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Isolation and Identification of Bacteria from Used Face Masks in Bauchi State University, Gadau

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Abstract

The most traditional and realistic technique to avert the blowout of respiratory contaminations is to wear face masks; quite a few research clusters have established its efficacy against the spread of respiratory viruses before the COVID-19 pandemic. The current study aimed to isolate and identify bacteria from disposable face masks after use in Bauchi state university, Gadau. A total of 100 used face masks were collected from students of the university and subjected to microbiological and biochemical tests in order to isolate and identify the organisms from both the inner and outer surfaces of the used face masks. Most isolated bacterial contamination on both the outside area of the used face masks were Staphylococcus aureus 41.6 %, Escherichia coli 45.8 %, Streptococcus pneumoniae 8.3 % and Streptococcus mutans with 4.1 % but the number of organisms found on the outside were much lower. This study shows that the most abundant bacteria found in used face masks was Escherichia coli with the highest percentage of occurrence. The findings from this study show that bacteria were isolated from both the inner and outer surfaces of all the face masks used for this study, majority of which were Gram positive cocci. Only one-gram negative rod was identified and isolated.

Keywords: COVID-19, pandemic, contamination, microbiological, biochemical.

1. Introduction

The most traditional and reasonable method to prevent the spread of respiratory infections is to wear face masks; several research groups have demonstrated its effectiveness against the transmission of respiratory viruses before the COVID-19 pandemic (Chughtaita et al., 2020;Park et al., 2022). Non-woven, polyurethane, and gauze or cloth masks are the most popular types of face masks that are available commercially worldwide(Martinelli et al., 2021). Non-woven masks are commonly used worldwide to prevent droplet infections by most respiratory microbes, including SARS-CoV-2 (Furnaz et al., 2022). Among environmental pathogens, viruses cannot replicate without infecting host cells, however most bacteria and fungi can survive and grow on various materials depending on the environmental conditions (Park et al., 2022). Bacteria and fungi are widely present on the surface of the materials used in our daily lives such as currency notes and in public transportation systems (Jefferson et al., 2020; Zhao et al., 2022). Although a few studies reported bacterial or viral contamination on masks in experimental and clinical settings, there is a

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need to study the characteristics of the various microbes that are present on face masks used daily in our day-to-day activities (Park et al., 2022; Gonçalves et al., 2018). Since masks can be a direct source of infection to the respiratory tract, digestive tract, and skin, it is pertinent to maintain their hygiene to prevent life potentially threatening bacterial and fungal infections (CDC, 2020).

2. Materials and methods

Sample collection

The partakers were males and females aged ≥18 years, and have voluntarily participated and signed the consent form. Furthermore, 100 disposable facemask samples were collected from school Gate, Faculty and Hostels in the same days during the 2 Weeks observations to investigate the microbial air quality (bacterial counts). The study was conducted with the ethical approval of the ethical committee of Bauchi State University Gadau. The 100 students were study from different departments such as: male and female school library, Male hostel, Female hostel, faculty of science, Faculty of medicine in the university.

A total of 100 disposable facemasks samples collected from the student study in university including: 60 samples from males and 40 samples from females.

Sampling was done during 12:00 to 15:00 p.m. in Monday, Wednesday and Friday for 1 week to assess bacterial counts. Sampling collection points includes school gate, male hostel, and female hostel, faculty of science faculty of medicine and school library.

2. Methods

A cross-sectional study was conducted to assess the bacterial contamination on 100 used disposable face masks from 100 students. The inside and outside area of the masks were analyzed.

The used disposable face masks, which were 100 in number, were collected to culture the bacterial counts. Inside and outside areas of the disposable face masks were and put in a sterile container consisting of distill water for 20 minutes. A spread plate method was used for determining total bacterial counts. General bacteria were cultivated in nutrient agar. The plates were incubated at 37°C for 48 hours to get the bacterial counts, and observation was done daily. After counting the isolated bacteria, they were preliminarily identified by Gram's stain and microscopic morphology (lacto-phenol cotton blue) following (Larone, 1995).

Characterization and identification of the Isolates

Cultural characteristics, gram stain reaction and biochemical tests were used to characterize and identify the isolates.

Gram Staining

A drop of water was placed on a sterile grease free slide, using a sterile wire loop, a small portion of inoculum were picked from 24 hours culture of isolate and emulsified on the drop of the distilled water until a thick homogeneous smear were made and it was heat fixed.

The smear was flooded with crystal violet for 60 seconds, then rinsed with water and afterwards a few drops of Lugol's iodine was added to the smear for 30 seconds and rinsed with water and 95 % alcohol was added drop wise until no drop of the crystal violet was seen dripping from the smear, it was then rinsed with water. Then the smear was flooded with safranin for 60 seconds then rinsed with water. It was air dried, then a drop of immersion oil placed on the smear and viewed under x100 objective lens. The gram reaction of each of the isolate was noted and recorded.

Biochemical Test

These include catalase test, coagulase test, oxidase test and indole test

Catalase test

Two (2) drops of hydrogen peroxide solution was placed on a clean grease free slide, using sterile wire loop, a small portion of the isolates picked and emulsified on the drop of hydrogen peroxide and the presence and absence of effervescence was noted and recorded.

Coagulase Test

A drop of distilled water was placed on a slide and the test colony was emulsified on the drop of the distilled water to make a thick suspension A loopful of plasma was added to the suspension and mixed gently and the presence or absence of agglutination was checked within 10 seconds

Oxidase Test

A filter paper was placed in a clean Petri dish and two (2) drops of freshly prepared oxidase reagent added using a glass rod. A colony of the test organism was removed and smeared on the filter paper the presence or absence of blue-purple colour was taken note of and recorded.

lndole test

The test organism was inoculated in a test tube containing 3ml of sterile tryptone water. It was incubated at 37°c for 48 hours. 0.5m1 Kovac's reagent was added and shaken gently. The reaction was observed for the presence or absence of red layer on the surface and was taken note of.

3. Results

A total of one hundred (100) used face masks were collected from one hundred students to isolate and identify bacterial contamination. Results revealed that the bacterial contamination on the inside and outside of the used face masks was 22-25 cfu/ml/piece.

Table 1. Bacterial contamination (cfu/ml/piece.) observed on inner surface of the used face masks (N = 100)

BACTERIA	FREQUENCY (CFU/ml)	% OF OCCURRENCE (%)
S. aureus	5	20.8
E. coli	6	25.0
S. pneumoniae	7	29.1
S. mutans	6	25.0
TOTAL	24	100

Key: E. coli = Escherichia coli, S. pneumonia = Streptococcus pneumonia, S. mutans = Streptococcus mutans, S. aureus = Staphylococcus aureus.

Table 2. Bacterial contamination (cfu/ml/piece.) observed on the outer surface of the used face masks (N = 100

BACTERIA	FREQUENCY (CFU/ml)	% OF OCCURRENCE (%)
S. aureus	10	41.6 %
E. coli	11	45.8 %
S. pneumoniae	2	8.3 %
S. mutans	1	4.1 %
TOTAL	24	100 %

Key: E. coli = Escherichia coli, S. pneumonia = streptococcus pneumoniae, S. mutans = streptococcus mutans, S. aureus=staphylococcus aureus

Table 3. Biochemical identification of bacteria & Gram staining

ORGANISM	CATALASE TEST	COAGULASE TEST	OXIDASE TEST	INDOLE TEST	GRAM REACTION	SHAPE
S. aureus	+	+	+	+	G+	Coccus
S. pneumoniae	-	-	-	-	G+	Coccus
E. coli	+	-	-	+	G-	Bacillus
S. mutans	-	-	-	-	G+	Coccus
Salmonella	+	-	-	-	G-	Bacillus

Key: E. coli=Escherichia coli. S. pneumoniae=streptococcus pneumoniae.
S. mutans=streptococcus mutants. S. aureus=staphylococcus aureus.

4. Discussion

This study was done to assess bacterial contamination on used face masks. The findings from this study show that bacteria were isolated from both the inner and outer surfaces of all the face masks used for this study, majority of which were Gram positive cocci. Only one-gram negative rod was identified and isolated. This is in agreement with similar studies conducted by Chughtai et al. (2019) and those of Delanghe et al. (2021), there were more organisms on the outer surface than those detected on the inner surface, this maybe because of the difference in exposure to the outside between th e two surfaces. Though the total number of organisms found where quite different, the findings were also in agreement with those of Gund et al. (2021), Pullangott et al. (2021) and those of Pullangott et al. (2021).

5. Conclusion

The present study revealed high bacterial contamination on the outside and inside areas of the used disposable masks. Wearing a mask is an easy way to reduce the risk of unknowingly spreading the infection. To reduce the load of bacterial contamination on the used masks, the university environments, especially microbial air quality in the student hostels, lecture theaters and general school premises should be improved.

References

CDC, 2020 – CDC. Life Cycle of Aedes aegypti and Ae. albopictus Mosquitoes. In U.S. Departement of Health and Human Services. 2020. Pp. 1-2. [Electronic resource]. URL: https://www.cdc.gov/

Chughtai et al., 2019 – *Chughtai, A.A., Stelzer-Braid, S., Rawlinson, W., Pontivivo, G., Wang, Q., Pan, Y., Zhang, D., Zhang, Y., Li, L., MacIntyre, C.R.* (2019). Contamination by respiratory viruses on outer surface of medical masks used by hospital healthcare workers. *BMC Infectious Diseases*. 19(1): 1-8. DOI: https://doi.org/10.1186/s12879-019-4109-x

Chughtaita et al., 2020 – *Chughtaita, A.A., Seale, H., MacIntyre, C.R.* (2020). Effectiveness of Cloth Masks for Protection against Severe Acute Respiratory Syndrome Coronavirus 2. *Emerging Infectious Diseases*. 26(10). DOI: https://doi.org/10.3201/EID2610.200948

Delanghe et al., 2021 – *Delanghe, L., Cauwenberghs, E., Spacova, I., De Boeck, I., Van Beeck, W., Pepermans, K., Claes, I., Vandenheuvel, D., Verhoeven, V., Lebeer, S*. (2021). Cotton and Surgical Face Masks in Community Settings: Bacterial Contamination and Face Mask Hygiene. *Frontiers in Medicine*. 8(September): 1-12. DOI: https://doi.org/10.3389/fmed.2021.732047

Furnaz et al., 2022 – *Furnaz, S., Baig, N., Ali, S., Rizwan, S., Khawaja, U.A., Usman, M.A., Haque, M.T.U., Rizwan, A., Ali, F., Karim, M*. (2022). Knowledge, attitude and practice of wearing mask in the population presenting to tertiary hospitals in a developing country. *PLoS ONE*. 17(3). DOI: https://doi.org/10.1371/journal.pone.0265328

Gonçalves et al., 2018 – *Gonçalves, C.L., Mota, F.V., Ferreira, G.F., Mendes, J.F., Pereira, E.C., Freitas, C.H., Vieira, J.N., Villarreal, J.P., Nascente, P.S.* (2018). Airborne fungi in an intensive care unit. *Brazilian Journal of Biology*. 78(2): 265-270. DOI: https://doi.org/10.1590/1519-6984.06016

Gund et al., 2021 – *Gund, M., Isack, J., Hannig, M., Thieme-Ruffing, S., Gärtner, B., Boros, G., Rupf, S*. (2021). Contamination of surgical mask during aerosol-producing dental treatments. *Clinical Oral Investigations*. 25(5): 3173-3180. DOI: https://doi.org/10.1007/s00784-020-03645-2

Jefferson et al., 2020 – *Jefferson, T., Del Mar, C.B., Dooley, L., Ferroni, E., Al-Ansary, L.A., Bawazeer, G.A., van Driel, M.L., Jones, M.A., Thorning, S., Beller, E.M., Clark, J., Hoffmann, T.C., Glasziou, P.P., Conly, J.M.* (2020). Physical interventions to interrupt or reduce the spread of respiratory viruses. *Cochrane Database of Systematic Reviews*. 11. DOI: https://doi.org/10.1002/14651858.CD006207.pub5

Larone, 1995 – *Larone, D.H.* (1995). Medically important fungi: A guide to identification, 3rd Edition, American Society for Microbiology.

Martinelli et al., 2021 – *Martinelli, L., Kopilaš, V., Vidmar, M., Heavin, C., Machado, H., Todorović, Z., Buzas, N., Pot, M., Prainsack, B., Gajović, S.* (2021). Face Masks During the COVID-19 Pandemic: A Simple Protection Tool With Many Meanings. *Frontiers in Public Health*. 8(January): 1-12. DOI: https://doi.org/10.3389/fpubh.2020.606635

Park et al., 2022a – *Park, A.M., Khadka, S., Sato, F., Omura, S., Fujita, M., Hashiwaki, K., Tsunoda, I.* (2022). Bacterial and fungal isolation from face masks under the COVID-19 pandemic. *Scientific Reports*. 12(1). DOI: https://doi.org/10.1038/s41598-022-15409-x

Park et al., 2022b – *Park, A.M., Khadka, S., Sato, F., Omura, S., Fujita, M., Hashiwaki, K., Tsunoda, I.* (2022). Bacterial and fungal isolation from face masks under the COVID-19 pandemic. *Scientific Reports*. 12(1): 1-19. DOI: https://doi.org/10.1038/s41598-022-15409-x

Pullangott et al., 2021 – *Pullangott, G., Kannan, U., Gayathri, S., Kiran, D.V., Maliyekkal, S.M.* (2021). A comprehensive review on antimicrobial face masks: An emerging weapon in fighting pandemics. *RSC Advances*. 11(12): 6544-6576). DOI: https://doi.org/10.1039/d0ra10009a

Zhao et al., 2022 – *Zhao, H., Jatana, S., Bartoszko, J., Loeb, M.* (2022). Nonpharmaceutical interventions to prevent viral respiratory infection in community settings: an umbrella review. *ERJ Open Research*. 8(2). DOI: https://doi.org/10.1183/23120541.00650-2021