

Excellent performance by avoiding microbial contamination (EPBAMC): A new portal and model for safety of respirator masks approved by bacterial contamination field research

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Article Info

Article type:
Research

Article History:

Received: 2022-01-05

Accepted: 2022-01-05

Published: 2022-02-01

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Keywords:

Safety

Effectiveness

Pandemic

Microbial Contamination

Respirator Masks

ABSTRACT

Introduction: Over the past decades, billions of people on Earth have used respirator masks to prevent animal-to-human and human-to-human virus transmission. Recent research has shown the low risk of surface transmission of COVID-19, which turned into a pandemic since January 2020. Social distancing and the use of masks indoors are the most important factors in breaking its transmission chain.

Material and Methods: However, the use of contaminated respirator masks can cause dangerous microbial and viral diseases. By adding the factor “avoiding microbial contamination”, the proposed model, called “Excellent Performance by Avoiding Microbial Contamination (EPBAMC)”, improves the WHO’s three-factor optimal-performance model of the respirator masks. In this study, to evaluate the need to add the factor of “avoiding contamination”, samples of brand-new respirator masks were collected from several countries and their microbial contamination was carefully studied. The research method was such that the research steps were performed with highest accuracy rate and no double infection was created.

Results: By culturing in sterilized medium, the bacterial load of the respirator masks was studied and the results were analyzed. By performing different cultures, a variety of pathogenic microorganisms were identified on half of the respirator mask samples. Some brand-new respirator mask samples contained more than one pathogen. A very important issue was that bacteria were found in brand-new respirators distributed by pharmacies that cause nosocomial infections and are resistant to antibiotics.

Conclusion: The results of this study made it necessary to review the standards of the production and distribution process and the procedures for controlling and inspecting respirator masks.

Cite this paper as:

Hashemi Taba N, Khatavakhotan AS. Excellent performance by avoiding microbial contamination (EPBAMC): A new portal and model for safety of respirator masks approved by bacterial contamination field research. *Front Health Inform.* 2022; 11: 103. DOI: [10.30699/fhi.v11i1.350](https://doi.org/10.30699/fhi.v11i1.350)

INTRODUCTION

With the outbreak of COVID-19 during 2020 and 2021, the use of respirator masks along with social distancing have become the most important measures to prevent the transmission of COVID-19. In addition to washing hands and observing a distance of one meter, as well as quarantining and tracking patients, The World Health Organization (WHO) considers the use of respirator masks as an important way to prevent the spread of SARS-CoV-2 virus, which causes COVID-19. By doing so, human-to-human transmission of the virus can be limited

and widespread vaccination can eradicate COVID-19.

Regardless of the type and extent of protection against the entry of the virus, the use of respirator masks has considerations such as how to use and how to dispose; but what has probably received less attention is the initial or double contamination of new respirator masks. Contamination of masks with respiratory agents and so-called airborne transmission is usually dangerous and important. Factors such as infection with tuberculosis and many viruses, as well as gastroenteritis, should all be considered.

The use of respirator masks is the most important tool to prevent the spread and involvement of people with respiratory diseases. Masks both prevent the spread of the virus by infected people and keep healthy people safe from infection. Using each other's masks or using masks inappropriately are all behavioral problems and can be solved through training; but if the new respirator masks are contaminated with microbes or viruses, users are unaware of the problem and this cannot be corrected with any behavioral etiquette. More specifically, contamination of brand-new respirator masks can make healthy people sick. Apart from the disease that occurred during the pandemic, this problem increases the risk of infection with the virus (for example, COVID-19) in those visiting medical centers and hospitals.

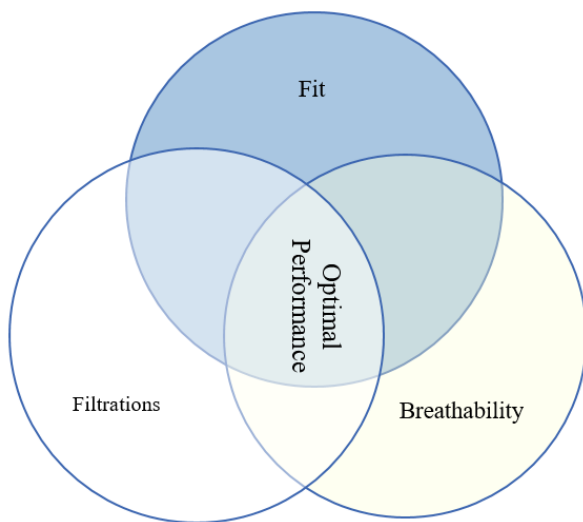


Fig 1: Essential parameters of filtration, breathability and fit

Studies conducted by academic researches and recommended by WHO show three criteria that are effective in the effectiveness of the respirator masks (Fig 1). As can be seen, the optimal performance of a respirator mask depends on three factors: the full fit of the mask on the face and the proper coverage, along with the possibility of comfortable breathing when using the mask and good filtering. Fit of masks currently is not defined by any standard except for the anthropometric considerations of facial dimensions (ISO/TS 16976-2). However, the face mask design improvement would be assumed as a good assistance in fighting with Covid-19 [1]. Table 1 shows the most important studies published in the articles of reputable scientific journals.

Suggested model

There are some optional parameters in addition to the essential performance parameters. The most important factor that is focused in this research is antimicrobial performance. The minimum threshold

for this performance criteria is elaborated by AATCC TM100 (for bacteria). According to Inhibition on microbial growth may take full effect after 2- or 24-hour contact time depending on the standard.

Fig 2 shows the proposed conceptual model of this study for the effectiveness of the respirator masks. In this model, which is called Excellent Performance By Avoiding Microbial Contamination (EPBAMC), the important criterion of “avoiding microbial contamination of respirator masks” has been added to the basic parameters.

The results of this study showed that another important factor, i.e. avoiding microbial contamination of the brand-new respirator masks, needs to be added to these three criteria. The three basic indicators of fit, breathability and proper filtration of bacteria and viruses require a precise definition of metrics and measurement methodologies, for which standards have been defined and presented, some of which are attached to this study.

Similarly, the proposed index of this research will require a series of complementary research to determine metrics and indicators as well as measurement methodology and comparison with standards. In order to implement this proposed conceptual model, it is necessary to define and explain the production process, especially the testing of masks, in detail. Static inspections and tests should cover walkthroughs and dynamic tests from raw material procurement to manufacturing and distribution. In this study, the main focus was on proving the need for this model and adding the fourth indicator of “avoiding microbial contamination”.

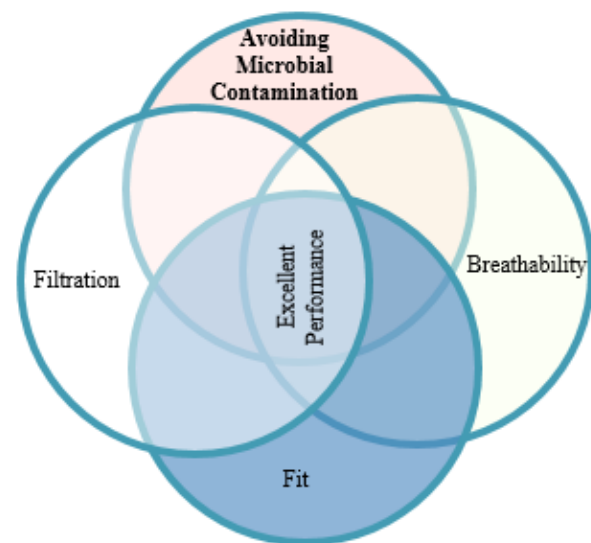


Fig 2: EPBAMC Excellent performance by avoiding microbial contamination

MATERIAL AND METHODS

The research method selected for this research is a mixed quantitative and qualitative research method. The systematic literature review conducted in this research shows that studying the microbial Contamination is mandatory. The various steps of the research method are summarized in Fig 3. The steps of conducting the research were as follows:

Table 1: Tabulated literature review

Seq.	Explanation	Ref.
1	Whether or not the respirator mask is used, infection prevention and control (IPC) metrics such as quarantine and isolation, testing, social distancing and tracking communications, etc. are very important and vital in preventing human-to-human transmission of the SARS-CoV-2 virus.	[2, 3]
2	Although the use of respiratory masks can be effective in preventing the transmission of the virus, the use of medical masks is essential for anyone with symptoms of COVID-19.	[2]
3	The results of many studies show that the highest rate of human-to-human transmission occurs through the close contact of an infected person with other people. Obviously, the amount of virus transmitted due to person to person contact depends on the type of they contact they make. The virus can be spread through the mouth and nose of carriers when they sneeze, cough, sing, talk, or even breathe deeply, and can be transmitted to nearby people. Studies have shown that a distance of less than one meter can transmit the virus through the mouth, nose and even eyes.	[4, 5]
4	Recent studies have shown that the virus transmission through objects such as medical equipment or furniture rarely occurs. 14-17	[6]
5	The use of respirator masks is very important because studies have shown that those who have no symptoms can be carriers. 25, 29-37	[7]
6	About 96 studies conducted on people with SARS-CoV-2 have shown that one in four or five people do not show any symptoms during the illness or being a carrier. 28 -29-30	[8]
7	According to the WHO recommendation, the use of various masks, both medical and non-medical masks, can prevent the spread of the virus. Obviously, those with positive test result for SARS-CoV-2 should wear a medical mask for 10 days after the test is positive. 119-120	[9, 10]
8	Depending on the type of respirator mask used, side effects such as headache and shortness of breath have been reported. 55 Using the mask for long hours can cause skin allergies and other side effects. 58-59-127	[11, 12]
9	Of course, respirator masks have other minor and major side effects, such as difficulty of use and communication problems with those who have hearing problems and understand speech through lip reading. 128- 129- 44- 59	[13, 14]
10	Research has shown that the use of masks by healthy people alone does not reduce the prevalence in society, unless sick and suspicious people wear respirator masks. 76	[15]
11	Gatherings and expanded bubbles are important reasons for the prevalence of COVID-19. Extensive research conducted in Beijing, China has shown that if family members wear respirator masks, the social transmission rate is reduced by about 80%.	[16]
12	Used masks can cause many diseases if their eradication or are not properly controlled.	[17]

Training session for students preparing samples

During the virtual sessions to comply with health

protocols and evidence recording standards, students participating in the test were introduced to the steps.

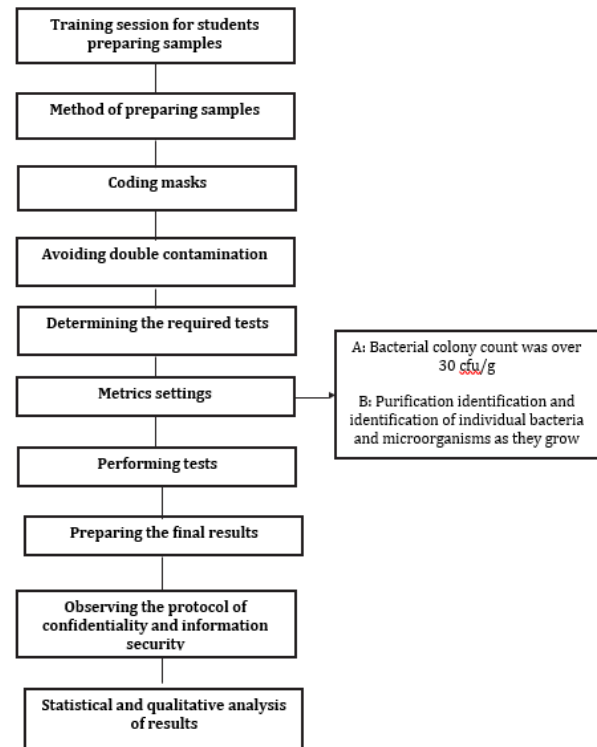


Fig 3: Research conducting steps

Method of preparing samples

Each student participating in the study got three to five samples from official shops and pharmacies. An essential condition for the sample masks was their complete packaging. Packaging of masks will not be opened until delivery to the lab and opening under the hood. As an evidence, photographs were taken from all locations and samples of masks. Fig 4 shows some examples of the respirator masks.

The photos of the samples were from all aspects of the box. The location of the preparation place was also captured and saved with GPS (Fig 5). Regarding these etiquettes, samples of new respirator masks were prepared.

Coding masks.

All masks were coded to protect the confidentiality of information and identity of persons. Each of the sample respirator masks was given a unique code according to the specified standard (manufacturer's name and serial number of the prepared respirator mask). Therefore, the code of each mask stands for the name of its manufacturer along with a one-digit series. All research operations, tests, results and reports were done with the same codes and no one but the researcher was aware of this information.

Avoiding double contamination.

Fig 5 shows sterilized nylons for carrying mask samples. Four hundred sterilized nylons were prepared to carry and test the masks. Laboratory and R&D department of a hospital sterilized and delivered 400 nylons. This was done to ensure that the samples were not contaminated during storage and transportation. Finally, the nylons were given to a laboratory for the diagnosis of medical infections. Fig 4 shows sterilized nylons for carrying mask samples.



Fig 4: Sample Masks

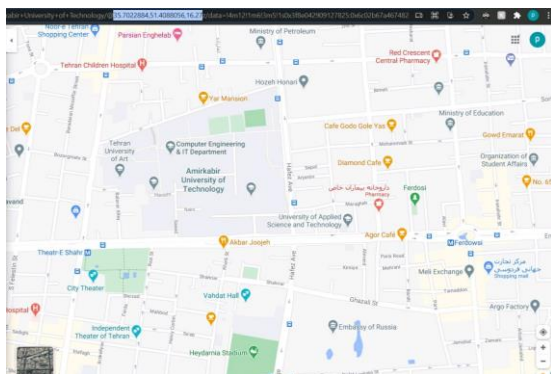


Fig 5: Sample mask location

Determining the required tests.

During meetings with researchers, physicians and experts of microbiology, the required tests on the respirator mask samples were determined according to ISO and WHO standards. General control indicators of respirator masks according to ISO 10993-1: 2018 can be seen in Fig 6. As can be seen in the standard metrics, the last row indicates less than 30cfu/g.

Metrics settings

The process of colony count and determination of the microbial load per gram of mask, was designed as follows and performed in two steps:

A: The test was performed as a primary culture to determine the total colony count and each type of bacteria in the case of count growth indicates the growth rate and number of colonies of each sample per gram. In all samples, bacterial colony count was below 30 cfu/g.

B: Purification identification and identification of individual bacteria and microorganisms as they grow.

Test	Type I ^a	Type II	Type IIR
Bacterial filtration efficiency (BFE), (%)	≥ 95	≥ 98	≥ 98
Differential pressure (Pa/cm ²)	< 40	< 40	< 60
Splash resistance pressure (kPa)	Not required	Not required	≥ 16,0
Microbial cleanliness (cfu/g)	≤ 30	≤ 30	≤ 30

^a Type I medical face masks should only be used for patients and other persons to reduce the risk of spread of infections particularly in epidemic or pandemic situations. Type I masks are not intended for use by healthcare professionals in an operating room or in other medical settings with similar requirements.

Fig 6: Performance requirements for medical face masks [18]

Performing tests

The required tests and cultures were performed over a period of three months. Each sample of respirator masks was opened under the hood and the tests were performed carefully in a clean room to ensure the absence of double contamination during testing and culture.

Preparing the final results

The final results related to the tests and output of the devices as well as the results of the cultures were prepared in the form of detailed and statistical reports.

Observing the protocol of confidentiality and information security

Because each of the sample producers was aware of the sample code and its specifications, another serial coding step was performed so that no one would be aware of the relationship between the results and the respirator mask sample. In this way, those who were in the laboratory were aware of the primary code and had no other information, and those who provided the sample masks saw the results with the secondary code.

Statistical and qualitative analysis of results.

The results of tests and cultures were then analyzed. The biomedical test data showed the pathogens found on samples. The diseases caused by microorganisms and their treatment options have been reviewed.

Limitations

This research has faced several limitations. The legal ban on the export of respirator masks from countries to control proper distribution has made it difficult to access a variety of respirator masks. The variety of respirator masks, i.e. home-made masks, medical masks and fabric masks, has also made it difficult to compare. Other limitations are the time limitation for preparing masks and the limitation of preparing masks.

Delimitation

For a variety of reasons, researchers have imposed restrictions on various phases and activities of research. Limiting the number of samples to 65 varieties is one of the restrictions. There are also a number of time constraints for the research to be completed over an 8-month period for the results to be effective in the COVID-19 pandemic period. All samples of masks were divided into sizes of one square centimeter for high accuracy of testing operations and covering the entire surface of the mask. Bacterial testing and culture of each of these sections has been costly and time consuming. Only fully packaged masks have been selected as a sample to distinguish manufacturing contaminants from possible secondary contaminants due to improper transportation or improper supply.

Research time

The research lasted from October 2020 to September 2021. It took three months to prepare the respirator masks, two months to develop the FMA (Face Mask Analytics) portal, three months to test the masks, and three months to analyze the data obtained from the tests and devices. Some activities, such as the preparation of sample masks and the FMA portal of mask samples' information, were performed simultaneously.

The production place of sample respirator masks

Most of the masks were purchased from pharmacies and stores or shops in Iran. Some of the masks were made in New Zealand, Japan, and the United Arab Emirates, from where they arrived with passengers.

Microbiological test of masks

Specialized and consulting meetings were held with the presence of specialists of the Peyvand laboratory, which is one of the most prestigious medical diagnostic laboratories. During this meeting, the research plan was explained and the desired tests were determined. A contract was also signed, based on which the laboratory undertook to observe all the

principles and requirements of the research method in testing the samples, including the requirements that all samples are required to be delivered to the laboratory along with sterilized nylons, and the experts and technicians are required to open each sample under the hood and divide it into small pieces for testing. The tests should also be performed in a clean room environment and the researchers should fulfill their financial obligations.

Respirator masks information portal

A portal was developed to store sample information. Information about the manufacturer, brand and place of purchase was entered in the database of this portal. To ensure the accuracy of the information, data were entered twice and inconsistencies were checked. Fig 7 shows a snapshot of a portal designed to record test information. The exact specifications of the masks, including the brand and license of the manufacturer or producer, as well as the information of the purchase location, along with photos of all six sides of the respirator mask boxes and the masks themselves were taken and stored.

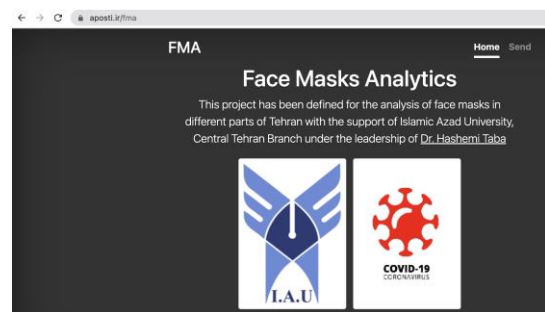


Fig 7: Snapshot of the sample's information website

RESULTS

Statistical analysis of the obtained results

The first finding was that some samples of respirator masks contained more than one microorganism as a pathogen. Tables 2 to 15 are examples of the tables related to the types of biochemical tests of masks in which pathogenesis has been detected. These tests were performed by the related device to detect the phenotype of the bacterium, each of which has been abbreviated. Negative cases mean that the result of the biochemistry test is negative and positive means that the test result is positive. Finally, according to the positive and negative results obtained by the device and their comparison with the database, bacteria were diagnosed and reported in terms of genus and species and sometimes strain.

Table 2: Biochemical tests of AMF02 sample

Identification Information																	
Sample Code	Selected Organism					Analysis Time	Probability			Bionumber							
AMF02	Acinetobacter Lwoffii					9.75 hours	98%			0040000100000002							
Biochemical Details																	
2	APPA	-	3	ADO	-	4	PyrA	-	5	lARL	-	7	dCEL	-	9	BGAL	-
10	H2S	-	11	BNAG	-	12	AGLTp	+	13	dGLU	-	14	GGT	-	15	OFF	-
17	BGLU	-	18	dMAL	-	19	dMAN	-	20	dMNE	-	21	BXYL	-	22	BAlap	-
23	ProA	-	26	LIP	-	27	PLE	-	29	TyrA	+	31	URE	-	32	dSOR	-
33	SAC	-	34	dTAG	-	35	dTRE	-	36	CIT	-	37	MNT	-	39	5KG	-
40	lLATk	-	41	AGLU	-	42	SUCT	-	43	NAGA	-	44	AGAL	-	45	PHOS	-
46	GlyA	-	47	ODC	-	48	LDC	-	53	lHISa	-	56	CMT	-	57	BGUR	-
58	O129R	-	59	GGAA	-	61	lMLTa	-	62	ELLM	-	64	lLATa	+	-	-	-

Table 3: Biochemical tests of SAR02sample

Identification Information																	
Sample Code	Selected Organism					Analysis Time	Probability			Bionumber							
SAR02	Staphylococcus hominis ssp hominis					6.00hours	96%			000002012320231							
Biochemical Details																	
2	AMY	-	4	PIPLC	-	5	dXYL	-	8	ADH1	-	9	BGAL	-	11	AGLU	-
13	APPA	-	14	CDEX	-	15	AspA	-	16	BGAR	-	17	AMAN	-	19	PHOS	-
20	LeuA	-	23	ProA	-	24	BGURr	-	25	AGAL	-	26	PyrA	+	27	BGUR	-
28	AlaA	-	29	TyrA	-	30	dSOR	-	31	URE	+	32	POLYB	-	37	dGAL	-
38	dRIB	-	39	lLATk	+	42	LAC	-	44	NAG	+	45	dMAL	+	46	BACI	-
47	NOVO	-	50	NC6.5	+	52	dMAN	-	53	dMNE	-	54	MBdG	-	56	PUL	-
57	dRAF	-	58	O129R	+	59	SAL	-	60	SAC	+	62	dTRE	+	63	ADH2s	-
64	OPTO	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 4: Biochemical tests of ADP05 sample

Identification Information																	
Sample Code	Selected Organism					Analysis Time	Probability			Bionumber							
ADP05	Staphylococcus hominis ssp hominis					6.00 hours	88%			070002246220221							
Biochemical Details																	
2	AMY	-	4	PIPLC	-	5	dXYL	-	8	ADH1	+	9	BGAL	+	11	AGLU	+
13	APPA	-	14	CDEX	-	15	AspA	-	16	BGAR	-	17	AMAN	-	19	PHOS	-
20	LeuA	-	23	ProA	-	24	BGURr	-	25	AGAL	-	26	PyrA	+	27	BGUR	-
28	AlaA	-	29	TyrA	+	30	dSOR	-	31	URE	-	32	POLYB	-	37	dGAL	+
38	dRIB	-	39	lLATk	+	42	LAC	+	44	NAG	-	45	dMAL	+	46	BACI	-
47	NOVO	(-)	50	NC6.5	+	52	dMAN	-	53	dMNE	-	54	MBdG	-	56	PUL	-
57	dRAF	-	58	O129R	+	59	SAL	-	60	SAC	-	62	dTRE	+	63	ADH2s	-
64	OPTO	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 5: Biochemical tests of SAR02 sample

Identification Information																	
Sample Code	Selected Organism				Analysis Time	Probability				Bionumber							
SAR02	Sphingomonas paucimobilis				5.00 hours	92%				1211335150200200							
Biochemical Details																	
2	APPA	+	3	ADO	-	4	PyrA	-	5	lARL	-	7	dCEL	+	9	BGAL	-
10	H2S	+	11	BNAG	-	12	AGLTp	-	13	dGLU	+	14	GGT	-	15	OFF	-
17	BGLU	+	18	dMAL	+	19	dMAN	-	20	dMNE	+	21	BXYL	+	22	BAlap	-
23	ProA	+	26	LIP	-	27	PLE	+	29	TyrA	+	31	URE	-	32	dSOR	-
33	SAC	+	34	dTAG	-	35	dTRE	+	36	CIT	-	37	MNT	-	39	5KG	-
40	lLATk	-	41	AGLU	+	42	SUCT	-	43	NAGA	-	44	AGAL	-	45	PHOS	-
46	GlyA	-	47	ODC	-	48	LDC	-	53	lHISa	-	56	CMT	+	57	BGUR	-
58	O129R	-	59	GGAA	-	61	lMLTa	-	62	ELLM	-	64	lLATa	-	-	-	-

Table 6: Biochemical tests of ADP03 sample

Identification Information																	
Sample Code	Selected Organism				Analysis Time	Probability				Bionumber							
ADP03	Sphingomonas paucimobilis				8.00 hours	85%				4201610550000200							
Biochemical Details																	
2	APPA	-	3	ADO	-	4	PyrA	+	5	lARL	-	7	dCEL	+	9	BGAL	-
10	H2S	-	11	BNAG	-	12	AGLTp	-	13	dGLU	+	14	GGT	-	15	OFF	-
17	BGLU	-	18	dMAL	+	19	dMAN	+	20	dMNE	+	21	BXYL	-	22	BAlap	-
23	ProA	-	26	LIP	-	27	PLE	(-)	29	TyrA	+	31	URE	-	32	dSOR	+
33	SAC	+	34	dTAG	-	35	dTRE	+	36	CIT	-	37	MNT	-	39	5KG	-
40	lLATk	-	41	AGLU	-	42	SUCT	-	43	NAGA	-	44	AGAL	-	45	PHOS	-
46	GlyA	-	47	ODC	-	48	LDC	-	53	lHISa	-	56	CMT	+	57	BGUR	-
58	O129R	-	59	GGAA	-	61	lMLTa	-	62	ELLM	-	64	lLATa	-	-	-	-

Table 7: Biochemical tests of AMH02 sample

Identification Information																	
Sample Code	Selected Organism				Analysis Time	Probability				Bionumber							
AMH02	Sphingomonas paucimobilis				5.00 hours	93%				0400130100220600							
Biochemical Details																	
2	APPA	-	3	ADO	-	4	PyrA	-	5	lARL	-	7	dCEL	-	9	BGAL	+
10	H2S	-	11	BNAG	-	12	AGLTp	-	13	dGLU	-	14	GGT	-	15	OFF	-
17	BGLU	+	18	dMAL	-	19	dMAN	-	20	dMNE	+	21	BXYL	+	22	BAlap	-
23	ProA	-	26	LIP	-	27	PLE	-	29	TyrA	+	31	URE	-	32	dSOR	-
33	SAC	-	34	dTAG	-	35	dTRE	-	36	CIT	-	37	MNT	-	39	5KG	-
40	lLATk	-	41	AGLU	+	42	SUCT	-	43	NAGA	-	44	AGAL	+	45	PHOS	-
46	GlyA	-	47	ODC	-	48	LDC	-	53	lHISa	-	56	CMT	+	57	BGUR	+
58	O129R	-	59	GGAA	-	61	lMLTa	-	62	ELLM	-	64	lLATa	-	-	-	-

Table 8: Biochemical tests of ADP05 sample

Identification Information																	
Sample Code	Selected Organism					Analysis Time	Probability				Bionumber						
ADP05	Sphingomonas paucimobilis					7.00 hours	93%				0601711150020200						
Biochemical Details																	
2	APPA	-	3	ADO	(-)	4	PyrA	-	5	lARL	-	7	dCEL	+	9	BGAL	+
10	H2S	-	11	BNAG	-	12	AGLTp	-	13	dGLU	+	14	GGT	-	15	OFF	-
17	BGLU	(+)	18	dMAL	+	19	dMAN	+	20	dMNE	+	21	BXYL	-	22	BAlap	-
23	ProA	+	26	LIP	-	27	PLE	-	29	TyrA	+	31	URE	-	32	dSOR	-
33	SAC	+	34	dTAG	-	35	dTRE	+	36	CIT	-	37	MNT	-	39	5KG	-
40	lLATk	-	41	AGLU	-	42	SUCT	-	43	NAGA	-	44	AGAL	+	45	PHOS	-
46	GlyA	-	47	ODC	-	48	LDC	-	53	lHISa	-	56	CMT	+	57	BGUR	-
58	O129R	-	59	GGAA	-	61	lMLTa	-	62	ELLM	-	64	lLATa	-	-	-	-

Table 9: Biochemical tests of AMH02 sample

Identification Information																	
Sample Code	Selected Organism					Analysis Time	Probability				Bionumber						
AMH02	Cronobacter sakazakii group					4.00 hours	95%				0631734150220401						
Biochemical Details																	
2	APPA	-	3	ADO	-	4	PyrA	-	5	lARL	-	7	dCEL	+	9	BGAL	+
10	H2S	+	11	BNAG	+	12	AGLTp	-	13	dGLU	+	14	GGT	-	15	OFF	-
17	BGLU	+	18	dMAL	+	19	dMAN	+	20	dMNE	+	21	BXYL	+	22	BAlap	-
23	ProA	-	26	LIP	-	27	PLE	+	29	TyrA	+	31	URE	-	32	dSOR	-
33	SAC	+	34	dTAG	-	35	dTRE	+	36	CIT	-	37	MNT	-	39	5KG	-
40	lLATk	-	41	AGLU	+	42	SUCT	-	43	NAGA	-	44	AGAL	+	45	PHOS	-
46	GlyA	-	47	ODC	-	48	LDC	-	53	lHISa	-	56	CMT	-	57	BGUR	+
58	O129R	-	59	GGAA	-	61	lMLTa	-	62	ELLM	+	64	lLATa	-	-	-	-

Table 10: Biochemical tests of ADP01 sample

Identification Information																	
Sample Code	Selected Organism					Analysis Time	Probability				Bionumber						
ADP01	Pantoea spp					5.00 hours	93%				0601530171130000						
Biochemical Details																	
2	APPA	-	3	ADO	-	4	PyrA	-	5	lARL	-	7	dCEL	+	9	BGAL	+
10	H2S	-	11	BNAG	-	12	AGLTp	-	13	dGLU	+	14	GGT	-	15	OFF	-
17	BGLU	+	18	dMAL	-	19	dMAN	+	20	dMNE	+	21	BXYL	+	22	BAlap	-
23	ProA	-	26	LIP	-	27	PLE	-	29	TyrA	+	31	URE	-	32	dSOR	-
33	SAC	+	34	dTAG	+	35	dTRE	+	36	CIT	+	37	MNT	-	39	5KG	-
40	lLATk	+	41	AGLU	-	42	SUCT	-	43	NAGA	+	44	AGAL	+	45	PHOS	-
46	GlyA	-	47	ODC	-	48	LDC	-	53	lHISa	-	56	CMT	-	57	BGUR	-
58	O129R	-	59	GGAA	-	61	lMLTa	-	62	ELLM	-	64	lLATa	-	-	-	-

Table 11: Biochemical tests of ADP04 sample

Identification Information																	
Sample Code	Selected Organism					Analysis Time	Probability					Bionumber					
ADP04	Pantoea spp					5.00 hours	93%					0601730171101010					
Biochemical Details																	
2	APPA	-	3	ADO	-	4	PyrA	-	5	IARL	-	7	dCEL	+	9	BGAL	+
10	H2S	-	11	BNAG	-	12	AGLTp	-	13	dGLU	+	14	GGT	-	15	OFF	-
17	BGLU	+	18	dMAL	+	19	dMAN	+	20	dMNE	+	21	BXYL	+	22	Balap	-
23	ProA	-	26	LIP	-	27	PLE	-	29	TyrA	+	31	URE	-	32	dSOR	-
33	SAC	+	34	dTAG	+	35	dTRE	+	36	CIT	+	37	MNT	-	39	5KG	-
40	ILATk	+	41	AGLU	-	42	SUCT	-	43	NAGA	-	44	AGAL	-	45	PHOS	-
46	GlyA	+	47	ODC	-	48	LDC	-	53	IHISa	-	56	CMT	-	57	BGUR	-
58	O129R	+	59	GGAA	-	61	IMLTa	-	62	ELLM	(-)	64	ILATa	-	-	-	-

Table 12: Biochemical tests of AMT01 sample

Identification Information																	
Sample Code	Selected Organism					Analysis Time	Probability					Bionumber					
AMT01	Pseudomonas luteola					5.00 hours	99%					4401331141500210					
Biochemical Details																	
2	APPA	-	3	ADO	-	4	PyrA	+	5	IARL	-	7	dCEL	-	9	BGAL	+
10	H2S	-	11	BNAG	-	12	AGLTp	-	13	dGLU	+	14	GGT	-	15	OFF	-
17	BGLU	+	18	dMAL	+	19	dMAN	-	20	dMNE	+	21	BXYL	(+)	22	Balap	-
23	ProA	+	26	LIP	-	27	PLE	-	29	TyrA	+	31	URE	-	32	dSOR	-
33	SAC	-	34	dTAG	-	35	dTRE	+	36	CIT	+	37	MNT	-	39	5KG	-
40	ILATk	+	41	AGLU	-	42	SUCT	+	43	NAGA	-	44	AGAL	-	45	PHOS	-
46	GlyA	-	47	ODC	-	48	LDC	-	53	IHISa	-	56	CMT	+	57	BGUR	-
58	O129R	+	59	GGAA	-	61	IMLTa	-	62	ELLM	-	64	ILATa	-	-	-	-

Table 13: Biochemical tests of AMF01 sample

Identification Information																	
Sample Code	Selected Organism					Analysis Time	Probability					Bionumber					
AMF01	Staphylococcus epidermidis					5.00 hours	99%					030000076620211					
Biochemical Details																	
2	AMY	-	4	PIPLC	-	5	dXYL	-	8	ADH1	+	9	BGAL	+	11	AGLU	-
13	APPA	-	14	CDEX	-	15	AspA	-	16	BGAR	-	17	AMAN	-	19	PHOS	(-)
20	LeuA	-	23	ProA	-	24	BGURr	-	25	AGAL	-	26	PyrA	-	27	BGUR	-
28	AlaA	-	29	TyrA	-	30	dSOR	-	31	URE	+	32	POLYB	+	37	dGAL	+
38	dRIB	-	39	ILATk	+	42	LAC	+	44	NAG	-	45	dMAL	+	46	BACI	+
47	NOVO	-	50	NC6.5	+	52	dMAN	-	53	dMNE	-	54	MBdG	-	56	PUL	-
57	dRAF	-	58	O129R	+	59	SAL	-	60	SAC	+	62	dTRE	-	63	ADH2s	-
64	OPTO	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 14: Biochemical tests of AMF01 sample

Identification Information																	
Sample Code	Selected Organism					Analysis Time	Probability			Bionumber							
AMF01	Rhizobium radiobacter					4.00 hours	94%			0611715570200200							
Biochemical Details																	
2	APPA	-	3	ADO	-	4	PyrA	-	5	IARL	-	7	dCEL	+	9	BGAL	+
10	H2S	+	11	BNAG	-	12	AGLTp	-	13	dGLU	+	14	GGT	-	15	OFF	-
17	BGLU	+	18	dMAL	+	19	dMAN	+	20	dMNE	+	21	BXYL	-	22	Balap	-
23	ProA	+	26	LIP	-	27	PLE	+	29	TyrA	+	31	URE	-	32	dSOR	+
33	SAC	+	34	dTAG	+	35	dTRE	+	36	CIT	-	37	MNT	-	39	5KG	-
40	ILATk	-	41	AGLU	+	42	SUCT	-	43	NAGA	-	44	AGAL	-	45	PHOS	-
46	GlyA	-	47	ODC	-	48	LDC	-	53	IHISa	-	56	CMT	+	57	BGUR	-
58	O129R	-	59	GGAA	-	61	IMLTa	-	62	ELLM	-	64	ILATa	-	-	-	-

Table 15: Biochemical tests of ADP07 sample

Identification Information																	
Sample Code	Selected Organism					Analysis Time	Probability			Bionumber							
ADP07	Pasteurella testudinis					10.25 hours	86%			0601700550120200							
Biochemical Details																	
2	APPA	-	3	ADO	-	4	PyrA	-	5	IARL	-	7	dCEL	+	9	BGAL	+
10	H2S	-	11	BNAG	-	12	AGLTp	-	13	dGLU	+	14	GGT	-	15	OFF	-
17	BGLU	+	18	dMAL	+	19	dMAN	+	20	dMNE	-	21	BXYL	-	22	Balap	-
23	ProA	-	26	LIP	-	27	PLE	-	29	TyrA	+	31	URE	-	32	dSOR	+
33	SAC	+	34	dTAG	-	35	dTRE	+	36	CIT	-	37	MNT	-	39	5KG	-
40	ILATk	+	41	AGLU	-	42	SUCT	-	43	NAGA	-	44	AGAL	+	45	PHOS	-
46	GlyA	-	47	ODC	-	48	LDC	-	53	IHISa	(-)	56	CMT	+	57	BGUR	-
58	O129R	-	59	GGAA	-	61	IMLTa	(-)	62	ELLM	-	64	ILATa	-	-	-	-

As mentioned in the metric explanation in paragraph three of the research method, the total count should be less than 30, except for two cases where no growth of bacteria was observed and were so-called negative. In 63 samples, i.e. about 97%, bacterial growth was observed; and therefore, they were considered contaminated. Of course, being sterilized was not a requirement for respirator masks. Therefore, culture test was performed for those with bacterial growth.

Out of 65 samples of cultured respirator masks, 33 samples did not show bacterial growth after 48 hours; but of the 63 samples of masks with bacterial growth, 30 samples, or nearly 48%, contained a variety of bacteria and pathogens. It is noteworthy that out of 65 samples of masks, only 9 samples, i.e. less than 14%, had gram negative bacilli.

Table 16 shows the types of micro-organisms seen after culture on the sample respirator masks. In this table, the first column shows the sample number, the second shows the sample mask code, and the third one shows the type of contamination and pathogen

identified.

Accurate analysis of pathogens

Some mask samples contained more than one pathogen. For example, the SAR02 mask carried both Staphylococcus hominis and Sphingomonas paucimobilis. The ADP05 mask sample also carried the pathogen Pantoea spp along with the bacterium Staphylococcus hominis. Rhizobium radiobacter and Staphylococcus epidermidis were also detected on the AMF01 mask sample with 94% and 99% probabilities, respectively.

The AMH02 mask sample also carried Staphylococcus hominis at the probability rate of 93% and was infected with the pathogenic bacterium Cronobacter sakazakii group. Finally, the AMF01 mask sample was both 99% more likely to carry Staphylococcus epidermidis and 94% more likely to be infected with the Rhizobium radiobacter.

Description of detected pathogens

In this section, the pathogens detected on tested respirator mask samples are described in detail. Specifically, for each sample mask, diseases related to the identified microorganisms are described. Some treatments and medications are also briefly listed for some possible pathogen-related diseases.

Acinetobacter pylori.

Acinetobacter pylori microorganism found on the AMF02 three-dimensional respirator masks is one of the most important causes of nosocomial infections that can cause infection of the blood, lungs or urinary

tract [19, 20]. This bacterium causes nosocomial infections [21]. It is highly resistant to almost all types of antibiotics, including a powerful group called Carbapenems, and is resistant to treatment [22].

Staphylococcus hominis.

The bacterium Staphylococcus hominis, which is found on SAR02 and ADP05 respirator masks with respective 96% and 88% rate of probability and produces biosurfactants [23]. Biosurfactants are biologically active surface molecules used to control larvae, beetles and pests [24].

Table 16: Types of micro-organisms seen on sample respirator masks

Line	Code	Direct smear by gram staining	Colony count CFU/g	Final result
1	STD01	Gram positive bacilli like to <i>Bacillus</i> species.	< 30	No pathogenic bacteria growth After 48 hours.
2	AMH03	Gram positive <i>filamentous</i> bacilli liketo <i>Bacillus</i> species.	< 30	No pathogenic bacteria growth after 48 hours.
3	M2R01	Gram positive bacilli like to <i>Bacillus</i> species. Gram positive <i>filamentous</i> bacilli like to <i>Bacillus</i> species.	< 30	No pathogenic bacteria growth after 48 hours.
4	PMD03	Gram Negative Bacilli	< 30	Gram positive bacilli like to <i>Bacillus</i> species.
5	SAR02	1- Gram positive cocci & diplococci 2- Gram negative bacilli 3-Gram positive bacilli like to <i>Bacillus</i> species.	< 30	I: <i>Staphylococcus hominis</i> II: <i>Sphingomonas paucimobilis</i>
6	PMD02	Gram variable bacilli like to <i>Bacillus</i> species.	< 30	No pathogenic bacteria growth after 48 hours.
7	AL705	Gram positive bacilli like to <i>Bacillus</i> species.	< 30	No pathogenic bacteria growth after 48 hours.
8	NF201	Gram positive bacilli like to <i>Bacillus</i> species.	< 30	No pathogenic bacteria growth after 48 hours.
9	SMN01	Gram positive bacilli like to <i>Bacillus</i> species.	< 30	No pathogenic bacteria growth after 48 hours.
10	ADP01	Gram negative bacilli	< 30	<i>Pantoea species</i>
11	AMH02	Gram negative bacilli	< 30	I: <i>Cronobacter sakazakii</i> group II: <i>Sphingomonas paucimobilis</i>

Sphingomonas paucimobilis

According to Yabuuchi the bacterium Sphingomonas paucimobilis, carried on SAR02, ADP03, ADP05, and AMH02 sample masks [25], is an alpha class of bacterial proteins which is involved in various types of clinical infections [26]. Although studies have shown that this opportunistic pathogen is less likely to cause death, it causes bloodstream infections, bacteremia and sepsis, and can be very dangerous in

patients with weakened immune systems or infants [24]. Especially, since over the past decades, this microorganism has shown that it is resistant to many antibiotics such as Ampicillin, Cephalothin, and Streptomycin. According to Devenci et al. study, an uncommon cause of Meningitis could be assumed as the sideeffect of the aforementioned pathogen [27].

Cronobacter sakazakii

On AMH02 respirator mask sample, the Cronobacter

sakazakii pathogen was detected with a very high probability of 95%. This microorganism can cause various diseases including meningitis, bacteremia, sepsis and enterocolitis. In adults, it can also cause infections, including septicemia, pneumonia, osteomyelitis, splenic abscess, and wound infection. Its mortality rate (40-80%) is high and survivors suffer from severe neurological complications [7].

Pantoea stewartii subsp

On ADP01 and ADP04 respirator mask samples, the *Pantoea stewartii* subsp pathogen was diagnosed with a high probability of 93%. This bacterium, which is considered a serious agricultural pest, is emphasized in the quarantine of many countries [28]. The main host of this bacterium is sweet corn. It should be noted that improper return of used respirator masks containing this bacterium to nature can lead to the spread of agricultural pests. This, in addition to economic damage, is a threat to human health itself. This pathogen, formerly known as *Enterobacter*, has been shown to be able to host humans and cause various joint and bone diseases [9]. According to Hu et al. antibiotics are a good treatment for this pathogen.

Pseudomonas luteola

The AMT01 respirator mask sample hosted the very dangerous bacterium *Pseudomonas luteola*. According to Ozdemir and *Pseudomonas luteola* is an opportunistic pathogen that can cause bacteremia, meningitis, artificial endocarditis, peritonitis in humans and animals [29, 30]. Most strains of this pathogen are sensitive to broad-spectrum antibiotics, such as cephalosporins, amino acids, and ciprofloxacin. However, infections related to artificial devices or substances are very resistant [31]. For example, prostheses that are found to be infected with this bacterium should be removed.

Staphylococcus epidermidis.

The AMF01 respirator mask sample had *Staphylococcus epidermidis* pathogen with a very high probability of 99%. This bacterium is a very common germ in the hospital area because it is common in those who have a catheter for a long time, as well as in people who have a prosthesis [32]. This bacterium also grows in the environments that do not have oxygen. Although some sources and studies do not consider this bacterium to be a pathogenic bacterium, *Staphylococcus epidermidis* is an important methicillin-resistant pathogen that causes infectious diseases that are very difficult to treat [33]. Obviously, when the natural balance of these bacteria is disturbed, they begin to multiply uncontrollably and damage various tissues in the body. If the bacterium is transmitted to the bloodstream and reaches the heart, it can damage the brain, heart,

lungs, muscles and bones [34]. According to Kleinschmidt (2015), wound infections with this bacterium include abscesses, sepsis, and endocarditis for those with artificial valves.

DISCUSSION

As mentioned earlier, another pathogen called *Rhizobium radiobacter* was detected on the AMF01 respirator mask sample with a probability of 94%. *Rhizobium radiobacter* is a pathogen found in soil and plants and causes plant diseases such as crown gall. The causative agent comes out of the scab and infects healthy roots or surrounding soil. Bacteria from these infected roots or soils are easily spread in different ways [16]. Research has shown that in some cases the bacterium has been a pathogen for humans, especially in those who have had immunocompromised or those who have used plastic derivatives for prosthetics [35]. Recent studies by Amaya and Edwards and Lai et al. have shown that this bacterium can cause brain abscess and septic shock. It is also resistant to many antibiotics such as gentamicin [36]. Medical research has shown that effective drug treatments for this pathogen are cefoperazone/sulbactam and sequential oral levofloxacin [37].

The ADP07 respirator mask sample was tested and the pathogenic bacterium *Pasteurella testudines* was detected with a probability of 86%. This bacterium has been detected in host turtles [38]. The bacterium is found in the saliva of rabbits, dogs and cats and infects [39]. Over the years, this microorganism has rarely been reported to be pathogenic to humans except for those who have immunocompromised [40]. However, oral penicillin and amoxicillin are recommended for children who develop respiratory disease and lung involvement due to bites by the pets infected with this bacterium [41].

Examination of the findings in terms of pathogens identified by this study on new masks showed that another important factor, i.e. avoiding contamination of the brand-new respirator masks should be added to the three criteria recommended by the World Health Organization. Among these, the cleanliness and non-contamination of the respirator masks have a higher priority than the three important factors mentioned by the WHO. Making respiratory masks in non-standard and contaminated workshops can lead to dangerous microbial and viral infections. In this study, 50% contamination of different samples of brand-new respirator masks, which are also offered by pharmacies, shows a potential and very close danger. People think that these masks are standard and are widely used. Putting contaminated masks on the nose and mouth can lead to various diseases of the digestive and respiratory systems and target various organs such as the ear, nose and throat.

CONCLUSION

This study was a detailed review of the respirator masks that are widely sold in the community. The results indicated widespread contamination of respirator mask samples with a variety of bacteria and pathogens. Some of these bacteria, as discussed before, are dangerous nosocomial infections that are less likely to be treated with antibiotics.

Some of these bacteria have symptoms similar to other respiratory illnesses during the COVID-19 pandemic and can be mistaken. If the use of respirator masks leads to illness, people with respiratory diseases will occupy hospital beds, which makes it difficult to fight the pandemic. Having these diseases can lead to physical weakness. Going to hospitals and being treated, as well as using antibiotics to deal with possible diseases caused by these masks themselves lead to further weakening of patients and weakening of their morale, which increases the risk of developing COVID-19 disease [6]. On the other hand, going to hospital increases the risk of infection [42]. Finally, it is necessary that patients with weakened immune systems and the elderly have a priority in receiving safe respirator masks with no microbial contamination.

Future research

This research mainly focused on proving the need for a concept model of excellent performance and adding the fourth indicator of "avoiding microbial contamination". A series of other studies are needed to define and explain the indicators for determining non-microbial, non-toxic and non-viral contamination. At the same time, it is necessary to develop a methodology for measuring the current situation in the process of producing respirator masks and comparing it with the desired situation. Finally, static and dynamic case tests as well as technical and scientific inspections must be developed.

Determining the exact type of pathogens on most respirator mask samples requires further extensive studies that can be considered as a continuation of this research. In addition, the samples of the prepared masks can be improved in terms of variety and classification. Finally, this research focused on the product, while in qualitative research, especially to

find the cause of problems and difficulties, it is necessary to focus on the process, as well. Therefore, researchers can study the tasks and activities of the mask production process from the stage of preparation of raw materials to the stages of cutting, manufacturing and distribution in terms of observing health protocols by conducting field research.

Another important research can be done on the used respirator masks to find out what bacterial and viral contaminants they may find after use.

As mentioned in the literature review of this study, as far as the effectiveness of respirator masks is considered, other criteria, such as the degree of fit of the mask, the amount of filtering of harmful viruses or bacteria, and ease of breathing are also considered. Complementary research can compare the criterion discussed in this study with other three mentioned criteria. Numerous other studies are needed to develop the process of eradicating contaminated masks, both brand-new and used. For the success of these studies, in addition to the law and procedure, development of an "action plan", as well as high monitoring procedures are important and necessary.

ACKNOWLEDGMENTS

Authors thank students of system analysis and design course in IAU University, Tehran central branch, for gathering the mask samples and developing the portal based on the specifications we have given them. We would also like to show our gratitude to the Payvand laboratory for testing the samples in this research.

AUTHOR'S CONTRIBUTION

All authors contributed to the literature review, design, data collection and analysis, drafting the manuscript, read and approved the final manuscript.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest regarding the publication of this study.

FINANCIAL DISCLOSURE

No financial interests related to the material of this manuscript have been declared.

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