ISSN: 3102-0402 (print)

PADMAKANYA JOURNAL OF SCIENCE AND TECHNOLOGY (PJST)
Volume 1, Issue 1
September 2025

Research Article

Microbiological Analysis of Single Use and Reused Surgical Facemasks and Antibiotics Susceptibility Test of Isolates

Amrita Sharma[1], Gita Ghimire[1], Amrit Acharaya[1]
[1] Department of Microbiology, Padmakanya Multiple Campus, Tribhuvan University
Correspondence: amrit.acharya@pkmc.tu.edu.np and amritnep@gmail.com

Article details:

Received: July 14, 2025
Revised: August 27, 2025
Accepted: August 29, 2025

***Keywords*:**
Antimicrobial resistance,
Bacterial Load,
Fungal Contamination,
MRSA,
Surgical facemask

Cite this document as:
Sharma, A., Ghimire, G. & Acharya, A. (2025), Microbiological Analysis of Single Use and Reused Surgical Facemasks and Antibiotics Susceptibility Test of Isolates, *Padmakanya Journal of Science and Technology*, 1(1), 25-34.

Abstract

The use of facemask as a protection measures against the dust and communicable diseases has been a common practice. Surgical mask, in particular provide some form of protection against these threats. However, if used improperly or reused, these masks could become carriers of diseases, counteracting their protective measures. The relation of the mask use frequency and extent of microbial contamination has not been properly explored. This study aims to determine the bacterial and fungal profile, bacterial load and assess the antimicrobial susceptibility of bacteria isolated from the one day and three day used mask. The research was conducted from December 2023 to August 2024 at the Microbiology Laboratory of Padmakanya Multiple Campus. Samples from the inner surface of surgical masks were processed using serial dilution on Plate Count Agar (PCA) for bacterial counts by the spread plate technique, and incubated at 37ºC for 24 hours. Fungi were cultured on Potato Dextrose Agar (PDA) at 25ºC for 5 days. Additional media such as Mannitol Salt Agar, Blood Agar, Chocolate Agar, M-Endo Agar, and Cetrimide Agar were used for bacterial and fungal analysis. Out of 20 masks used for one day, no fungal growth was detected, and 45% (9 masks) showed the presence of *Staphylococcus* spp., and no bacteria were found on the remaining masks. In contrast, 20 facemasks reused for three days yielded 22 bacterial isolates. The predominant bacterial species was *Staphylococcus aureus* (14 isolates, 63.64%), and the most frequent fungal species was *Aspergillus* spp. (12 isolates, 60%). Compared to one-day used masks, the three-day reused masks showed a higher microbial load and diversity. Notably, all *S. aureus* (100%) were methicillin –resistant (MRSA) and showed resistant to multiple β-lactam antibiotics including Penicillin-G, Oxacillin, and Amoxicillin. Gram-negative isolates, however, remained largely sensitive to tested antibiotics. The study highlights increased microbial contamination with prolonged facemask reuse, underscoring the importance of timely replacement and proper mask hygiene to prevent potential health risks.

Introduction

A facemask is a protective covering for the mouth and nose, typically made of cotton or polypropylene (a type of plastic), used by people to protect themselves from infectious agents such as bacteria, viruses, and fungi. After COVID-19, facemasks became a vital piece of personal protective equipment. When the COVID-19 pandemic broke out, many nations introduced the requirements of wearing masks in public areas such as hospitals, colleges, and marketplaces (Martinelli *et al.,* 2021). Facemasks were traditionally used in surgical departments of hospitals to prevent post-surgery infections from spreading through the mouth and nasal cavities of doctors and staff. Before COVID-19, the use of facemasks was not as common among the general public (Ding *et al.,* 2023). The effectiveness of facemasks in controlling respiratory viral transmission has been well documented (Chhabra *et al.,* 2024).

Despite their protective role, facemask can act as reservoirs for microbial contamination. However, frequent use of the mask creates the ideal humidity and temperature for microbial growth. Among the micro-organisms found in masks *Staphylococcus aureus*, *Streptococcus* spp., *Pseudomonas* spp., endospore forming *Bacillus* spp., *Acinetobacter* spp. and *Escherichia coli* are the common causes of serious infections in communities and hospitals. Moreover, fungi like *Aspergillus* spp., *Candida* spp., *Penicillium* spp., *Cladosporium* spp. have also been seen frequently isolated from masks (Kisielinski *et al.,* 2024). This risk increases when marks are often adjusted by hand, transferring microbes from surroundings (Ibarra-Estrada *et al.,* 2022). Cotton masks, in particular retain more moisture than surgical masks, creating conditions favorable microbial growth and persistence (Delanghe *et al.,* 2021).

Although, the presence of microbes on masks is established, research has largely focused on viral protection and respiratory droplets prevention, while microbial contamination and resistance pattern have received less attention. The role of masks as potential fomites, especially when used multiple days without proper cleaning or disposal, has not been adequately explore (Ibarra-Estrada *et al.,* 2022). Given that human saliva actually includes 100 million bacteria per milliliter and is home to several pathobionts, the potential for masks harbor and transmit harmful microorganisms is significant (Delanghe *et al.,* 2021). Yet the bacteria load, species diversity, and antimicrobial resistance of contaminants on reused masks remain poorly studied. Monitoring microbial diversity and antimicrobial resistance in masks is crucial to address the emerging threat of antimicrobial resistance.

Therefore, this study aims to address gap investigating microbial diversity on facemasks worn for a single day and those reused for three consecutive days. Additionally, the study aims to quantify and compare the bacterial load between one-day-used and three-day-reused facemasks. Furthermore, it assesses the antimicrobial susceptibility patterns of the bacterial isolates to understand their potential resistance profiles shedding light on potential health. The findings also provide evidence on the health risks associated with reused facemasks and raise public awareness about safe facemask practices.

Methods

A cross-sectional, descriptive study was conducted among the students of Padmakanya Multiple Campus. A total of surgical mask samples were collected, 20 masks used for a single day (6-10 hours), and 20 masks reused over three consecutive days (Photograph 1 and 2). Used Surgical masks were collected from the students of Padmakanya Multiple Campus. The Masks were carefully folded inward and hold by the ties aside. The collected sample was kept in a zip-lock plastic bag. The collected sample was labeled accordingly by sample code.

The collected sample from the student was brought into the microbiology lab. The inner and outer layer of the masks was separated. The inner part of the mask was taken and kept in 10 ml saline water for 10 minutes. The collected water was further processed to a serial dilution from 10^{-1} to 10^{-3}. Dilution of 10^{-1} was inoculated in Mannitol Salt Agar (MSA), Blood Agar (BA), Chocolate Agar (CA), M-Endo Agar (photograph 3, 4, & 5), Cetrimide Agar and dilution of 10^{-3} was inoculated in Potato Dextrose Agar (PDA) and Plate Count Agar (PCA) by spread plate technique (Cheesbrough, 2006)

Bacteriological Identification

The sample was inoculated in the culture plate and incubated at 37ºC for 24 hours for bacteria. Enumeration of bacteria was done on Plate Count Agar (PCA). All types of colonies were counted on both one day used and three days reused facemask. Then, a comparison of one day used and three days reused facemasks was performed accordingly. Blood Agar was used for the isolation and identification of *Streptococcus* spp. The hemolytic colony was selected, and subculture was done on nutrient agar. Gram staining and biochemical tests were performed to confirm the presence of specific bacteria. For the identification of *Staphylococcus aureus,* Mannitol Salt Agar was used. The yellow fermented colony was selected, and subculture was done on Nutrient Agar. After 24 hours of incubation at 37ºC the subculture plates were taken out and Gram's Staining, Catalase, and Oxidase tests were performed. The Coagulase test was performed as confirmatory test for *Staphylococcus aureus*. Similarly, for the identification of *E.coli,* M-Endo agar was used. The green metallic colony was selected, and a subculture was done on Nutrient Agar. After 24 hours of incubation at 37ºC, the subculture plates were taken out and Gram Staining, catalase, oxidase, and biochemical test were performed. The presence of MR positive, SIM positive, TSIA A/A, which is yellow in colour, was observed in both slant and butt. H2S gas was produced, confirming that the bacteria are *E. coli*. Similarly, for the identification of *Pseudomonas* spp, Cetrimide agar was used. The green colony was selected and subcultured on a Nutrient Agar plate. After 24 hours of incubation at 37ºC, the subculture plate was taken out and Gram staining, catalase, oxidase, and biochemical tests were performed. The presence of Citrate positive and TSIA alkaline/alkaline that is in both slant and butt red colour was observed, and also H_2S gas was produced, which confirms the organisms were *Pseudomonas* spp. (Cheesbrough, 2006).

Fungal Identification

After culturing the sample on PDA, the plates were incubated at 20ºC for 5 days. Fungal colonies were collected using cellophane tape and transferred onto a glass slide containing 2-3 drop of lactophenol cotton blue stain. The prepared slides were observed under a microscope. *Aspergillus* spp., Yeast and *Mucor* spp. were identified from the reused facemask. Fungal identification was done according to hypha and spore morphology. Although some fungi were not identified due to a lack of spore formation (Cheesbrough, 2006).

Antibiotic susceptibility testing

In this process, all identified *Staphylococcus aureus, Streptococcus* spp., *E.coli, Pseudomonas* spp. isolates were subjected to antibiotic susceptibility tests by the modified Kirby-Bauer disc diffusion method. Three to five colonies of bacteria were taken and transferred to a tube containing 5 ml of nutrient broth, mixed gently until a homogenous suspension formed, and then turbidity was observed in the bacterial suspension. Then the tube was incubated for 4 hours at 37ºC. After 4 hours, the tube was taken out from the incubator and the bacterial suspension was then swabbed over the entire surface of the MHA plate using a sterile cotton swab. Antibiotic discs were applied within 15 minutes using sterile forceps on the surface of the medium and

incubated at 37ºC for 24 hours. The zone of inhibition of growth around each disc was then measured in millimeters, and zone diameter interpreted in accordance with standard as sensitive, intermediate, and resistant antibiotics. In this study, the antibiotic disc used are Nitrofurantoin (300mcg), Ampicillin (10mcg), Norfloxacin (10mcg), Cefoxitin (30mcg), Amoxycillin (10mcg), Oxacillin (1mcg), Penicillin-G (10mcg), and Chloramphenicol (30mcg) (CLSI, 2024).

Screening of Methicillin Resistant *S. aureus* (MRSA)

Screening of MRSA was based on disc diffusion method using Cefoxitin (30 mcg). Diameter of zone of inhibition of ≤ 21 mm was considered as methicillin resistant (MRSA) whereas diameter of zone of inhibition ≥ 22 mm was considered as Methicillin Susceptible (MSSA)(Magiorakos *et al.,* 2012 and CDC, 2006).

Screening of MDR

When the organisms show resistance to more than three different class of antibiotics was taken as multi-drug-resistant isolates (Magiorakos *et al.,* 2012 and CDC, 2006). In this study, three different classes of antibiotics were chosen. They are Nucleic acid inhibitors (Nitrofurantoin, Norfloxacin), Protein Synthesis (Gentamicin), Cell-wall inhibitor (Ampicillin, Cefoxitin).

Table 1: Total bacterial count in 1 day used mask and three days reused mask

Sample (Code No.)	One day used mask (cfu/ml)	Three days reused mask (cfu/ml)
(M01)	4.6×10^4	3.48×10^5
(M02)	4.8×10^4	3.28×10^5
(M03)	5.5×10^4	2.88×10^5
(M04)	6.0×10^4	2.64×10^5
(M05)	4.8×10^4	2.56×10^5
(M06)	4.7×10^4	2.52×10^5
(M07)	6.1×10^4	2.96×10^5
(M08)	6.2×10^4	2.88×10^5
(M09)	4.4×10^4	2.72×10^5
(M10)	4.2×10^4	2.56×10^5
(M11)	4.7×10^4	3.12×10^5
(M12)	4.9×10^4	3.00×10^5
(M13)	5.2×10^4	2.68×10^5
(M14)	5.5×10^4	2.64×10^5
(M15)	5.2×10^4	2.52×10^5
(M16)	5.0×10^4	2.48×10^5
(M17)	4.9×10^4	2.48×10^5
(M18)	5.1×10^4	3.08×10^5
(M19)	4.8×10^4	2.64×10^5
(M20)	4.4×10^4	2.52×10^5

Growth status of bacteria in used masks

Out of 20 samples of facemask used for one day that was collected for the study, 45% showed bacteria growth positive. All the growth-positive isolated bacteria were *Staphylococcus* spp. Similarly, in three-day reused surgical masks, bacterial growth was observed in all samples (100%).

Quality control

Quality control and regular evaluation of laboratory equipment, reagents, and media were done before performing experiments. For the standardization of the Kirby-Bauer test for the performance testing of the antibiotics and MHA, the control strain of *S. aureus* was tested primarily. The strict aseptic condition was maintained while carrying out all the procedures (Cheesbrough, 2006).

Data analysis

All the raw data obtained from the laboratory investigations were tabulated and presented in a defined table and were statistically analyzed by using MS Excel for frequency calculation. The collected samples were categorized into one day used mask and three days reused mask.

RESULTS

Total bacterial count in surgical mask

For the counting of bacteria, serial dilution was done from 10^{-1} to 10^{-3}. From 10^{-3} the bacteria were inoculated in plate count agar. The total bacterial count in one day used masks ranged from 4.2×10^4 to 6.2×10^4 CFU/ml and three days reused masks ranged from 2.48×10^5 to 3.48×10^5 CFU/ml (Table 1).

Out of 20 reused surgical mask for three days, in which 22 bacteria were isolated and identified, from the sample, 4 different species of bacteria were detected. Among all the samples, the highest number of bacteria isolated was *Staphylococccus aureus* 14/22 (63.64%) followed by *Streptococcus* spp. 3/22 (13.64%) (Figure 1 and photograph 6).

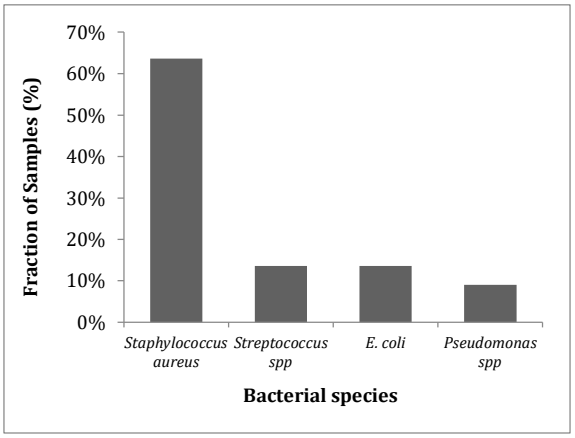


Figure 1: Frequency of bacteria in three days reused mask

Isolation of fungi

In all the 20 samples of facemask that were used for a day, no fungal growth was reported. However, the fungus grew in the mask that were used for three days. Among all the colonies observed, three species of fungi were observed. They were *Aspergillus* spp., (Photograph 8) *Mucor* spp. and Yeast. Among isolated species of fungi, the highest number of fungi was

Aspergillus spp. 12/20 (60%) followed by *Mucor* spp. 5/20 (25%) (Table 2).

Table 2: Number of samples with isolated fungi

Organism	No. of sample
Aspergillus spp.	12/20 (60%)
Mucor spp.	5/20 (25%)
Yeast	1/20 (5%)
No fungal growth	2/20 (10%)

Antibiotic susceptibility pattern of Staphylococcus aureus isolates

The most susceptible drugs for *S. aureus* were Gentamicin (100%) (Table 3). Likewise, *S. aureus* was resistant to Cefoxitin (100%), Penicillin-G (100%), Oxacillin (92.85%), and Amoxycillin (92.85%). Similarly, *S. aureus* was intermediate to Amoxycillin (7.14%) and Oxacillin (7.14%). This confirms Gentamicin is significantly more effective while others show high resistance.

Table 3: Antibiotic Susceptibility pattern for *Staphylococcus aureus* isolates

Organism	Antibiotics	Disc content	Antibiotic Susceptibility Pattern		
			Sensitive	Intermediate	Resistant
S. aureus (n=14)	Gentamicin	(30mcg)	14(100%)	-	(0%)
	Amoxycillin	(10mcg)	(0%)	(7.14%)	13(92.85%)
	Oxacillin	(1mcg)	(0%)	(7.14%)	13(92.85%)
	Cefoxitin	(30mcg)	(0%)	-	14 (100%)
	Penicillin-G	(10mcg)	(0%)	-	14 (100%)

Antibiotic susceptibility pattern of *Streptococcus* spp. isolates

The most susceptible drugs for *Streptococcus* spp. were found Gentamicin (100%). Likewise, *Streptococcus* spp. was resistant to Cefoxitin (100%), Penicillin-G (100%), Oxacillin (100%) and, Amoxycillin (100%) (Photogragh 7). This confirms *Streptococcus* spp. is significantly more resistant to Amoxycillin, Oxacillin, Cefoxitin, and Penicillin-G compare to Gentamicin (Table 4).

Table 4: Antibiotic Susceptibility pattern for *Streptococcus* spp isolates

Organism	Antibiotics	Disc content	Antibiotic Susceptibility Pattern		
			Sensitive	Intermediate	Resistant
Streptococcus	Gentamicin	(30mcg)	3(100%)	-	(0%)
Spp N=3	Amoxycillin	(10mcg)	(0%)	-	3(100%)
	Oxacillin	(1mcg)	(0%)	-	3(100%)
	Cefoxitin	(30mcg)	(0%)	-	3(100%)
	Penicillin-G	(10mcg)	(0%)	-	3(100%)

Antibiotic susceptibility pattern of *Pseudomonas* spp. isolates

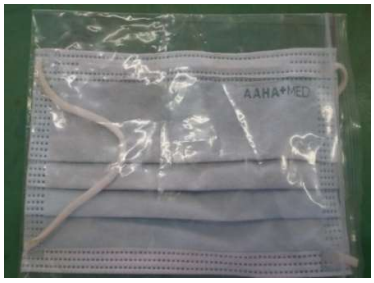
The most susceptible drugs for *Pseudomonas* spp. were found Gentamicin (100%) and Norfloxacin (100%). Likewise, *Pseudomonas* spp. was resistant to Oxacillin (100%), Ampicillin (100%), and Chloramphenicol (50%). Similarly, *Pseudomonas* spp. was intermediate to Chloramphenicol (50%). *Pseudomonas* spp. exhibits high variability in resistance. Gentamicin and norfloxacin are significantly more effective compared to Oxacillin and Ampicillin (one way –ANOVA, P=0.0025, F=21.00) as shown below in Table 5.

Table 5: Antibiotic Susceptibility pattern for *Pseudomonas* spp. Isolates

Organism	Antibiotics	Disc content	Antibiotic Susceptibility Pattern		
			Sensitive	Intermediate	Resistant
Pseudomonas	Gentamicin	(30mcg)	2(100%)	-	(0%)
Spp. N=2	Chloramphenicol	(30mcg)	(0%)	1(50%)	1(50%)
	Norfloxacin	(10mcg)	2(100%)	-	(0%)
	Oxacillin	(1mcg)	(0%)	-	2(100%)
	Ampicillin	(10mcg)	(0%)	-	2(100%)

Photographs

Photograph 1: 1 day used mask sample

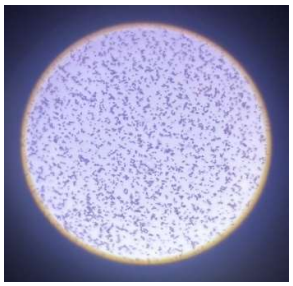


Photograph 2: 3 days reused mask sample

Photograph 3: Beta-hemolytic colony observed in blood agar

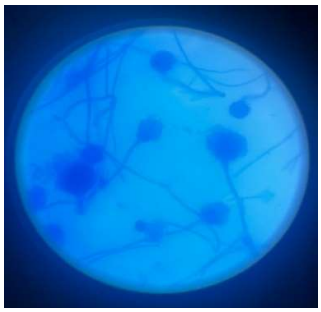
Photograph 4: Yellow fermented colony of *S. aureus* in MSA

Photograph 5: Green metallic colony of *E. coli* in M-Endo agar



Photograph 6: *Streptococcus* spp. observed under 100x oil immersion

Photograph 7: AST of isolated *Streptococcus* Spp. with antibiotic Gentamicin, Cefoxitin, Amoxycillin, Oxacillin and Penicillin-G



Photograph 8: *Aspergillus* spp. observed under 40x

Antibiotic susceptibility pattern of *E. coli* isolates

Norfloxacin (100%), Nitrofurantoin (66.67%) and Gentamicin (66.67%) were found to be the most effective drugs against *E. coli* (Table 6). Likewise, *E. coli* was resistant to cefoxitin (100%), Ampicillin (100%). Similarly, *E. coli* was intermediate to Nitrofurantoin (33.33%) and Gentamicin (33.33%). *E. coli* shows varying resistance levels to different antibiotics. (F-statistic= 21.75, p=0.00006, One way-ANOVA). Ampicillin and Cefloxitin both show significantly higher resistance to Gentamicin, Norfloxacin and Nitrofurantoin, with mean difference 0.0 (95% CI: -1.5193 to 1.5193 (turkey's post hoc test).

Table 6: Antibiotic Susceptibility pattern for *E. coli* isolates

Organism	Antibiotics	Disc content	Antibiotic Susceptibility Pattern		
			Sensitive	Intermediate	Resistant
	Gentamicin	(30mcg)	2(66.67%)	1(33.33%)	(0%)
	Norfloxacin	(10mcg)	3(100%)	-	(0%)
E. coli	Ampicillin	(10mcg)	(0%)	-	3(100%)
N=3	Nitrofurantoin	(300mcg)	2(66.67%)	1(33.33%)	(0%)
	Cefoxitin	(30mcg)	(0%)	-	3(100%)

Distribution of MRSA among *S.aureus* isolates

Among 22 isolates, 14(63.64%) were *S.aureus*. Out of total *S. aureus* isolates, 14 (100%) were Methicillin Resistant *S. aureus* (MRSA).

Distribution of MDR among Gram negative isolates

Among 22 positive isolates, 5(22.7%) were Gram negative bacteria. All the Gram-negative isolates 5/5; 100%) shows no multidrug resistance.

DISCUSSION

Facemasks are used worldwide and become daily used article of clothing accessories. Even in today, Facemasks continue to be widely manufactured and marketed in broad range of forms, shapes, and materials. They are still regarded as one of the primary measures for protection and control over the disease. This can be accomplished with a variety of masks available on the market. Simplified masks that cover the mouth and nose and retain droplets are the main means of avoiding transmission. This is useful when it is not feasible to keep the 1.5 m minimum gap. Only a small level of self-defense is provided by the masks when worn properly (Matuschek *et al.,* 2020).

In this study, *Staphylococcus* spp. was isolated from 45% of the samples of facemask used for a single day. This was only the bacterial species that was isolated in the study from a single day used mask. Staphylococcus spp. was the commonly collected bacteria in a study conducted by Lee *et al.* (2021), where he identified *Staphylococcus* spp. as the most common pathogenic bacterium in the 27 k-94 mask used for a single day by 208 healthy adults. This suggests that short-term masks used facilitate the growth of skin-derived bacteria such as *Staphylococcus* spp. which are naturally present on human skin and mucous membranes.

In this study, out of 20 reused surgical masks for three days, 22 bacteria were isolated and identified, and 4 different species of bacteria were detected. Among all the samples, the highest number of bacteria isolated was *Staphylococccus aureus* 14/22 (63.64%), followed by *Streptococcus* spp. 3/22 (13.64%), *E. coli* 3/22 (13.63%), and least isolated were *Pseudomonas* spp. 2/22 (9.09%). *Staphylococcus aureus* is a well-known bacterium linked to various diseases, especially in immunocompromised individuals, making this a remarkable discovery. Because *E. coli* and *Pseudomonas* species are not usually associated with the human skin microbiome and are frequently linked to more serious illnesses, their presence is especially worrying. Their existence implies that prolonged use of masks may introduce and harbor germs from external sources, possibly because of improper handling or storage of the mask. In contrast, according to Nightingale *et al.* (2022), 69 facemasks were collected from the staff. The employment categories of facemask users were nursing assistant (22.73%), nurse (12.12%), and administrative or other (37.88%). Among 69 facemasks, 14.3% were contaminated with *Klebsiella pneumoniae*, 13.0% with *Enterobacter* spp., and 4.2% with *Escherichia coli*. In nursing home healthcare workers, facemasks were contaminated with many pathogenic and non-pathogenic microorganisms. This contamination causes a risk for transmission, if facemasks are not properly used or not properly disposed of after wearing. Similarly, according to Yousefimashouf *et al.* (2023), research conducted in 175 masks from staff working in Sina hospital, nine species were detected in which 471 bacterial isolates were found. The most isolated bacteria were coagulase-negative *Staphylococcus* spp. (28%) followed by *Acinetobacte*r spp. (20.8%), *Pseudomonas* spp. (13.8%), *Klebsiella* spp. (3.8%) and *Enterococcus* spp. (1.2%) were the least frequently found species. Comparing this research with research done in this study, out of 20 sample masks, 22 isolates of bacteria including four species were detected in which *Staphylococcus aureus* (63.64%) were followed by *Streptococcus* spp. (13.64%), *E. coli* was (13.63%), and *Pseudomonas* spp. (9.09%).

According to Monalisa *et al.* (2017), research conducted in 36 surgical masks, there were the highest isolates of bacteria (54%) of *E. coli* followed by *S. aureus* (25%), *Klebsiella* spp. (5%), *Enterococcus* spp. (4%), *Pseudomonas* spp. (3%), *Enterobacter* spp. (2%) were found.

Moreover, the study observed no fungal growth in one day used masks, but three day reused masks exhibited the growth of three fungal species in 20 sample. There are 12/20(60%) *Aspergillus* spp., 5/20(25%) *Mucor* spp., 1/20 (5%) yeast and 2/20(10%) have no fungal growth. In the fungal isolates, 60% were *Aspergillus* spp., making it the most common species. According to studies, fungi, especially molds such as *Aspergillus*, grow vigorously in warm, humid environments, which are often found in masks worn for extended periods. Particularly in people with underlying medical conditions like asthma or chronic obstructive pulmonary disease (COPD), these fungi may be harmful to lung health. In contrast, according to Merad *et al.* (2023), 52 forensic healthcare practitioners who used facemasks were collected for fungal isolation and identification. 1-2 hours used facemasks contain 4% of fungal contamination, whereas 5-6 hours used facemasks contain 36% of fungal contamination. The most isolated fungi were *Alternaria* spp. (32%), *Penicillium* spp. (20%) *Aspergillus* spp. (16%) found inside the area of the masks. According to Monalisa *et al.,* (2017), research conducted on 36 surgical masks found that the highest isolates of fungi were *Candida* (6%) and *Aspergillus* (1%).

Using masks for one day resulted in a total bacterial count of 4.2×10^4 to 6.2×10^4 CFU/ml; however, after three days, the counts were much higher, ranging from 2.48×10^5 to 3.48×10^5 CFU/ml. These findings align with research showing a gradual increase in microbial colonization in Facemasks. The sharp increase in bacteria highlights the importance of regularly replacing masks and practicing good hygiene to lower the risk of microbial contamination. In contrast, research conducted by Ding *et al.,* (2023) The total number of bacteria on the inner surface of the mask worn for 0.5 hours, 1 hour, 2 hours, 4 hours, and 5 hours was found to be 69 CFU/ml, 91.3 CFU/ml, 159.6 CFU/ml, 219 CFU/ml, and 879 CFU/ml, respectively. Similarly, research conducted by Delanghe *et al.* (2021) in 13 healthy volunteers facemask after 4 hours of wearing were taken. Cotton mask contains 1.46×10^5 CFU/mask and surgical masks contains 1.32×10^4 CFU/mask. This suggests that when masks are reused for long period of time shows more number of bacteria.

Similarly comparing the result of antimicrobial susceptibility from Yousefimashouf *et al.* (2023) 75% *Staphylococcus aureus* isolates were resistant to Erythromycin, and they show very good susceptibility pattern to ciprofloxacin (81.2%) but in this research (63.64%) of *Staphylococcus aureus* showed completely resistant to Cefoxitin (100%), Penicillin-G (100%), Oxacillin (92.85%) and Amoxycillin (92.85%). Similarly, 9.09% of *Pseudomonas* spp. showed completely resistant to oxacillin (100%), Ampicillin (100%), and Chloramphenicol (50%) and sensitive to Gentamicin (100%) and Norfloxacin (100%). Comparing these results with result of Yousefimashouf *et al.* (2023) shows that *Pseudomonas* shows the highest resistance to Erythromycin (73.8%) and are sensitivity to Gentamycin (61.5%). For *E. coli* out of (13.63%) isolates, it shows completely resistant to cefoxitin (100%) and Ampicillin (100%) and sensitive to Norfloxacin (100%), Nitrofurantoin (66.67%), and Gentamicin (66.67%). Comparing these results with Yousefimashouf *et al.* (2023), *E. coli* shows highest resistance by Ciprofloxacin (66.6%), and lowest resistance is shown by Ampicillin (9.08%). Likewise, (78.4%) of the *E. coli* strains were susceptible to Gentamycin and Ampicillin antibiotics and lowest sensitivity is shown by Ciprofloxacin (21.5%).

Among 22 isolates, 14 (63.64%) were *S. aureus*. Out of the total *S. aureus* isolates 14(100%) were Methicillin Resistant *S. aureus* (MRSA) and 0% were Methicillin Sensitive *S. aureus*. Among 22 positive isolates, 5 were Gram-negative organisms. Out of the total Gram-negative organism isolates, 5 (100%) show no MDR.

Masks that are only used once have sparked concerns. It has been discovered that masks can retain a considerable number of germs even after only one use. Bacterial retention on masks may be influenced by external factors, respiratory moisture content, and the length of usage, resulting in significant bacterial loads that have been found on masks after repeated usage. These investigations have unsettlingly shown that these masks have a substantial incidence of germs, including *Streptococcus* and *Staphylococcus* species. This implies that bacterial contamination may worsen if repurposed masks are handled, cleaned, or stored improperly (Delanghe *et al.,* 2021).

This study acknowledges certain limitations. Variation in mask types, sample sizes, and research designs may limit the generalizability of the findings. There absence of selective media for *Hemophilus* restricted the detection of this organism. Further research with larger samples and broader microbial screening is required to better comprehend the relationship between bacterial load and mask reused.

Conclusion

This study demonstrates that microbial contamination is significantly higher in reused surgical masks compared to those used for a single day. Prolonged use increases both the diversity and quantity of bacterial and fungal pathogens, including *Staphylococcus aureus*, *Streptococcus* species, *E. coli* and *Pseudomonas* spp., *Aspergillus* spp., *Mucor* spp. and Yeast. These findings highlight the importance of proper mask hygiene and caution against extended or repeated use of disposable masks due to increased risk of microbial exposure and potential health hazards. The greater contamination on reused facemasks indicates their potential role in

disease transmission. In the inner layer of a surgical mask, detection of Methicillin-Resistant *S. aureus* (MRSA) suggests the possibility of nasal MRSA carriage, so it is necessary to examine and give proper treatment to eliminate antibiotic-resistant bacteria.

A High rate of contamination occurs in mask after repeated used of the same mask; therefore, public should avoid reusing mask. Public health authorities should establish thorough guidelines on proper mask use, emphasizing correct practices, following hygienic measures and timely changing out single-use masks. Additionally, both public and healthcare professionals should be informed about the potential risks associated with reusing masks.

Acknowledgements

We would like to express our heartfelt gratitude to Mrs. Bindra Devi Shakya, Coordinator, and Mrs. Neena Karmacharya, Assistant Campus Chief for granting us this invaluable opportunity. Our sincere thanks also go to Asst. Prof. Charu Arjyal, Director, and all the faculty members and staffs of Department of for their unweaving support, generosity and cooperation throughout this journey.

CRediT Author Statement

AS: Conceptualization, Methodology, Software, Formal analysis, Investigation, Resources, Data Curation, Writing- Original Draft, Writing- Review & Editing, Visualization, Project administration; **GG**: Conceptualization, Writing- Review & Editing, Visualization, Supervision; **AA**: Conceptualization, Software, Formal analysis, Resources, Data Curation, Writing- Original Draft, Writing- Review & Editing, Visualization

Conflicts Of Interest

The author declare there are no conflicts of interest regarding the publication of this paper.

References

Bard, J. D., Hindler, J. A., Gold, H. S., & Limbago, B. (2014). Rationale for Eliminating Staphylococcus Breakpoints for β-Lactam Agents Other Than Penicillin, Oxacillin or Cefoxitin, and Ceftaroline. *Clinical Infectious Diseases : An Official Publication of the Infectious Diseases Society of America*, *58*(9), 1287–1296. https://doi.org/10.1093/cid/ciu043

Cheesbrough, M. (2006). *District Laboratory Practice in Tropical Countries* (2nd ed.).Cambridge University Press. https://doi.org/10.1017/CBO9780511543470

Cheng, V. C.-C., Wong, S.-C., Chuang, V. W.-M., So, S. Y.-C., Chen, J. H.-K., Sridhar, S., To, K. K.-W., Chan, J. F.-W., Hung, I. F.-N., Ho, P.-L., & Yuen, K.-Y. (2020). The role of community-wide wearing of Facemask for control of coronavirus disease 2019 (COVID-19) epidemic due to SARS-CoV-2. *The Journalof Infection*, *81*(1), 107–114. https://doi.org/10.1016/j.jinf.2020.04.024

Chhabra, M. (2024). Microbial Detection from Used Facemasks and Hygiene Practices. *Journal of Communicable Diseases,55,111-118*. https://doi.org/10.24321/0019.5138.202363

Chughtai, 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),e200948. https://doi.org/10.3201/eid2610.200948

Clinical and Laboratory Standards Institute. (2024). *Performance standards for antimicrobial susceptibility testing* (34th ed., CLSI supplement M100). Wayne, PA: CLSI. Retrieved from https://clsi.org/about/news/clsi-publishes-m100-performance-standards-for-antimicrobial-susceptibility-testing-34th-edition.

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 Facemasks in Community Settings: Bacterial Contamination and Facemask Hygiene. *Frontiers in Medicine*, *8*, 732047. https://doi.org/10.3389/fmed.2021.732047

Ding, G., Li, G., Liu, M., Sun, P., Ren, D., Zhao, Y., Gao, T., Yang, G., Fang, Y., & Li, W. (2023). Bacterial contamination of medical Facemask wearing duration and the optimal wearing time. *Frontiers in Cellular and Infection Microbiology*, *13*, 1231248. https://doi.org/10.3389/fcimb.2023.1231248

Hiramatsu, K., Ito, T., Tsubakishita, S., Sasaki, T., Takeuchi, F., Morimoto, Y., Katayama, Y., Matsuo, M., Kuwahara-Arai, K., Hishinuma, T., & Baba, T. (2013). Genomic Basis for Methicillin Resistance in Staphylococcus aureus. *Infection&Chemotherapy*,*45*(2),117–136. https://doi.org/10.3947/ic.2013.45.2.117

Ibarra-Estrada, M. Á., García-Salas, Y., Mireles-Cabodevila, E., López-Pulgarín, J. A., Chávez-Peña, Q., García-Salcido, R., Mijangos-Méndez, J. C., & Aguirre-Avalos, G. (2022). Use of Airway Pressure Release Ventilation in Patients With Acute Respiratory Failure Due to COVID-19: Results of a Single-Center Randomized Controlled Trial*. *Critical Care Medicine*, *50*(4), 586. https://doi.org/10.1097/CCM.0000000000005312

Kisielinski, K., Wojtasik, B., Zalewska, A., Livermore, D. M., & Jurczak-Kurek, A. (2024). The bacterial burden of worn Facemasks-observational research and literature review. *Frontiers in Public Health*, *12*, 1460981. https://doi.org/10.3389/fpubh.2024.1460981

Kumar, N. R., Balraj, T. A., Kempegowda, S. N., & Prashant, A. (2024). Multidrug-Resistant Sepsis: A

Critical Healthcare Challenge. *Antibiotics*, *13*(1), Article 1. https://doi.org/10.3390/antibiotics13010046

Kumar, V. V., Thomas, A. M., Saji, L. A., & Sebastian, S. R. (2023). Facemasks: Boon or Bane? A Multimethod Study on Utilization Pattern and Short-term Effects of Facemasks on Adults in Central Kerala. *Dentistry and Medical Research*, *11*(1), 12. https://doi.org/10.4103/dmr.dmr_14_23

Lee, A. W. L., Neo, E. R. K., Khoo, Z.-Y., Yeo, Z., Tan, Y. S., Chng, S., Yan, W., Lok, B. K., & Low, J. S. C. (2021). Life cycle assessment of single-use surgical and embedded filtration layer (EFL) reusable Facemask. *Resources, Conservation and Recycling*, *170*, 105580. https://doi.org/10.1016/j.resconrec.2021.105580

Lucke, K., Hombach, M., Hug, M., & Pfyffer, G. E. (2010). Rapid Detection of Methicillin-Resistant Staphylococcus aureus (MRSA) in Diverse Clinical Specimens by the BD GeneOhm MRSA Assay and Comparison with Culture. *Journal of Clinical Microbiology*, *48*(3), 981–984. https://doi.org/10.1128/JCM.01990-09

Luksamijarulkul, P., Aiempradit, N., & Vatanasomboon, P. (2014). Microbial Contamination on Used Surgical Masks among Hospital Personnel and Microbial Air Quality in their Working Wards: A Hospital in Bangkok. *Oman Medical Journal*, *29*(5), 346–350. https://doi.org/10.5001/omj.2014.92

Magiorakos, A.-P., Srinivasan, A., Carey, R. B., Carmeli, Y., Falagas, M. E., Giske, C. G., Harbarth, S., Hindler, J. F., Kahlmeter, G., Olsson-Liljequist, B., Paterson, D. L., Rice, L. B., Stelling, J., Struelens, M. J., Vatopoulos, A., Weber, J. T., & Monnet, D. L. (2012). Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: An international expert proposal for interim standard definitions for acquired resistance. *Clinical Microbiology and Infection*, *18*(3), 268–281. https://doi.org/10.1111/j.1469-0691.2011.03570.x

Manager, J. (2023). Evaluation of the Microbiota and Integrity of Respirators Reused by Health Professionals in a Hospital Environment. *Aerosol and Air Quality Research*, *23*(4), 220057. https://doi.org/10.4209/aaqr.220057

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

Matuschek, C., Moll, F., Fangerau, H., Fischer, J. C., Zänker, K., van Griensven, M., Schneider, M., Kindgen-Milles, D., Knoefel, W. T., Lichtenberg, A., Tamaskovics, B., Djiepmo-Njanang, F. J., Budach, W., Corradini, S., Häussinger, D., Feldt, T., Jensen, B., Pelka, R., Orth, K., … Haussmann, J. (2020). Facemasks: Benefits and risks during the COVID-19 crisis. *European Journal of Medical Research*, *25*(1), 32. https://doi.org/10.1186/s40001-020-00430-5

Merad, Y., Belmokhtar, Z., Hadjazi, O., Belkacemi, M., Matmour, D., Merad, Z., Bassaid, A., & Megherbi, O. (2023a). Fungal contamination of medical masks among forensic healthcare workers in the COVID19 era. *New Microbes and New Infections*, *53*. https://doi.org/10.1016/j.nmni.2023.101134

Merenstein, C., Fitzgerald, A. S., Khatib, L. A., Graham-Wooten, J., Bushman, F. D., & Collman, R. G. (2023). Effects of Mask Reuse on the Oropharyngeal, Skin, and Mask Microbiome. *The Journal of Infectious Diseases*, *228*(4), 479–486. https://doi.org/10.1093/infdis/jiad167

Methicillin-resistant Staphylococcus aureus carrying the mecC gene: Emergence in Spain and report of a fatal case of bacteraemia | Journal of Antimicrobial Chemotherapy | *Oxford Academic*. (n.d.). Retrieved August 25, 2024, from https://academic.oup.com/jac/article/69/1/45/854702

Milton, D. K., Fabian, M. P., Cowling, B. J., Grantham, M. L., & McDevitt, J. J. (2013). Influenza virus aerosols in human exhaled breath: Particle size, culturability, and effect of surgical masks. *PLoS Pathogens*, *9*(3), e1003205. https://doi.org/10.1371/journal.ppat.1003205

Nightingale, M., Mody, M., Rickard, A., & Cassone, M. (2022). Bacterial contamination on used Facemasks in healthcare personnel. *Antimicrobial Stewardship & Healthcare Epidemiology*, *2*(S1), s86–s87. https://doi.org/10.1017/ash.2022.220

Nikaido, H. (2009). Multidrug Resistance in Bacteria. *Annual Review of Biochemistry*,*78*,119–146. https://doi.org/10.1146/annurev.biochem.78.082907.145923

Nrior, R. R., Egbuchelem, A., & Dike, V. N. (2022). Microbiological Assessment of Used Facemasks in Port Harcourt Secondary Schools in COVID-19 Era. *Microbiology Research Journal International*, 22–30. https://doi.org/10.9734/mrji/2022/v32i430381

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

Soleman, S. R., Lyu, Z., Okada, T., Sassa, M. H., Fujii, Y., Mahmoud, M. A. M., Ebner, D. K., & Harada, K. H. (n.d.). Efficacy of personal protective equipment to prevent environmental infection of COVID-19 among healthcare workers: A systematic review. *Environmental Health and Preventive Medicine*, *28*, 1. https://doi.org/10.1265/ehpm.22-00131

Tcharkhtchi, A., Abbasnezhad, N., Zarbini Seydani, M., Zirak, N., Farzaneh, S., & Shirinbayan, M. (2020). An overview of filtration efficiency through the masks: Mechanisms of the aerosols penetration. *Bioactive Materials*, *6*. https://doi.org/10.1016/j.bioactmat.2020.08.002

Tough Year 2006 for the USEPA and the CDC: Leadership Lapses?: Human and Ecological Risk Assessment: An International Journal: Vol 13 , No 1—Get Access. (n.d.). Retrieved August 25, 2024, from https://www.tandfonline.com/doi/full/10.1080/10807030601179758

Yousefimashouf, M., Yousefimashouf, R., Alikhani, M. S., Hashemi, H., Karami, P., Rahimi, Z., & Hosseini, S. M. (2023a). Evaluation of the bacterial contamination of Facemasks worn by personnel in a center of COVID 19 hospitalized patients: A cross-sectional study. *New Microbes and New Infections*, *52*, 101090. https://doi.org/10.1016/j.nmni.2023.101090