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Assesment of microbiological growth on surgical face mask

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Abstract

Wearing surgical face masks for a long time has a number of physiologic and psychological consequences, as well as the potential to reduce work efficiency. Physical side effects of long-term usage of surgical masks include headaches, trouble breathing, acne, skin breakdown, rashes, and reduced memory. It also obstructs eyesight, communication, and thermal balance. The objective of this research was to investigate the effects of bacteria growth and type of bacteria morphology present on the face mask. Serial dilutions were used to calculate the concentration of microorganisms. As it would usually be impossible to actually count the number of microorganisms in a sample, the sample was diluted and plated to get a reasonable number of colonies to count. Since the dilution factor was known, the number of microorganisms Log CFU per mL was calculated. The CFU count results clarify the growth curve pattern of bacteria formed on agar plate surfaces and revealed some details of bacterial life after their adhesion onto surfaces in the presence of agar. This growth curve exhibited distinct phases: the lag phases, the exponential (log) phases, the stagnant phases and the dead phases. Gram staining and hanging drop method was used to identify the gram positive, gram negative and the bacteria morphology. This study provides insightful on the investigation of bacteria present on surgical face masks which is little available in literature.

Keywords :
Surgical mask, bacteria, gram staining, bacteria growth curve

1 Introduction

Mask usage is increasing due to COVID-19 virus (Wang et al., 2020; Sangkham 2020). As a respiratory infectious disease, the airborne transmission of COVID-19 is one of significant pathways that are transported over a large region by air currents and then inhaled by susceptible individuals. Millions of people have been affected by the current COVID-19 pandemic and with no vaccine available, actions to prevent transmission are urgently needed (Malik et al., 2020). Even there is a widespread agreement on travel restrictions and social isolation in reducing disease transmission in reducing the transmission of disease, suggestion on using face masks varies across countries (Eikenberry et al., 2020; Goh et al., 2020).

The use of surgical face masks is strongly recommended in countries with high COVID-19 cases (Worby and Chang 2020; Liao et al., 2021). Therefore, the use of face masks has been increased due to the high demand of people around the world who are aware of the risk of getting infected by COVID-19 (Worby and Chang 2020). High usage of face masks imposed on the daily cost needed for masks (Silva et al., 2020; Ganesapillai et al., 2022). This causes people to tend to reuse used masks or else use a face mask for long time no matter what types of mask available (Das et al., 2021).

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Wearing surgical face masks for a long time has a number of physiologic and psychological consequences, as well as the potential to reduce work efficiency (Scheid et al., 2020; Delanghe et al., 2021). Physical side effects of long-term usage of surgical face masks include headaches, trouble breathing, acne, skin breakdown, rashes, and reduced memory (Chowdhury et al., 2022; Rohani Shirvan et al., 2022). It also obstructs eyesight, communication, and thermal balance (Elisheva, 2020). Furthermore, headaches caused by prolonged surgical face mask use can be attributed to mechanical factors, hypercapnia, and hypoxemia (Kumar and Singh 2022; Ramirez-Moreno et al., 2021). Tight straps and pressure on the superficial facial and cervical nerves are mechanical factors that cause headaches (Ong et al., 2021; Ong et al., 2020). Other causes of headaches among healthcare professionals during prolonged mask use include cervical neck strain from wearing PPE, sleep deprivation, irregular mealtimes, and emotional stress (Ganesapillai et al., 2022).

The above discussion suggests that surgical face masks also have some negative impact on health even they have a modest protective effect, can lower total infections and deaths, as well as delay the epidemic's peak (Worby and Chang 2020; Liao et al., 2021). One of the easiest things to know about the negative impact of wearing masks including epidemiological impact is an increase in the amount of natural microbiology that grows due to the use of masks for a certain period of time. An increase in the amount of natural microbiology can contribute to healthy skin problem in areas covered by face masks. It is likely that a large number of people will be negatively affected by epidemiological impact regarding to their healthy skin. Thus, this study investigates the epidemiological impact of face masks using mathematical models, taking into account resource constraints and a variety of supply and demand dynamics. This study contributes significantly on the investigation of bacteria present on surgical face masks which is little available in literature.

2 Materials and method

2.1 Materials

The materials are CFU glassware include screw-capped test tubes, sterile pipettes, glass rod spreader (bent in the shape of a hockey stick). Meanwhile, hanging drop and gram staining the materials will include glass slide (glass slide that has depression) or regular glass slide with adhesive or paraffin rings, paraffin wax loop, coverslip, microscope, bunsen burner and young broth cultures.

2.2 CFU count

The spread plate method is a method to count the number of colonizing bacteria present in a liquid sample. Place a fixed inoculum of 0.1 mL of sample in the center of the agar plate using a micropipette. The sample was then finally spread on the agar using a hockey stick. This step is repeated for samples from each dilution. Plates are then inverted and incubated at 37° C. for 24-48 h. Bacterial numbers were expressed as colony forming units per mililiter (CFU/mL).

2.3 Gram staining

Gram staining began with smear preparation. A very small sample of the bacterial colony was taken using a sterile and refrigerated loop and gently stirred into a drop of water on the slide to create an emulsion. It is very important to avoid creating thick, dense smears containing excess bacterial samples. A very thick smear can reduce the amount of light that can pass and make it difficult to visualize the morphology of individual cells. Swabs typically require only a small amount of bacterial culture. After heat curing, an effective lubricating film appears as a thin whitish layer or film. Heat-fixing then kills the bacteria in the smear, allowing the smear to adhere firmly to the slide and allow the sample to pick up the stain more easily. After the smear was allowed to air dry, the entire slide was passed 2-3 times through the flame of a Bunsen burner, holding the slide by one end, smear side up. The smear is then ready for staining. Third, slides containing heat-fixed swabs of bacterial specimens from dragons were placed in staining dishes.

Crystal violet was gently dipped into the smear and left for 1 min. The slides were then tilted slightly and rinsed gently with distilled water. The smear was then gently dipped in gram iodine and left for 1 min. The slide was tilted slightly and gently rinsed with distilled water using a wash bottle. The smear will appear as a purple circle on the slide. It was then destained with 95% ethyl alcohol or acetone. The slide was tilted and the alcohol was dripped over 5-10 s until the alcohol was almost clear. Then wash off with water immediately. Finally, safranin was gently dipped and left for 45 s counter-staining. The slides were tilted slightly and rinsed gently with tap or distilled water using a wash bottle. Blot the slide dry with a tissue and finally the smear can be observed using a light microscope under oil immersion.

3 Results and discussion

3.1 Growth phases of microbiological

Figs. 1-3 are graph samples of surgical face masks used for 2, 4, and 6 h, respectively. The results clarify the growth curve pattern of bacteria generated on agar plate surfaces and disclose certain aspects of bacterial life following adherence to agar-coated surfaces. This growth curve appears to display numerous stages, including lag periods, exponential (log) phases, stationary phases, and dead phases.

During the lag phase, at point zero h until 23 h, all the bacteria growth samples shows no specific log CFU/mL data (Tessarolo et al., 2022). This is because upon inoculation into the new medium, bacteria do not immediately reproduce, and the population size remains constant. The cells are metabolically active and increase only in cell size. They are also synthesizing the enzymes and factors needed for cell division and population growth under their new environmental conditions.

3.2 Microbiological presence in surgical face mask

Most of the samples increase uniformly within 24 h to 120 h this means that the bacteria population has enters the log phase, in which cell numbers increase in a logarithmic fashion, and each cell generation occurs in the same time interval as the preceding ones, resulting in a balanced increase in the constituents of each cell (Scaglione et al., 2022). Among all the samples, sample of 12 h showed the highest log CFU/mL which is 2.29E+00 while sample that shows the least is the 2 hours sample which is 2.15E+00. The log phase continues until nutrients are depleted or toxic products accumulate, at which time the cell growth rate slows, and some cells may begin to die (Rodriguez-Palacios et al., 2020).

When using the hanging drop method, the form, motility, and arrangement of bacteria are all easily observable. On the other hand, it is more difficult to analyse the movement of bacteria when the microbial suspension in the well of the cavity slide is crushed by the cover slip. The hanging drop method, smear preparation, heat fixing, and stain films are utilised in the process of identifying an organism through the use of light microscopy. According to morphological assessments using gram staining and hanging drop technique, the bacteria have a coccus shape. It indicates that coccus bacteria, such as *Staphylococci* sp. and *Streptococci* sp., have the potential to grow in face masks.

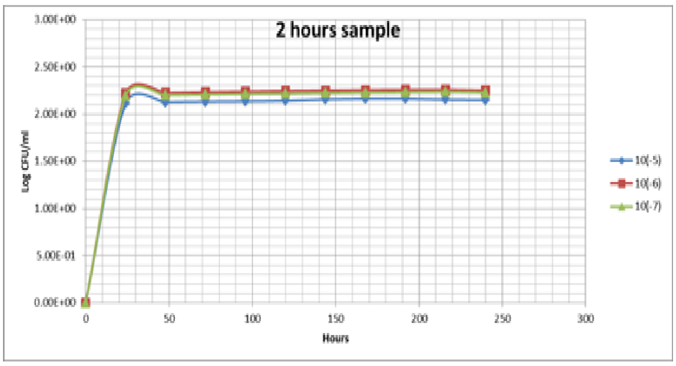

Figure 1 Sample face mask that has been used for 2 h

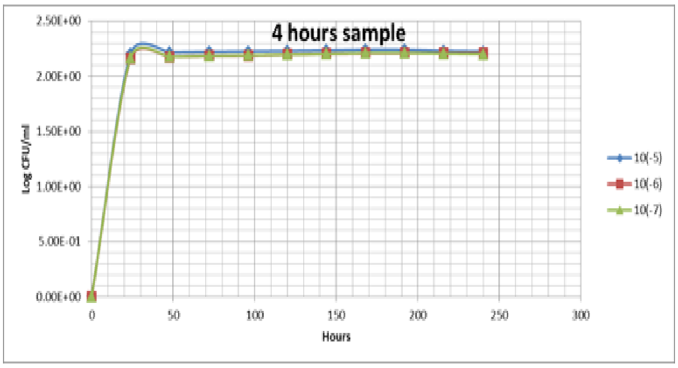

Figure 2 Sample face mask that has been used for 4 h

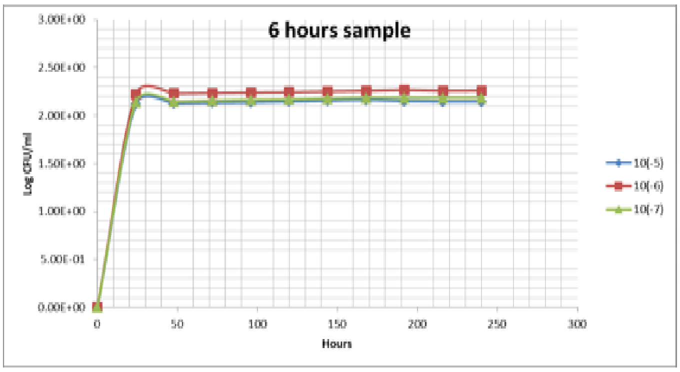

Figure 3 Sample face mask that has been used for 6 h

Similarly, previous study presented that bacteria *Staphylococci* 26.35% as a predominant species followed by *Pseudomonas* 17.82% and *Streptococci* 15.50%. Aspergillus fungal species was also present in 6.97%. Mean ± SD of bacterial and fungal contamination on inside/outside area of the used masks was 48 ± 26 and 180 ± 110 CFU/mL/piece and 14 ± 6 and 32 ± 13 CFU/mL/piece (Sachdev et al. 2020). Organisms' morphology were also reported included *Staphylococcus* (42; 95.45%), *Bacilli* (18; 40.91%), *Diphtheroid* (5; 11.36%), and *Streptococcus* (4; 9.09%) with 15 masks (34.09%) showing more than 1 organism's morphology (Abreu-Irizarry et al. 2022). It indicated that surgical mask is possibility contaminated by microorganism.

4 Conclusion

The aim of this study was to investigate the epidemiological impact of face masks using mathematical models, taking into account resource constraints and a variety of supply and demand dynamics. The efficiency of the face mask depended on presence of bacteria population on the surgical face mask. The sample of 12 h used surgical face mask showed the highest bacteria count. It means that the surgical face mask is a one-time used face mask. The population of bacterial growth based on the length of time on face mask usage was determined by the growth rate from all samples during the 10 days of incubation. The presence of bacteria approximately 100-190 colonies/10 days proved that the surgical face mask should not be utilized for more than a day. Thus, the bacteria morphology on face mask was similar to the shape of coccus.

Declaration of competing interest

The authors declare no known competing interests that could have influenced the work reported in this paper.

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