Prevalence of Pathogenic Bacteria on Face Masks from Wet Markets in Makkah during the COVID-19 Pandemic

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ABSTRACT

The Coronavirus disease 2019 (COVID-19) pandemic compelled people worldwide to use face masks to limit the spread of the disease. Improper use of masks may increase the transmission of pathogenic bacteria, causing co-infection that may result in an increase in complications of COVID-19 and a rise in the death rate. This study aims to determine the presence of pathogenic bacteria on the surfaces of masks worn by workers while working at several markets (meat, fish, fruit, and vegetable markets) in Makkah, Saudi Arabia, during the COVID-19 pandemic. The pathogenic bacteria on the masks of some workers in different markets in Makkah were identified and confirmed by growth media and an automated system. Pathogenic bacteria were detected in 99% of the studied samples. The most commonly detected bacteria on masks were *E. coli*, followed by *Staphylococcus epidermidis*, and *Staphylococcus aureus*. A few samples included additional bacterial species, such as *Klebsiella pneumoniae*. To the best of our knowledge, this is the first study to investigate harmful bacteria on the masks of a random group of non-healthcare workers. Improper mask use by various populations might result in significant cross-contamination with pathobionts.

1. Introduction

Coronavirus disease 2019 (COVID-19) is caused by a novel virus (SARS-CoV-2). This disease emerged in Wuhan, China, and was identified in December 2019 (Li *et al.*, 2020; Phelan *et al.*, 2020). The pandemic has had noticeable effects on people, cultures, lifestyle, and economic and public health (Mukhtar, 2020; Yoosefi *et al.*, 2021; Giuntella *et al.*, 2021; Azuma *et al.*, 2021). Consequently, various regulations and habits were changed worldwide to prevent the spread of the disease (Zarocostas, 2020). The public health sector is one of the most sensitive areas affected by COVID-19, which is considered an extremely infectious disease (Alimohamadi *et al.*, 2020). COVID-19 is transmitted by droplets loaded with viruses released from infected individuals through sneezing, coughing, and exhaling, similar to other respiratory viral infections (Anderson *et al.*, 2020; Setti *et al.*, 2020; and Somsen *et al.*, 2020). A single infected person can infect up to three people (Chen *et al.*, 2019). Globally, approximately 409 million confirmed cases and over 5.8 million deaths had been documented as of February 13, 2022 (World Health Organization (WHO), 2022). To limit the spread of this virulent virus, wearing face masks was recommended by health authorities in many countries, such as the USA, the UK, Germany, China, Singapore, and Hong Kong (Feng *et al.*, 2020). According to WHO (2022), using face masks can help prevent the spread of COVID-19 and consequently limit the number of deaths. However, other factors can play a role in the spread of COVID-19 infection.

Bacterial co-infection is very common in various infections, such as viral respiratory infections, and can significantly affect the rate of morbidity and mortality (Gupta *et al.*, 2008; Morris *et al.*, 2017; Westblade *et al.*, 2021). Undesirable effects of several cases associated with COVID-19 infections showed that the viral infection was not the only cause of the adverse outcomes. Bacterial co-infection can be a critical factor in the patient health situation (Nasir *et al.*, 2021; Singh *et al.*, 2021), and the possibility of death is

amplified when patients with COVID-19 have bacterial co-infection (Silva *et al.*, 2021; Shafran *et al.*, 2021). Bacterial pathogens can infect humans through food, water, or living vectors (Doron and Gorbach, 2008). Hand contact is considered one of the most common modes of pathogens transmission (Pittet *et al.*, 2006; Kutter *et al.*, 2018). It can spread pathogens among people and on surfaces such as food, tools, clothes, and personal belongings (Sze-To *et al.*, 2014; Mitchell *et al.*, 2015; Aljamali *et al.*, 2021).

Although masks are supposed to be a preventing tool (Dehaghi *et al.*, 2020; Dehgani-Mobarak *et al.*, 2020; Saijonkari *et al.*, 2020), they could be one of the harmful pathogen vehicles (Chughtai *et al.*, 2019). Improper use of masks might lead to bacterial transmission, which can cause co-infection cases in patients (Simatupang *et al.*, 2021). Furthermore, several unsuitable practices have been observed in various communities using masks. Such practices include touching the outer surface of masks with bare hands while working, attempting to put masks on and take them off multiple times while performing assigned work, and reusing masks repeatedly for extended periods, in some cases for days, with no regard for sanitation or contamination (Simatupang *et al.*, 2021). These diverse behaviors could be salient factors since they could transmit pathogenic organisms from individual to individual, product to person, and surface to surface.

During the pandemic, few studies have been conducted on the prevalence of pathogenic bacteria on the surfaces of face masks, and there is a need to demonstrate the diversity of pathogenic bacteria on the surfaces of masks worn while working. Therefore, this study aims to detect the prevalence of pathogenic bacteria on the surfaces of masks worn by workers while working at different markets (meat, fish, fruit, and vegetable markets) in Makkah, Saudi Arabia, during the COVID-19 pandemic.

2. Material and Methods

2.1. Collection of Samples:

Cotton swabs (Sigma-Aldrich, Bangalore, India) were used to swab the outer surfaces of face masks worn by workers in the central market of Makkah, which is one of the main and biggest cities in the western region of Saudi Arabia. The central market was divided into four individual sections (fruits, vegetables, fish, and meat). Samples were collected between 10:30 am and 1:30 pm on Friday, as this is when the marker is the busiest and most crowded. It is the first day of the weekend in Saudi Arabia.

Samples were collected in April 2021, when wearing masks was mandatory for the whole country as per government guidelines due to the rise in COVID-19 infections. During the collection of samples, data such as mask type (surgical or textile), wearing duration, and market type were recorded. A total of 100 samples were taken from the central market's four sections (27 from the fruit market, 26 from the vegetable market, 27 from the fish market, and 20 from the meat market).

2.2. Detection of Bacterial Pathogens:

All the experiments were conducted following the methods proposed by Saikia and Joshi (2014). First, within 5 hours, all the swabs were directly streaked onto four different types of culture media: blood agar, MacConkey agar, HiCrome Staph Selective Agar, and eosin methylene blue (EMB) agar (HiMedia, Mumbai, India)). Second, plates were incubated at 37°C for 24 hours. In the third stage, the colonies grown on EMB agar and HiCrome Staph Selective Agar were identified based on their cultural characteristics by following the manufacturer's instructions. Metallic colonies on EMB were classified as E. coli. Green colonies and blue colonies on HiCrome Staph Selective Agar were classified as Staphylococcus aureus and Staphylococcus epidermidis, respectively. Blood agar and MacConkey agar media were used to verify the hemolytic pattern of staphylococci and the lactose fermentation ability of lactose fermenters, respectively.

2.3. MicroScan Automated Identification:

The Gram staining step was performed on samples (n = 13) that showed no characteristic features on culture media, such as metallic colonies on EMB or green and blue colonies on HiCrome Staph Selective Agar. All the samples (n = 13) were Gram-negative. These samples were prepared for MicroScan analysis according to the manufacturer's instructions. Briefly, using the rapid inoculation technique, a single fresh colony from the overnight cultures on blood agar was rapidly standardized for MicroScan antimicrobial sensitivity testing (AST) and identification tests. After inoculation, the solution was transferred onto the N66 panels and loaded into the instrument for overnight treatment. Finally, MicroScan (Walkaway 96 plus) Gram-positive and -negative cards automatic identification technology was used to identify all the isolates (Osei et al., 2020).

2.4. Statistical Analysis:

Statistical Package for the Social Sciences (SPSS) version 26 was used to code and input the data (IBM Corp., Armonk, NY, USA). The mean, standard deviation, median, minimum, and maximum were used to describe the quantitative data. Conversely, the frequency (count) and relative frequency (%) were used to summarize the categorical data. The non-parametric Kruskal–Wallis and Mann–Whitney tests were utilized to compare quantitative variables (Chan, 2003a). The Chi-square (c2) test was used to compare categorical data. The exact test was employed instead when the anticipated frequency was less than 5 (Chan, 2003b). Statistical significance was defined as p-values less than 0.05.

3. Results and Discussion

The existence of bacterial species on the surfaces of surgical and textile face masks was examined in 100 samples collected from four different local markets in Makkah, Saudi Arabia. The distribution of bacterial species on the masks collected from the markets is shown in **Table 1**.

Table 1: Distribution of bacterial strains in different markets

Bacterial strain	Fish (n = 27)	Fruits (n = 27)	Vegetables (n = 26)	Meat (n = 20)	Total (n = 100)
E. coli	21	13	18	16	68
Staphylococcus epidermidis	15	17	23	7	62
Staphylococcus aureus	13	10	17	9	49
Klebsiella pneumoniae	0	1	1	1	3
Klebsiella rhinoscleromatis	0	0	1	1	2
Klebsiella ozaenae	0	1	0	1	2
Acinetobacter baumanii	0	0	1	0	1
Acinetobacter lwoffii	0	0	0	1	1
Mannheimia haemolytica	1	0	0	0	1
Providencia rustigianii	1	0	0	0	1
Pantoea agglomerans	0	0	1	0	1
Pseudomonas putida	0	1	0	0	1

Of the 100 samples, 99 positive samples revealed the existence of at least one bacterial species. Only one sample showed no bacterial growth on any of the growth media used. The maximum recorded number of bacterial species on a single mask (four species) was collected from the vegetable market. The total number of bacterial species detected per mask sample was one, two, and three, representing 26%, 53%, and 19% of the collected samples, respectively.

The most prevalent pathogenic bacteria was E. coli, detected in 68% of the samples, followed by Staphylococcus epidermidis, detected in 62% of the samples, and Staphylococcus aureus, detected in 49%. Klebsiella pneumoniae was detected in 3% of samples, while Klebsiella rhinoscleromatis and Klebsiella ozaenae were recorded in only 2% of the samples. Some bacterial species, such as Acinetobacter baumanii, Acinetobacter lwoffii, Mannheimia haemolytica, Providencia rustigianii, and Pseudomonas putida, were detected in only 1% of the samples. It was found that the mask-wearing duration did not affect the mask bacterial flora. Some samples with a short mask-wearing duration showed the existence of many bacterial species, whereas others with a long mask-wearing duration contained just a few bacterial species, as shown in **Figure 1**.

Figure 1: Prevalence of pathogens on textile and surgical masks
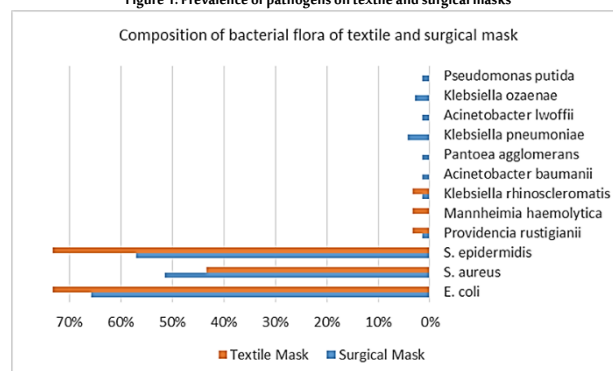
The total number of bacterial isolates per market were 62, 51, 43, and 36 in the vegetable market (26 samples), fish market (27 samples), meat market (20 samples), and fruit market (27 samples), respectively, as shown in **Chart 1**.

Chart 1: Total number of bacterial isolates per each market
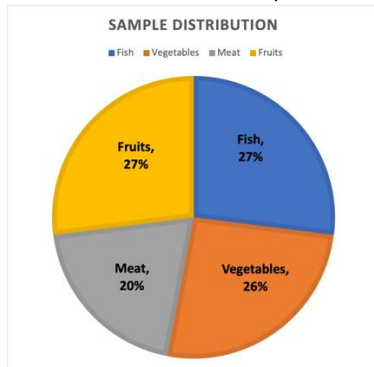
Among the studied samples, it was found that the most commonly used mask was the surgical mask, representing 70% of the collected samples, while textile masks represented 30%, as shown in **Chart 2**.

Chart 2: Most commonly used mask: surgical and textile masks
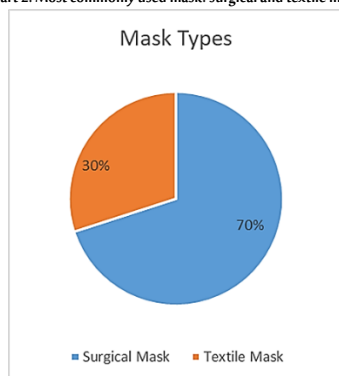
The statistical analysis showed no significant effect of mask type on bacterial flora. However, some bacterial species, namely, *Acinetobacter baumanii, Acinetobacter lwoffii, Pantoea agglomerans*, and *Pseudomonas putida*, were detected on surgical masks but not on textile masks. Conversely, *Mannheimia haemolytica* was detected on textile masks but not on surgical masks, as shown in **Figure 1**. The statistical analysis also showed a significant variation ($p<0.05$) in S. epidermidis among the studied markets, and it was more commonly found in the vegetable market. However, no significant changes were observed in the distribution of other bacterial isolates in all the analyzed markets. Furthermore, the statistical analysis revealed that surgical masks were ($p<0.05$) utilized much more commonly than textile masks.

Several studies have demonstrated the usefulness of face masks in limiting the spread of airborne viruses (Liang *et al.*, 2020; MacIntyre and Chughtai, 2015; Suess *et al.*, 2012). However, a limited number of studies have investigated their bacterial loads in the population, particularly during the COVID-19 pandemic (Delanghe *et al.*, 2021). The present study indicated that *E. coli* was the most prevalent bacterium. There are several reports about the involvement of E. coli in some skin diseases, such as necrotizing fasciitis (Afifi and El-Hindawi, 2008; Krebs *et al.*, 2001; Li *et al.*, 2006), cellulitis of upper or lower limbs (Brzozowski and Ross, 1997; Corredoira *et al.*, 1994; Yoon *et al.*, 1998), neonatal omphalitis (Fraser *et al.*, 2006), burn injuries (Rodgers *et al.*, 2000), and surgical site infections (Tourmousoglou *et al.*, 2008). Moreover, *E. coli* is involved in other internal infections, such as urinary tract infections (Bannon *et al.*, 2016; Flores-Mireles *et al.*, 2015) and acute diarrheal disease (Torres *et al.*, 2001). *Staphylococcus epidermidis* and *Staphylococcus aureus* are among the normal human and animal skin flora involved in skin health. However, some of them are also known as skin pathogens that cause skin diseases such as acne vulgaris and atopic dermatitis

(Findley and Grice, 2014; Bjerre *et al.*, 2017; Byrd *et al.*, 2017).

Furthermore, three detected species belonging to the genus *Klebsiella*, namely, *Klebsiella pneumoniae, Klebsiella rhinoscleromatis,* and *Klebsiella ozaenae*, are involved in many respiratory diseases, such as *pneumonia, ozena*, and *rhinoscleroma*, respectively (Janda and Abbott, 2006). The existence of bacterial species such as *E. coli, Staphylococcus aureus*, and *Klebsiella pneumoniae* on face masks is a critical finding. The detection of bacterial flora recorded in this study partially agrees with that recorded by Delanghe *et al.* (2021). They recorded the existence of strains belonging to the genera *Bacillus, Staphylococcus*, and *Acinetobacter*. The difference in the findings may be due to the variation in population and mask-wearing duration.

A previous study showed variations in the composition of the bacterial flora on textile and surgical masks (Delanghe *et al.*, 2021). However, we observed no significant difference between the two types of masks. The conflicting results may be due to differences in mask-wearing duration. In the current study, in several cases, most masks were worn for more than 6 hours or more than 24 hours, whereas the mask-wearing duration in the study conducted by Delanghe *et al.* (2021) was only 4 hours.

Another reason for pathobiont transmission is speech volume (Patel *et al.*, 2020), and it has been demonstrated that high speech volumes increase the possibility of pathogen transmission, including COVID-19. The finding of the current study is in accordance with that of Patel *et al.* (2020). The chosen markets being considered places with high speech volumes for prolonged periods is another reason behind having 99% positive samples. The mask itself can be a favorable habitat for bacterial accumulation (Abbasi *et al.*, 2020), particularly in confined and congested areas with inadequate ventilation (Tuñón-Molina *et al.*, 2021). Furthermore, improper habits associated with using masks observed during the study, such as touching the mask surface and beneath the mask to scratch the face or nose, may increase the microbial load of the mask (Simatupang *et al.*, 2021). It can consequently transfer bacteria to other organs, such as the eyes and mouth, or the objects present in various markets, such as food, shopping bags, bills, payment machines, and tools. On average, humans touch their face, including the mouth, nose, and eyes, more than 20 times per hour (Kwok *et al.*, 2015). That could be the main reason for infection (Shen *et al.*, 2020) with a wide range of different viruses such as Coronavirus, Ebola virus, swine flu, and seasonal flu (Sudharsan *et al.*, 2020).

There is no doubt about the importance of using masks, especially during the COVID-19 pandemic. Several studies have highlighted masks' essential role in protecting the public from different infections and diseases (Greenhalgh *et al.*, 2020; Liang *et al.*, 2020; MacIntyre and Chughtai, 2015). However, the current study's findings show that the primary purpose of using masks might be influenced by community behavior (Simatupang *et al.*, 2021). The public still needs to be more educated about the proper use of masks and their necessity to achieve the main purpose of using masks.

The pathogenic bacteria detected in this study are related to fecal contamination, hand washing, or personal hygiene issues. This highlights the need to educate communities about the proper methods of washing hands and self-hygiene. Health authorities worldwide and other related local organizations must take serious actions to encourage the public at different levels, specifically non-educated communities, to focus on the adverse effects of bad habits that might lead to serious health issues. The guidance and information should be simplified and clarified to be easily understood. For instance, in the current study, most workers in the chosen market do not speak the native or local language of the

country fluently, making it exceedingly difficult to understand the essential role of safety and hygiene guidance. Many people and workers might be wearing masks only because of the new rules and systems that have been implemented in some countries since the start of this pandemic, and they might not know the reasons for and benefits of those rules. We noticed several inappropriate actions from our observation, such as wearing a "half mask" and touching the mask before handling products and shopping bags. According to Lee *et al.* (2020), in a study of 1500 participants, more than 90% did not follow hand hygiene before using masks, and more than 95% did not follow the correct hand hygiene after taking masks off.

All these behaviors can affect the purpose of using masks. Consequently, masks can be a transmission tool for several pathogens. People and communities must learn and follow the proper use of masks to avoid spreading COVID-19 and other pathogens. All the health sectors worldwide must educate communities and consider the variety of backgrounds, cultures, and education.

4. Conclusion

Face masks have been used increasingly during the COVID-19 pandemic to prevent the spread of this lethal disease. Improper use of masks may lead to contamination of the mask with pathogenic bacteria. The findings of this study confirmed the existence of bacterial species on 99% of the samples collected from some workers in wet markets, including vegetable, fruit, fish, and meat markets. Some of these bacteria, such as *Klebsiella pneumoniae, Klebsiella ozaenae, Klebsiella rhinoscleromatis,* and *Staphylococcus aureus*, are strong pathogens and may cause dangerous diseases. Bacteria prevalence on the masks may be due to the crowded nature of these markets and workers' lack of awareness about the right way to use a mask. Educating people about the proper way to use a mask and providing instructions in different languages within multilingual and multicultural communities may reduce the contamination of masks with bacteria. Statistical studies about the prevalent respiratory and skin bacterial diseases during the COVID-19 pandemic are needed to evaluate their relation to mask use.

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