiological quality of their used face masks. It was aimed to increase awareness about the importance of maintaining masks properly and ensuring proper hygienic management. We also tried to educate people about the importance of face masks in this current situation and how they might be free from microbial contamination.

Materials and Methods

Study area and sample collection

Seventy face masks were collected from people of different occupations and ages at Mawlala Bashani Science and Technology University campus premises and markets in Tangail district, Bangladesh. More importantly, we were trying to collect these masks, which were used for several days to months. We conducted this study from January to March 2022. Before collecting and processing the samples, collectors were taken appropriate personal protective equipment. Each mask collected from people was kept in a plastic bag labeled with different important parameters (e.g., sampling source, no., date, and time) and stored separately. After that, the samples were taken immediately to the microbiology laboratory of the Department of Biotechnology and Genetic Engineering, MBSTU, for further processing and experiments. We also ensured that the people from whom we received mask samples were not flu or flu-type diseases infected.

Primary data collection

At the time of sampling, some basic and important data were collected from the selected people. A questionnaire-based interview was taken containing different questions related to our current study such as respiratory disease conditions, frequency of occurrence of these diseases, the age of masks and their time of use, and so on (Sareen *et al*., 2005).

Clinical information about the participants

During the sample collection, we ensured that the participants were clinically healthy and were not infected with flu-like diseases at that time. But they were affected with fever and typical flu-like symptoms randomly several times. Some participants were patients with different respiratory infections, although uninfected with Covid-19. One common thing for all the participants is that they ignored the hygienic face mask issue during or after use.

Total time of mask usage by the participants

Among the participants, most of them consecutively used face masks, even the one-time surgical face mask. For surgical face masks, the participants use them for about 1-3 days; for the cloth masks, participants use them for several weeks to months.

Sample processing

The masks were cut into 1inch^2 diameter shaped circles and submersed into 2 ml saline water in a falcon tube. Then they were kept for 15 to 20 minutes at room temperature. Finally, vortex them carefully, and then standard serial dilution was performed to obtain the sample up to 10^{-3} consistently diluted for the microbiological analysis.

Determination of Total Viable Count (TVC) and Total Coliform Count (TCC)

One hundred µl of the sample (raw and diluted) was shifted by a micropipette, spread on fresh Mac-Conkey and nutrient agar plates with a U-shaped glass rod, and inoculated at 37°C for 18 hours. The total viable count is expressed as the number of colony-forming units in each nutrient agar plate (Sharifi-Rad *et al.*, 2016). The total coliform count is the number of bacterial colony-forming units in each Mac-Conkey agar plate (Yannick, 2013).

Assessment of the fungal presence

Small pieces of mask samples (1inch^2 in diameter) were swabbed on Potato Dextrose Agar (PDA) media and then incubated for 4-5 days at 28°C for fungal growth (Prapagdee *et al*., 2008).

Biochemical experiments

For the characterization of bacterial isolates, we performed several types of biochemical experiment. Kligler Iron Agar (KIA), Motility Indole Urease (MIU), Citrate, Voges Proskauer (VP), Oxidase, Catalase, Mannitol, Eosin Methylene Blue (EMB), Starch, Methyl Red (MR), Glucose, Lactose fermentation test, were accomplished according to Bergey's Manual Determinative Bacteriology (Deshwal *et al*., 2013; Karmaker *et al*., 2016).

Molecular identification by Polymerase Chain Reaction (PCR)

DNA extraction from bacterial isolates. The single bacterial colony from semi-solid culture media was cultured on liquid media (TSB+YE) overnight. From liquid media, 1 ml of liquid media containing bacterial cells was taken to 1.5 ml micro-centrifuge tube and centrifuged at 13000 rpm for 7 minutes. The bacterial cells were precipitated and removed from the supernatant. Then, 200µl of PCR water and vortex finely mix the bacterial cells with PCR water. After that, the extraction of DNA was performed by boiling for 10 minutes at 100°C

and then exposing cold shock by placing them under ice (Matsunaga *et al*., 2003)such as the Yersinia pseudotuberculosis invasin and Escherichia coli intimin, are surface-expressed proteins that mediate host mammalian cell invasion or attachment. Here, we report the identification and characterization of a new family of Big domain proteins, referred to as Lig (leptospiral Ig-like. Then again, vortex and centrifuged each sample for seven minutes at 13000 rpm. Then the fine supernatant (containing bacterial DNA) was collected with a micropipette and stored in a separate micro-centrifuge tube. Quantification and purity of this bacterial DNA were determined by DeNovix (DS-11 Spectrophotometer). And finally, these bacterial DNA were used as PCR templates.

PCR program was performed for the characterization of bacteria. We used five PCR primers to detect *E. coli* species (Table 1). In this PCR amplification program, different kinds of genes e.g., LT, bfpA, aaiC, Stx1, and IpaH were targeted for the detection of five *E. coli* species e.g. enterotoxigenic *E. coli* (ETEC); enteropathogenic *Escherichia coli* (EPEC); enteroaggregative *E. coli* (EAEC), enterohemorrhagic *E. coli* (EHEC), and enteroinvasive *E. coli* (EIEC) (Stacy-Phipps *et al*., 1995; Chassagne *et al*., 2009; Zhang *et al*., 2013; Rogawski *et al*., 2017; Sethabutr *et al.*, 2000).

After performing PCR, the product of gene-specific PCR was analyzed in 1.8% ultra-pure agarose gel (stained with Ethidium bromide) and exposed to UV illumination. An electric current of 95V was applied for the gel run. Along with the PCR product, a 50 bp ladder was used as an indicator of PCR products.

Antibiotic susceptibility testing

Biochemically and PCR-confirmed isolates (*E. coli*, *Pseudomonas* spp., *Proteus* spp.) were undergone antibiogram experiment. Here we followed the standard protocol of the disk diffusion method (according to the Kirby-Baur method). Antibiotic disks that we collected were commercially available and placed on Mullar-Hinton agar (inoculated with selected isolates) to reveal their effectiveness on the selected bacterial isolates (Maharjan and Mahawal, 2020). Commercially available antibiotics such as Ciprofloxacin (5), Gentamicin (10), Tetracycline (30), Chloramphenicol (30), Miconazole (30), Ampicillin (10), Ceftriaxone (30), Nalidixic acid (30), Erythromycin (30) were used for this antibiogram experiment.

Statistical analysis

SPSS version 20, MS Excel 2016, and Graph-pad Prism 8 were used for data analysis and illustration of graphs.

Results and Discussion

It has been unanimously accepted that a face mask is an effective protective tool against air-transmitted viral pathogens and prevents the expansion of infectious diseases. However, filters of face masks aren't capable of inactivating microbes like bacteria or viruses. Sometimes the consecutive use of the same face mask several times may influence secondary bacterial contaminations. A previous study found different bacterial pathogens responsible for the SARS-CoV-2-mediated pneumonia disease complex, and the situation becomes miserable when these bacterial isolates become resistant to antibiotics used for pneumonia treatment (Martí *et al*., 2021). This study was trying to determine the microbial quality of consecutively used face masks among general people, and the scenario of bacterial load in face masks is shown in Table 2. The highest TVC in the surgical mask was 2.48×10^4 CFU/inch^2, and TCC was 1.98×10^4 CFU/inch^2. In cloth face masks, the highest TVC and TCC were 2.93×10^4

Table 1. Primers for detection of virulent genes of different *E. coli*.

Organism	Gene	Primer	Oligonucleotide sequence (5' to 3')	Product size (bp)
ETEC	LT	Forward	CACACGGAGCTCCTCAGT	508
		Reverse	CCCCCAGCCTAGCTTAGTTT	
EPEC	bfpA	Forward	GGAAGTCAAATTCATGGGGGTAT	300
		Reverse	GGAATCAGACGCAGACTGGTAGT	
EAEC	aaiC	Forward	ATTGTCCTCAGGCATTTCAC	215
		Reverse	ACGACACCCCTGATAAACAA	
EHEC	Stx1	Forward	CAGTTAATGTGGTGGCGAAGG	348
		Reverse	CACCAGACAATGTAACCGTAACCGCTG	
EIEC	IpaH	Forward	TGGAAAAACTCAGTGCCTCT	423
		Reverse	CCAGTCCGTAAATTCATTCT	

Table 2. Total Viable Count (TVC) and Total Coliform Count (TCC) of surgical and cloth's face musk CFU/inch^2

No. of samples	Surgical musk			Cloth's musk		
	TVC	TCC	Fungi	TVC	TCC	Fungi
01	$1.38\times10^4 \pm 0.124$	$1.20\times10^3 \pm 0.005$	+	$1.52\times10^4 \pm 0.154$	$7.20\times10^3 \pm 0.111$	-
02	$4.80\times10^3 \pm 0.022$	$4.00\times10^3 \pm 0.008$	+	$5.10\times10^3 \pm 0.009$	$3.80\times10^3 \pm 0.005$	-
03	$1.65\times10^4 \pm 0.043$	$4.56\times10^3 \pm 0.100$	-	$2.90\times10^4 \pm 0.233$	$2.00\times10^4 \pm 0.167$	+
04	$4.80\times10^3 \pm 0.007$	$1.30\times10^3 \pm 0.006$	-	$9.00\times10^3 \pm 0.202$	$7.20\times10^3 \pm 0.007$	-
05	$1.12\times10^4 \pm 0.121$	-	-	$1.28\times10^4 \pm 0.181$	$8.64\times10^3 \pm 0.022$	-
06	$9.52\times10^3 \pm 0.009$	$7.36\times10^3 \pm 0.102$	-	$1.04\times10^4 \pm 0.104$	$3.80\times10^3 \pm 0.005$	-
07	$4.80\times10^3 \pm 0.120$	$1.26\times10^3 \pm 0.122$	-	$2.88\times10^4 \pm 0.146$	$2.08\times10^4 \pm 0.120$	+
08	$1.86\times10^4 \pm 0.140$	$3.12\times10^3 \pm 0.112$	+	$1.90\times10^4 \pm 0.105$	$4.80\times10^3 \pm 0.112$	-
09	$1.60\times10^4 \pm 0.128$	$8.00\times10^3 \pm 0.130$	+	$2.48\times10^4 \pm 0.109$	$1.35\times10^4 \pm 0.111$	+
10	$2.44\times10^4 \pm 0.113$	$5.60\times10^3 \pm 0.087$	-	$2.10\times10^4 \pm 0.133$	$1.64\times10^4 \pm 0.115$	+
11	$7.20\times10^3 \pm 0.104$	$5.04\times10^3 \pm 0.085$	-	$2.88\times10^4 \pm 0.129$	$1.00\times10^4 \pm 0.189$	+
12	$1.12\times10^4 \pm 0.126$	-	-	$2.60\times10^4 \pm 0.084$	$1.84\times10^4 \pm 0.119$	+
13	$4.24\times10^3 \pm 0.090$	-	-	$2.12\times10^4 \pm 0.109$	$1.64\times10^4 \pm 0.127$	+
14	$2.40\times10^4 \pm 0.110$	$4.80\times10^3 \pm 0.090$	-	$7.80\times10^3 \pm 0.103$	$4.25\times10^3 \pm 0.088$	-
15	$2.48\times10^4 \pm 0.124$	-	-	$8.40\times10^3 \pm 0.101$	$3.60\times10^3 \pm 0.067$	-
16	$1.60\times10^2 \pm 0.002$	-	-	$4.40\times10^3 \pm 0.103$	$2.06\times10^3 \pm 0.088$	-
17	$4.08\times10^3 \pm 0.102$	$4.00\times10^3 \pm 0.004$	-	$2.70\times10^4 \pm 0.089$	$1.72\times10^4 \pm 0.122$	+
18	$1.61\times10^4 \pm 0.129$	$6.00\times10^3 \pm 0.004$	-	$2.49\times10^4 \pm 0.155$	$7.20\times10^3 \pm 0.102$	-
19	$2.32\times10^3 \pm 0.008$	$8.8\times10^3 \pm 0.009$	-	$1.90\times10^4 \pm 0.120$	$1.70\times10^4 \pm 0.142$	+
20	$1.88\times10^3 \pm 0.118$	$1.00\times10^3 \pm 0.101$	-	$2.64\times10^4 \pm 0.135$	$7.68\times10^3 \pm 0.121$	-
21	$8.2\times10^2 \pm 0.004$	$3.60\times10^2 \pm 0.010$	-	$2.30\times10^4 \pm 0.111$	$1.96\times10^4 \pm 0.102$	-
22	$3.8\times10^2 \pm 0.002$	$2.96\times10^2 \pm 0.020$	-	$2.13\times10^4 \pm 0.140$	$1.56\times10^4 \pm 0.103$	+
23	$6.16\times10^3 \pm 0.101$	$6.00\times10^3 \pm 0.095$	-	$2.93\times10^4 \pm 0.142$	$1.32\times10^4 \pm 0.130$	+
24	$6.24\times10^3 \pm 0.101$	$6.80\times10^3 \pm 0.090$	-	$4.80\times10^3 \pm 0.120$	$2.44\times10^3 \pm 0.100$	-
25	$4.46\times10^3 \pm 0.119$	$4.00\times10^3 \pm 0.103$	-	$1.16\times10^4 \pm 0.144$	$4.60\times10^3 \pm 0.102$	-
26	$1.80\times10^4 \pm 0.145$	$1.20\times10^4 \pm 0.141$	+	$6.00\times10^3 \pm 0.101$	$2.90\times10^3 \pm 0.090$	-
27	$1.28\times10^4 \pm 0.161$	$1.04\times10^4 \pm 0.152$	+	$2.88\times10^4 \pm 0.102$	$1.42\times10^4 \pm 0.130$	-
28	$7.20\times10^3 \pm 0.098$	$4.60\times10^3 \pm 0.082$	-	$8.60\times10^3 \pm 0.090$	$2.40\times10^3 \pm 0.098$	-
29	$2.60\times10^4 \pm 0.130$	$1.98\times10^4 \pm 0.100$	-	$6.00\times10^3 \pm 0.105$	$2.30\times10^3 \pm 0.108$	-
30	$9.60\times10^3 \pm 0.105$	$6.60\times10^3 \pm 0.133$	-	$1.61\times10^4 \pm 0.146$	$8.80\times10^3 \pm 0.110$	-
31	$1.40\times10^4 \pm 0.133$	$1.14\times10^4 \pm 0.120$	+	$2.04\times10^4 \pm 0.138$	$1.80\times10^4 \pm 0.143$	+
32	$1.80\times10^4 \pm 0.140$	$1.71\times10^4 \pm 0.138$	+	$2.06\times10^4 \pm 0.144$	$6.40\times10^3 \pm 0.111$	-
33	$4.6\times10^2 \pm 0.004$	$2.6\times10^2 \pm 0.007$	-	$2.20\times10^4 \pm 0.145$	$8.80\times10^3 \pm 0.123$	-
34	$1.96\times10^3 \pm 0.060$	$7.0\times10^2 \pm 0.060$	-	$2.52\times10^4 \pm 0.143$	$1.34\times10^4 \pm 0.120$	-
35	$9.6\times10^2 \pm 0.050$	$4.6\times10^2 \pm 0.070$	-	$4.40\times10^3 \pm 0.110$	$1.30\times10^3 \pm 0.100$	+
Control-1	0	0	-	0	0	-
Control-2	0	0	-	0	0	-
Control-3	0	0	-	0	0	-
Mean*	$9.92\times10^3 \pm 0.088$	$5.38\times10^3 \pm 0.076$	-	$1.76\times10^4 \pm 0.125$	$9.82\times10^3 \pm 0.103$	

Fungal positive (+); Fungal negative (-); Control mask= Unused or finely washed mask; Mean*= Mean TVC & TCC without control masks. The average bacterial load of Surgical and Cloth face masks

and 2.08×10^4 CFU/inch^2, respectively. Moreever, 22.85% of surgical masks and 37.14% of cloth masks were fungus-contaminated (Table 2).

The average bacterial load in both face masks is shown in Table 2. Most significantly, the bacteria in cloth face masks was higher than in surgical face masks. Statistical analysis also showed a significant (*P*- value 0.0369) association between them (Fig. 1).

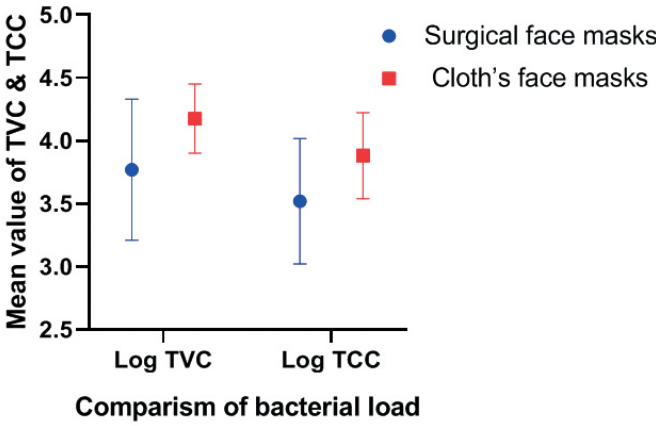

Fig. 1. Comparison of bacterial load in surgical and cloth face masks

Comparison between with or without washing of masks between uses

The microbial load in the face mask (used mask with washing between uses) was significantly high shown in Table 2. Still, the fresh surgical mask or finely washed face mask samples between uses were microbiologically safe (TCC-null). This study revealed that people use cloth face masks for a long time (consecutively even several months) without proper washing. So microbial quality of the used cloth mask is significantly (*P*- value 0.0369) less than the used (2/3 days) surgical mask.

Result of Biochemical test

Isolates were confirmed as *E. coli* (36%), *Pseudomonas* spp. (12%), and *Proteus* spp. (10%) based on morphological characteristics of different selected media and different standard Biochemical tests (Table 3). *E. coli* is a human pathogen responsible for hemolytic uremic syndrome (HUS) and different types of diarrhea and diarrhea-like diseases. But they do not affect lung infection (Nguyen and Sperandio, 2012)and approximately 75% of EHEC outbreaks are linked to the consumption of contaminated bovine-derived products. This review will discuss how EHEC causes disease in humans but is asymptomatic in adult ruminants. It will also analyze factors utilized by EHEC as it travels through the bovine gastrointestinal (GI. But it is a great concern that *P. aeruginosa* is an opportunistic pathogen responsible for lung infections and multi-decade bronchitis in cystic fibrosis-bearing patients. Besides, *P. aeruginosa* doesn't infect a healthy human lung, but when SARS-Cov2 infects the human lungs, a secondary infection may be created by *P. aeruginosa* (Williams *et al.*, 2010). Another study found that SARS-Cov2 emphasizes the growth of anaerobic bacteria and stimulates colonization inside the lungs. The presence of *P. mirabilis* (a facultative anaerobic bacteria) in the lungs may increase the cause of death during the Covid 19 pandemic (Chakraborty, 2020). Therefore, it is alarming that we confirmed the isolates as *E. coli, Pseudomonas* spp., and *Proteus* spp., which may cause different kinds of lung diseases along with *E. coli*-based diarrheal diseases.

Molecular identification of bacterial isolates

Biochemically verified *E. coli* was further confirmed through PCR analysis using LT, bfpA, aaiC, Stx1, and IpaH gene-specific primer for ETEC, EPEC, EAEC. EHEC, and EIEC where 2 isolates generated a single 300 bp (represent bfpA gene) amplified DNA band on 1.8% agarose gel. Thus the bacterial isolates were confirmed as enteropathogenic *Escherichia coli* (EPEC), respectively.

Table 3. Reaction of Biochemical test

	Biochemical reaction																	
		KIA			MIU													
Gram Staining	EMB plate	Slunt	Butt	Gas	Motility	Indole	Urease	Simon's Citrate	VP test	Oxidase	Catalase	Mannitol	Starch hydrolysis	Methyl Red	Glucose	Lactose fermentation test	Presumptive Bacteria
-Ve	+	A	A	+	+	+	+	-	-	-	+	A	-	+	A G	+	*E. coli*
-Ve	-	K	K	-	+	-	V	+	-	+	+	+	-	-	-	-	*Pseudomonas* spp.
-Ve	-	K	A	V	+	-	+	V	-	-	+	-	-	+	G	-	*Proteus* spp.

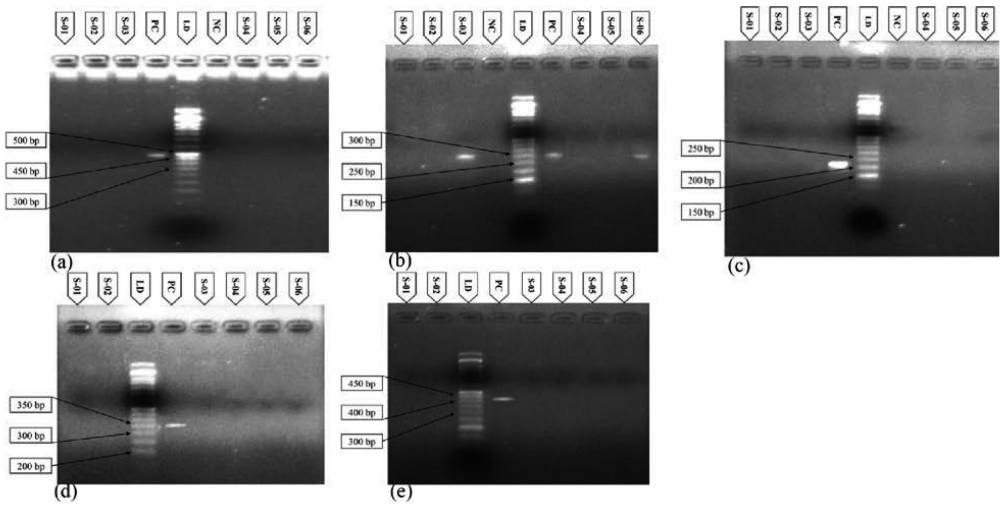

Fig. 2. Molecular identification of *Escherichia coli,* using LT, bfpA, aaiC, Stx1, and IpaH gene-specific primer for ETEC, EPEC, EAEC. EHEC, and EIEC.

PC: Positive Control; LD: Ladder; NC: Negative Control. (a) LT gene-specific PCR for ETEC: the result was negative, and no ETEC was found among the isolates; (b) bfpA gene-specific PCR for EPEC: two isolates showed a positive result for bfpA gene-specific PCR and thus confirmed enteropathogenic *Escherichia coli* presence among *E. coli* isolates; (c) aaiC, Stx1, and IpaH gene-specific primer for EAEC. EHEC, and EIEC: the result was negative, no EAEC. EHEC, and EIEC were found among the isolates.

Other *E. coli* bacterial species were not found in this molecular study (Fig. 2b).

Antibiotic resistance pattern

Antibiotic resistance is a threatening issue for modern antimicrobial medicine and treatment methods' key features. The current antibiotic treatment strategy is highly selective for specific microbes. However, the abuse or uncontrolled use of these antibiotics creates antibiotic-resistant microorganisms or microbes, making themselves more powerful against these antimicrobial agents. So it is more important to improve the treatment approach, and the use of existing antibiotics should be precise and well-regulated (McAdams *et al*., 2019). On the other hand, if we fail to control antibiotic-resistant microbes, more dangerous pathogenic strains may be generated, such as methicillin-resistant *Staphylococcus aureus* (Bootsma *et al*., 2006). In this study, *E. coli, Pseudomonas* spp., and *Proteus* spp. were undergone antibiogram experiment. Here we revealed that more than 80% of *E. coli* isolates were resistant to Tetracycline, Ciprofloxacin, and Ampicillin. *Pseudomonas* spp. were resistant to 100% of Ampicillin, and 86.67% of Chloramphenicol while 93.33% of *Proteus* spp. isolates were resistant to Miconazole. Antimicrobial resistance is a great threat to public health around the world. The face masks were contaminated with antibiotic-resistant pathogenic microbes which should be a great concern. The antibiogram experiment *of E. coli*, *Pseudomonas* spp., and *Proteus* spp. and their antibiotic susceptibility pattern are shown in the following statements.

Antibiotic susceptibility test of E. coli

In this study, the *E. coli* isolates were sensitive to Tetracycline (86.67%), Ciprofloxacin (80%), and Chloramphenicol (66.67%). But they were resistant to Ampicillin (80%), Miconazole (66.67%), and Erythromycin (66.67%) (Fig. 3). Many other studies described that *E. coli* are resistant to commonly used antibiotics. A previous study described that 100% of *E. coli* isolates were resistant to Clindamycin, Erythromycin, Penicillin, and Novobiocin. Additionally, most *E. coli* isolates began to show resistance spectrum patterns to Amoxicillin, Tetracycline, Streptomycin, and Sulfamethoxazole, respectively (Furtula *et al*., 2010).

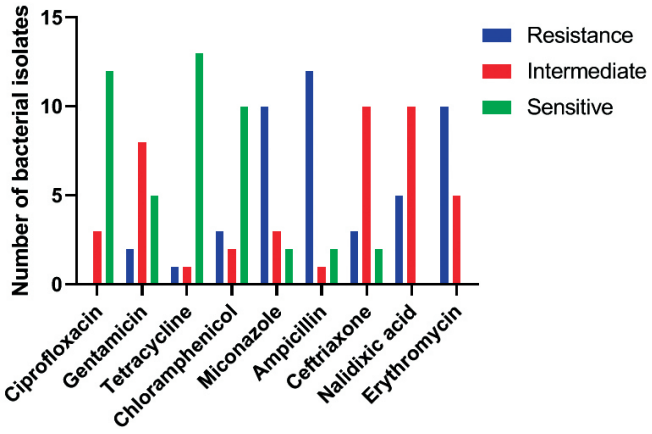

Fig. 3. Antibiotic susceptibility test of *E. coli*

Antibiotic susceptibility test of Pseudomonas spp.

Pseudomonas spp. were sensitive to Ceftriaxone (86.67%), Ciprofloxacin (66.67%), and Gentamicin (53.33%), but they were highly resistant to Ampicillin (100%), and Chloramphenicol

(86.67%). At the same time, *Pseudomonas* were immediately susceptible to Tetracycline (100%), Nalidixic acid (80%), and Erythromycin (66.67%), respectively (Fig. 4). Many other studies found that *Pseudomonas* spp. were mostly resistant to Amoxycillin (100%), Ampicillin (100%), Lincomycin (100%), Sulphamethoxazole (100%), and Erythromycin (80%) But they were sensitive to Doxycycline (100%), Nalidixic acid (100%), Chloramphenicol (60%), and Florfenicol (60%), respectively (Farid, 2013). Therefore, the result of the present study provides worthy information about diseases causing antibiotic-resistant *Pseudomonas* spp., and possible sept against them should be taken.

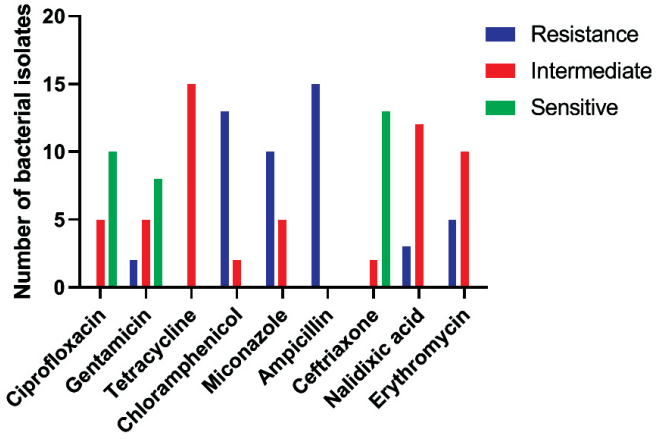

Fig. 4. Antibiotic susceptibility test of *Pseudomonas* spp.

Antibiotic susceptibility test of Proteus spp.

The antibiotic-resistant pattern of *Proteus* spp. is shown in Figure 5. *Proteus* spp. isolates were highly resistant to Miconazole (93.33%), and Ampicillin (66.67%), but they were sensitive to Ciprofloxacin (66.67%), Gentamicin (66.67%), Ceftriaxone (66.67%), and Tetracycline (60%). On the other hand, Chloramphenicol (73.33%), Nalidixic acid (66.67%), and Erythromycin (60%) had intermediate activity against *Proteus* spp. (Fig. 5). Previously described *P. mirabilis* isolates had variable degrees of antibiotic resistance towards different commercially used antibiotics. Such as Tetracyclin 100%, Erythromycin 100%, and Ampicillin 95%; Rifampicin 85%, Amoxicillin 70%, Cefotaxime, Gentamycin, and Piperacillin 60%, respectively

Face masks, including cloth masks, surgical masks, and N95 masks, provide essential protection against not only SARS-CoV-2 but also airborne infectious diseases (Brooks *et al*., 2021). However, repetitive usable masks are very susceptible to microbial (bacteria, viruses, and fungi) contamination. From medical grade to washable face masks, consumers want fashionable masks.

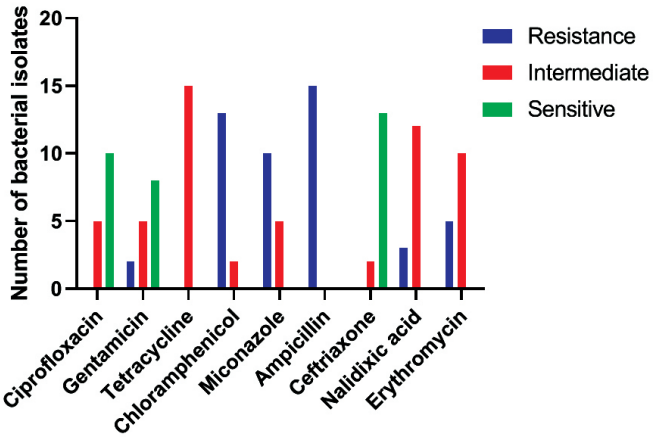

Fig. 5. Antibiotic susceptibility test of *Proteus* spp.

As masks come to face covering, consumers are more focused on fashionable masks rather than antimicrobial and protective face masks. The retailer also focused on public demand and choice. According to the latest fashion, they supply face masks in the market rather than upgrading some essential properties such as antimicrobial activity. A more protective filter system might be beneficial in reducing infectious diseases. This study focuses on microbial contamination of face masks, and the multiple-used masks showed a higher bacterial load that might influence respiratory infectious diseases. So people of all classes should be aware of using face masks carefully, and washable face wash carefully and regularly before use.

Anthropometric assessment

The questionnaire-based anthropometric assessment was performed among all 70 correspondents from whom we drew the samples. Among them, 71.42% were male, and 28.57% were female in different occupations. In this assessment, it was found that the mask-using time significantly (*P*-value 0.040) increased concerning different kinds of flu or flu-like disease conditions among the respondents (Table 4). Patients with different respiratory diseases use the same face masks for a long time (even several months for cloth masks) without or with minimal autoclaved. Hence, there is a great chance of microbial contamination of these masks, which may be responsible for secondary inflammations.

Conclusion

A face mask provides essential support against the expansion of COVID-19 and other airborne diseases. Although initially, it creates some problems, such as recognizing a person or speaking, we are now acclimatized. However, the continuous use of face masks may cause bacteria or

Table 4. General overview of the correspondents and correlations of different parameter

Gender	Occupation	Mask uses time * Flu like diseases Cross tabulation							
			Flu like diseases						
				None	1-2 times	3-4 times	5-7 times	More than 10 times	Patients of different respiratory diseases	*P*-value
Male (n=50)	Day labor (n=4) Businessman (n=28) Govt. job (n=15) Student (n=10)	Mask uses time	1-2 days	6	3	6	0	0	2	0.04
			3-4 days	5	6	6	1	0	0	
Female (n=20)	Housewife (n=11) Unemployed (n=2)		1 week	1	4	4	2	2	0	
			2 weeks	0	0	0	1	0	2	
			1 month	1	4	1	0	1	0	
			Several months	3	3	1	2	1	2	
Total N=70	N=70		Total	16	20	18	6	4	6	70

fungus contamination. The face mask may create diseases like respiratory tract infections when proper hygienic approaches are ignored. Alarmingly, the microbiological assessment of the generally used face masks was performed, and the result was anxiousness that these experimental reused masks had a significantly higher bacterial load (highest TVC, 2.93×10^4 and TCC, 2.08×10^4 CFU/inch^2). So we should be careful to use a reusable face mask, and an additional piece of advice is that one should carry 1-2 extra face masks and immediately wear a new mask if the old ones seem to be contaminated. After returning home, all the used reusable masks should be washed with an appropriate disinfectant and be used in the future.

Acknowledgement

We are thankful to Prof. Dr. Ashraf Hossain Talukder, Pl of the SLOs project, Dept. of the BGE, MBSTU, for his help by providing us necessary primers for the PCR.

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