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## Original article

# Antibiotic sensitivity profile of microorganisms isolate from used and unused nose maske

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## ABSTRACT

**Background:** Nose masks may harbor organisms found on the skin and nasal region that may become opportunistic or vehicles for disease transmission. This investigation was designed to identify the patterns of bacteria and fungi isolated from used and unused nose masks regarding antibiotic susceptibility. **Methods:** A total of 25 used nose mask samples and 50 unused nose masks were obtained from the Akure South region of Ondo State. Antimicrobial sensitivity testing was carried out on the isolated microorganisms. Most of the organisms isolated were known as human microflora. **Results:** The most predominant organisms isolated were *Bacillus* sp. (bacteria) and *Aspergillus* sp. (fungi). Results revealed that *Bacillus* was sensitive to the drugs administered for Gram-positive bacteria, while *Micrococcus* was resistant. Also, the Gram-positive bacteria showed high sensitivity to septrin and erythromycin but were resistant to zinnacef. *Proteus* exhibited high sensitivity to the antibiotics administered for Gram-negative bacteria, while *Yersinia* was resistant. The Gram-negative bacteria were highly sensitive to septrin, but resistant to ciprofloxacin and tarvid. **Conclusions:** Generally, antibiotics were more effective against Gram-positive bacteria than Gram-negative bacteria. The antifungal assay demonstrated that *Neurospora sitophila* was resistant to the administered antifungal drugs, while *Mucor mucedo* showed high susceptibility to antifungal drugs.

## Introduction

Any condition that results in the person experiencing discomfort, dysfunction, distress, social problems, or death or has a similar effect on those who come into contact with the person is referred to as a disease. When a human being's vital functions are interrupted or altered, it affects their natural conditions. Since the days of the human hunter-gatherer, infectious diseases have occasionally broken out. But as society moved toward stable community life, they became more noticeable. Infectious diseases are mostly caused by

pathogenic organisms such as viruses, bacteria, fungi, protozoa, and helminths. These pathogenic organisms are thought to be responsible for one-fourth of all annual deaths worldwide [1]. Flu and other viruses, including the human coronavirus, the flavivirus, the filoviridae, and the Russian flu of 1889, are mostly to blame for significant epidemics and pandemics. The four contagious diseases that shock the world were COVID-19, AIDS, Ebola, and Influenza. According to the World Health Organization [2], annual epidemics and sporadic pandemics are brought on by respiratory diseases

caused by seasonal influenza viruses three to four times per century. According to estimated studies, up to 50% of Ebola cases occurred in facilities, and AIDS, which still has no effective treatment, is still a significant global public health issue. Also, since COVID-19 began, there have now been more than 767 million cases worldwide (12 July 2023). When preexisting microbial strains undergo mutation or re-assortment, more potentially infectious resistant strains arise, occasionally rendering vaccinations and medicines ineffective [3].

Non-pharmaceutical interventions such as hand washing, personal protective equipment (PPE), isolation, quarantine, personal hygiene, use of disinfectants, and social distancing have been implemented to control or delay the spread of infection [4,5]. Most nations have mandated or encouraged their citizens to wear face masks in public areas [6]. However, there are several issues with using a conventional face mask, including the ability of microbes to survive on the surface of the mask, the re-aerosolization of settling particles, the proper handling and disposal of used face mask [7], and the transmission of fomite.

Before the development of COVID-19, face masks were commonly used based on significance in the workplace by construction workers, health workers, waste management agencies, etc. The face mask helps prevent dust, smell, and environmental contamination. Also, face masks have been used during winter to avoid contamination due to aerosols that are known to be widely spread during this period [7].

Face mask is being recommended more advised or even required in public places other than hospitals and care institutions [8] to lessen the spread of respiratory viruses from infected individuals to healthy individuals; surgical or cotton-manufactured devices are most frequently utilized [5]. Policymakers encourage the use of cotton-made masks as personal protective equipment. Non-surgical masks are regarded as non-standardized and not meant for usage by health care professionals in contrast to medical masks [9]. The effectiveness of face masks against various airborne transmission is best studied in controlled environments, such as when worn in hospitals by qualified personnel [10].

Face masks have been recognized as effective in preventing the spread of infectious diseases. The COVID-19 pandemic, which is still

ongoing, shows the same importance of use. Face masks play an important part in pandemic preparedness, and they have undergone material and design changes to meet evolving safety and comfort standards. However, most face masks sold commercially are made with a single use in mind. Due to the huge amount of dangerous waste generated, the possibility of secondary transmission, and the possibility of cross-contamination, the widespread usage of disposable face masks may present a serious environmental concern. The aim of this research was to assess the types and loads of bacteria and fungi isolated from used and unused nose masks and determine the patterns of the bacterial and fungal isolates to antimicrobials such as antibiotics and antifungals.

## Materials and methods

### Sampling site and collection of samples

The investigation was conducted in Akure, Ondo State, Nigeria (**Figure 1**). The capital and main city of Ondo State is Akure, located in southwest Nigeria, between latitudes 7° 15'0 N and 5°11'42 E, with a land area of 14,793 square kilometers.

In this study, 25 used nose mask samples, both sterile and cotton-made, were randomly collected from consenting individuals between December 2021 and January 2022 but were isolated within 4-12 hours of collection. Also, 50 unused nose masks were obtained from the FUTA south gate in Akure, including disposable and cotton-made masks.

### Media preparation and sterilization

The manufacturer's instructions were followed in preparing all the media used. Dehydrated nutrient agar (2.8 g), mannitol salt agar (11.1 g), MacConkey agar (5 g), potato dextrose agar (3.9 g), Sabouraud dextrose agar (3.9 g), Urease agar (2.451 g), Simmon citrate agar (2.428 g), sulphide, indole, motility (SIM) agar (3.623 g), Triple sugar iron (TSI) agar (6.403 g) was dissolved individually in 100 mL of distilled water in a conical flask. The mixture was sterilized in the autoclave for 15 minutes at 121°C, after which a 45°C cooling period was allowed for the media and then aseptically poured into sterile Petri dishes. Used and unused nose mask samples were immersed in 100 mL of sterilized water. The water suspension was allowed to stand for 30 minutes to make a stock culture, then homogenized for 30 seconds and serial dilutions were made.

Sterile techniques, including the use of sterile tools and gloves, were employed during handling of

samples. Equipment used for sample processing underwent thorough sterilization through autoclaving before each use. Regular cleaning and disinfection of work surfaces and tools were carried out to prevent any potential contamination. Sample processing was performed in a controlled condition to minimize the risk of cross contamination. The incubators used were properly monitored. These measures were taken to maintain the integrity of our samples and to ensure the accuracy of the antibacterial sensitivity profile presented in the manuscript.

#### **Isolation of bacteria from nose mask samples**

From the serially diluted samples, 1 mL each was aseptically taken from 10<sup>-2</sup> dilution of the nose mask samples with a syringe and dispersed into sterile petri dishes, after which mannitol salt, MacConkey and nutrient agar were poured in the plates aseptically. The poured plate was allowed to solidify, and after being turned over, the plates were kept at 37°C for 24 hours. The morphological characteristics of the isolates were recorded [11]. After 24 hours, individual colonies of bacterial isolates were picked from the plate using a sterile inoculating loop and subcultured to obtain pure bacterial culture using the streaking method into an already prepared agar plate [12]. The plates were incubated for 24 hours at 37°C. Pure cultures of the bacterial isolates obtained were maintained on nutrient agar slants in McCartney bottles. The cultures were kept in the refrigerator at 4°C for further investigations.

#### **Isolation of fungi from nose mask samples**

From the serially diluted samples, 1 mL each was aseptically taken from the 10<sup>-1</sup> dilution of the nose mask samples with a syringe and dispersed into the sterile Petri dishes, after which already prepared potato dextrose agar and Sabouraud dextrose agar were poured into plates aseptically. The poured plate was allowed to solidify and then incubated in an inverted position at 25°C for 48 hours [11]. After that, individual colonies of fungal isolates were picked from the plate using a sterile inoculating needle and subcultured to obtain pure isolates [12].

The pure fungal isolates obtained were maintained in potato dextrose agar slants for identification procedures using lactophenol cotton blue staining technique and microscopy [11].

#### **Identification of bacterial and fungal isolates from nose mask (Used and Unused)**

Biochemical tests, which included Gram staining, catalase test, coagulase test, urease test, triple sugar iron (TSI) test, methyl red and Voges proskauer test, motility, hydrogen, and indole test, were carried out on bacterial isolates. The bacterial isolates were identified using the Bergey's identification manual. Fungal isolates were identified on the basis of cultural characteristics of the colonies and morphological and microscopic examination [11].

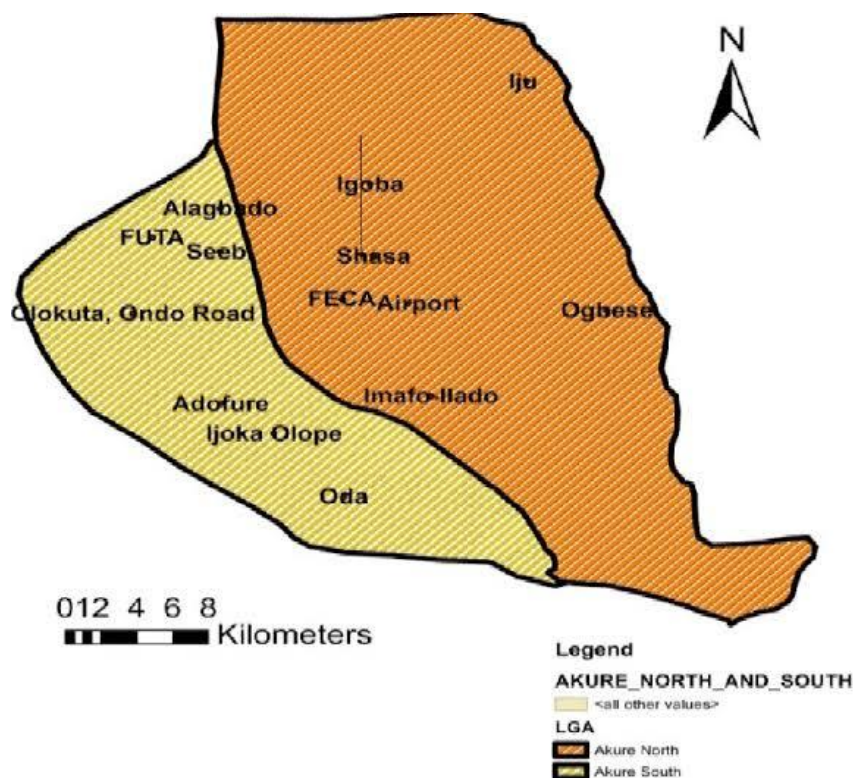
#### **Antimicrobial sensitivity testing**

All the inoculums were standardized to 0.5 MacFarland standard with a spectrophotometer (UV-VIS Spectrophotometer, 752Pro), adjusted to a wavelength of 620 nm, and set to zero using blank. The inoculum was examined at 0.08-0.1 absorbance [11].

The sensitivity test for antibiotic was performed using Kirby Bauer's disc diffusion method by placing the antibiotic disk on seeded plates. After incubation of 24 hours, inhibition zones were measured using a transparent ruler and then recorded in millimeters. The result was interpreted using a CLSI chart [12]. Antifungal sensitivity assay used the agar well diffusion method [11]. The antifungal drugs used included ketoconazole (100 mg/mL), nystatin (100 mg/mL), sivotokonazole (100mg/mL), and itraconazole (100 mg/mL). Inoculated plates were then incubated at 25°C for 48 hours. Measurements were made using a transparent ruler to determine how large the inhibitory zone was and then recorded in millimeters [13].

#### **Statistical analysis**

One way analysis of variance was performed on the data obtained, and Duncan's New Multiple Range test (DNMRT) was used to compare treatment means at  $p \leq 0.05$  significance level.

**Figure 1.** Map of Akure (study area) south local government area.

## Results and discussion

### Occurrence of microorganisms in

#### (a) Used nose mask

Result showed that *Bacillus subtilis*, *Micrococcus varians*, *Neisseria* sp., *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Streptococcus* spp., *Staphylococcus aureus*, *Vibrio* sp., and *Yersinia pseudotuberculosis* were found in nose masks. *Bacillus* and *Yersinia* were the most frequently occurring bacteria, and *Proteus*, *Vibrio*, and *Pseudomonas* were the least occurring bacteria (**Figure 2**). The bacteria present ranged from  $5.0 \times 10^2$  -Cfu/mL to  $2.17 \times 10^4$ -Cfu/mL (**Table 1**). Five fungal species, namely *Aspergillus flavus*, *A. niger*, *Mucor mucedo*, *Neurospora sitophila*, and *Rhizopus stolonifer* were isolated from the nose mask. *A. niger* was the most frequently encountered fungus. Isolated fungi ranged from  $1.0 \times 10^1$  -Sfu/mL to  $1.26 \times 10^3$ -Sfu/mL (**Table 2**).

Biochemical test results showed that four bacteria were identified as Gram positive (*Bacillus subtilis*, *Staphylococcus aureus*, *Micrococcus varians* and *Streptococcus pneumoniae*) and five as Gram negative bacteria (*Proteus mirabilis*, *Vibrio cholera*, *Yersinia pseudotuberculosis*, *Neisseria meningitides* and *Pseudomonas aeruginosa*).

Colonial growth on the surface of solid media was observed and characteristics, such as surface texture, transparency or opacity, color, size, elevation of the growth was described. Colonies of *Micrococcus* sp. were vibrant lemon yellow in color with rough surface texture and opaque, colonies were medium in size, irregular shaped with flat elevation and filamentous edges. *Streptococcus* sp. showed medium-sized creamy white colour colony with flat elevation, curled form, scalloped margin, opaque and with a dull surface. *Bacillus subtilis* exhibited a medium sized creamy flat colony with irregular form and lobate edges, the colony surface was smooth in texture and transparent. *Staphylococcus aureus* exhibited white medium sized, raised colonies with filamentous edges, circular form, smooth surface and opaque, *Proteus mirabilis* and *Vibrio cholerae* had the same characteristics except for differences in colony color. *Yersinia pseudotuberculosis* had a medium sized colonies of creamy colony with flat elevation, irregular form, convex edge with opaque and dull surface.

All fungal isolates displayed rapid growth rates except *R. stolonifer*, which had non-rapid growth rates. *Aspergillus flavus* and *Aspergillus niger* had granular texture; *Rhizopus stolonifera* and *Mucor*

*mucedo* had cotton like texture while *Neurospora sitophila* had fluffy texture. All spores were flat except those of *R. stolonifer*, which had raised spores, and *Neurospora sitophila*, which had umbonate spores.

#### (b) Unused nose mask

A surgical face mask was determined to be sterile and devoid of microorganisms. In contrast, an unused nose mask exhibited fewer microorganisms than a previously worn mask. Microorganisms isolated from cotton-made nose masks (unused) included *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*, while no fungal growth was recorded.

#### Occurrence of microorganisms in unused surgical and cotton nose masks

Bacteria isolated were *Bacillus* sp., *Pseudomonas* sp., and *Staphylococcus aureus*, whereas no fungal growth was recorded.

#### Antimicrobial susceptibility patterns of isolated microorganisms

The antibiotic susceptibility test showed that *Bacillus* was sensitive to antibiotics, while *Micrococcus* was resistant. Also, the Gram-positive bacteria showed high sensitivity to septrin and erythromycin, but resistant to zinnacef (Table 3). *Proteus* was highly sensitive to antibiotics, while

*Yersinia* was resistant. The Gram-negative bacteria showed high sensitivity to septrin but were resistant to ciprofloxacin and tarvid (Table 4). Generally, antibiotics were more effective against Gram-positive bacteria than Gram-negative bacteria.

The results of the antifungal assay demonstrated that *Neurospora sitophila* was resistant to the administered antifungal drugs, while *Mucor mucedo* showed high susceptibility to antifungal drugs (Table 5).

The antifungal susceptibility pattern revealed that *A. flavus* was resistant to sivoketoconazole and itraconazole as no result was gotten but was slightly susceptible to ketoconazole and highly susceptible to nystatin. *A. niger* was susceptible to all the drugs administered to it with sivoketoconazole having the highest susceptibility. *M. mucedo* was also susceptible to the drugs administered to it with nystatin and itraconazole having the highest susceptibility. *Neurospora sitophila* was resistant to the drugs but susceptible to nystatin alone. Lastly, *R. stolonifer* was susceptible to the drugs administered to it with nystatin having the highest sensitivity. Nystatin is the most effective drug for all the isolated fungi with itraconazole showing low effectiveness (Figure 5).

**Table 1.** Bacteria loads of selected nose mask.

Isolates	Bacterial Loads (cfu/mL)
<i>Bacillus subtilis</i>	$1.2 \times 10^5$
<i>Micrococcus varians</i>	$2.1 \times 10^2$
<i>Neisseria meningitidis</i>	$1.2 \times 10^2$
<i>Proteus mirabilis</i>	$2.0 \times 10^2$
<i>Pseudomonas aeruginosa</i>	$5.2 \times 10^5$
<i>Staphylococcus aureus</i>	$5.0 \times 10^5$
<i>Streptococcus pneumoniae</i>	$1.2 \times 10^2$
<i>Vibrio cholerae</i>	$1.5 \times 10^2$
<i>Yersinia pseudotuberculosis</i>	$1.9 \times 10^5$

**Table 2.** Fungal loads of selected nose mask.

Isolates	Fungal Loads (cfu/mL)
<i>Aspergillus flavus</i>	$3.0 \times 10^1$
<i>Aspergillus niger</i>	$1.3 \times 10^3$
<i>Mucor mucedo</i>	$5.0 \times 10^1$
<i>Neurospora sitophila</i>	$1.0 \times 10^1$
<i>Rhizopus stolonifera</i>	$1.0 \times 10^1$

**Table 3.** Antibiogram patterns of Gram-positive bacterial isolates.

Isolates	AM	APX	CN	CPX	E	PEF	R	S	SXT	Z
<i>Bacillus subtilis</i>	S	S	I	S	S	S	S	S	S	R
<i>Micrococcus varians</i>	R	R	I		I	I	I	S	I	R
<i>Staphylococcus aureus</i>	S	R	I	R	S	I	S	S	I	R
<i>Streptococcus pneumoniae</i>	S	R	I	S	S	S	I	S	I	R

Keys: S = Sensitive; I = Intermediate; R = Resistant, PEF = Pefloxacin, CN = Gentamycin, APX = Ampiclox, Z = Zinnacef, AM = Amoxicillin, R = Rocephin, CPX = Ciprofloxacin, S = Streptomycin, SXT = Septrin, E = Erythromycin

**Table 4.** Antibiogram patterns of Gram-negative bacterial isolates.

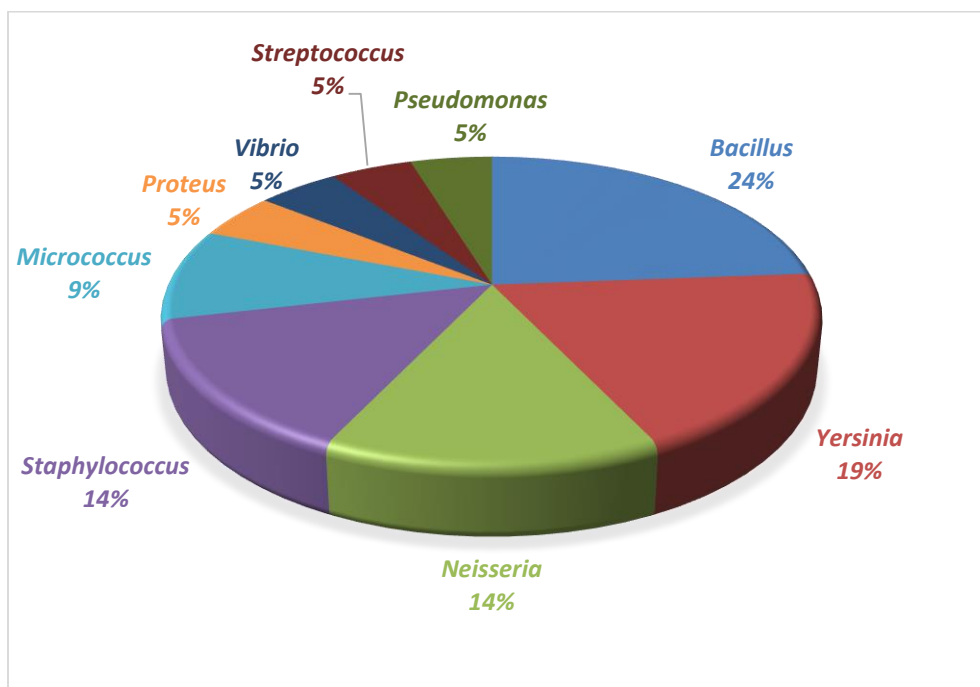
Isolates	AM	AU	CH	CN	CPX	OFX	PEF	S	SP	SXT
<i>N. meningitidis</i>	I	I	I	I	R	R	I	I	R	S
<i>P. mirabilis</i>	I	S	S	S	S	S	S	S	I	S
<i>P. aeruginosa</i>	I	R	I	I	R	R	I	S	R	S
<i>V. cholerae</i>	R	I	R	I	R	R	I	I	I	I
<i>Y. pseudotuberculosis</i>	I	I	I	I	R	R	R	R	I	S

Keys: S = Sensitive; I = Intermediate; R = Resistant, PEF = Pefloxacin, CN = Gentamycin, APX = Ampiclox, Z = Zinnacef, AM = Amoxicillin, R = Rocephin, CPX = Ciprofloxacin, S = Streptomycin, SXT = Septrin, E = Erythromycin.

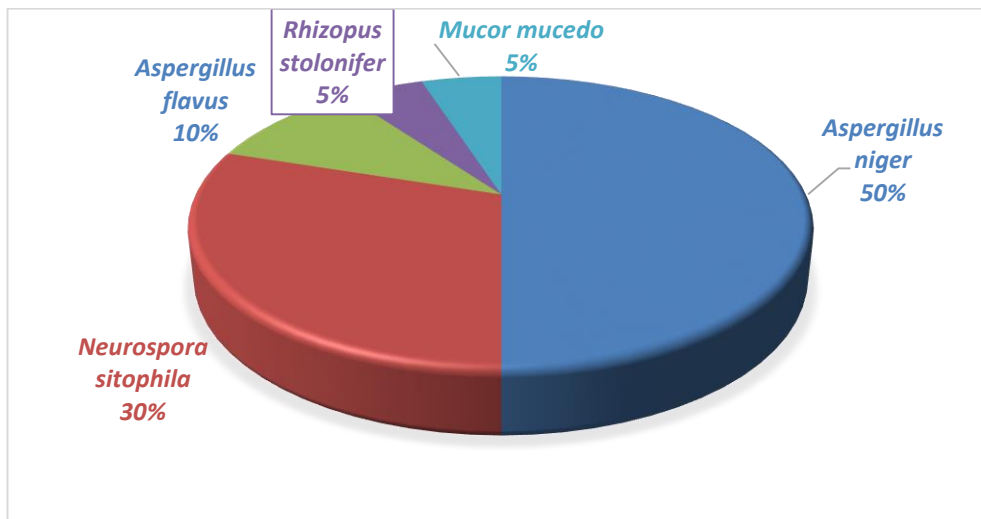
**Table 5.** Antifungal susceptibility patterns of fungal isolates.

Isolates	Zones of inhibition (mm)			
	Sivoketoconazole	Nystatin	Itraconazole	Ketoconazole
<i>Aspergillus flavus</i>	0	23	0	9
<i>Aspergillus niger</i>	30	20	15	17.5
<i>Mucor mucedo</i>	20	30	30	22
<i>Neurospora sitophila</i>	0	5	1	0
<i>Rhizopus stolonifer</i>	9	20	15	15

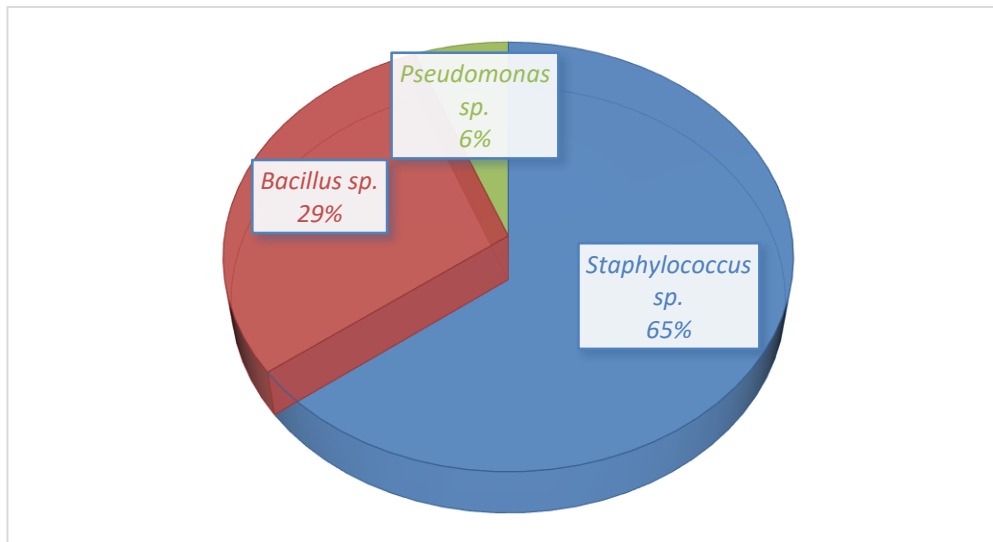
**Figure 2.** Percentage occurrence of bacteria isolated from used nose masks.



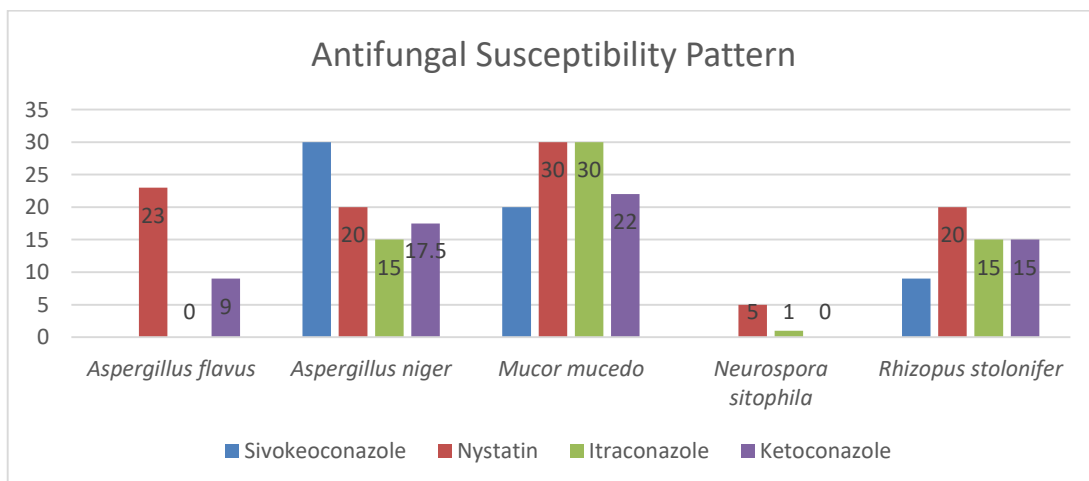
**Figure 3.** Percentage occurrence of fungal isolates from used nose masks.



**Figure 4.** Percentage occurrence of bacteria isolated from unused cotton nose masks.



**Figure 5.** Antifungal susceptibility patterns on used nose mask.





## Discussion

The general assumption is that medical and non-medical masks are safe [6]. Studies on mask efficacy [14] generally do not account for the fact that the microorganisms in human saliva and those in exhaled breath could be of biosafety concern, especially when masks are worn for too long, not properly stored or reused without proper disinfection. Therefore, it is necessary to monitor or study the microbial activities on used and unused face masks. In this study, 25 used nose mask samples; disposable and cotton-made masks were randomly collected from consenting individuals, but mostly among the students. In addition, 50 unused nose masks, including disposable and cotton, were evaluated in Akure. The microbial (bacterial and fungal) loads of used nose masks were significantly higher than those of unused nose masks. Used nose masks contained many microorganisms compared to unused nose masks.

It was suspected that used nose masks had been in contact with organisms on the skin, nose, mouth, and saliva. Human saliva has been reported to contain *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Candida albicans*, *Klebsiella pneumoniae*, *Neisseria*, *Prevotella*, and *Veillonella* spp., which are just a few of the many pathogenic microorganisms that exist. Human saliva contains up to 100 million bacteria per milliliter. Self-inoculation of mucous membranes in the mouth, nose, and eyes is an important transmission route of viruses [15], as people touch their face about 23 times an hour, with 44% of those touches involving contact with a mucous membrane [16]. Moreover, people may be unaware of other important measures, such as social distancing and hand hygiene [17].

Microorganisms isolated in this study were: *Aspergillus flavus*, *Aspergillus niger*, *Bacillus species*, *Micrococcus varian*, *Mucor mucedo*, *Neisseria species*, *Neurospora sitophila*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Rhizopus stolonifer*, *Staphylococcus aureus*, *Streptococcus specie*, *Vibrio sp.* and *Yersinia pseudotuberculosis*. Most of the organisms isolated are known to be human microflora. The most predominant microorganisms isolated from this research were *Bacillus* species (a bacterium) and *Aspergillus* species (a fungus). *Bacillus* is present in soil, air, and water, which humans usually come into contact with during their daily activities. **Lee et al.** identified

*Staphylococcus*, *Micrococcus*, *Vibrio*, and *Pseudomonas* as skin microbiome bacteria and some associated with skin infection [18].

The sterile disposable-unused nose mask was supposed to be free of microorganisms. However, minimal bacterial growth was observed on the unused cotton nose mask, whereas fungal growth was not observed in unused nose mask. The unused (sterile and cotton) nose masks were not expected to contain microorganisms, but this may be due to contamination from the environment and storage conditions. The microorganisms isolated from unused cotton nose mask were *Staphylococcus aureus* (65%), *Bacillus* specie (29%), and *Pseudomonas* specie (6%).

Therefore, used nose masks should not be reused and should be properly disposed of as it could be a means by which infection is spread to the environment. Nose masks should also be properly worn to avoid self-inoculation with pathogenic microorganisms. The general populace should also be educated on how to use and dispose of nose masks, as the general public does not use face masks properly. An observational checklist with 1,500 participants recruited in Hong Kong revealed that nearly none of them were able to implement all the correct procedures wearing a face mask, as 91.5% of them neglected to wash their hands before putting the mask on and 97.3% when taking it off [18]. Face mask misuse increases the risk of contracting and spreading viral and bacterial infections. Self-inoculation of nose, eyes, and mouth mucous membranes is a major virus transmission channel [19, 20]. Moreover, people may be unaware of other important measures, such as social distancing and hand hygiene [17]. Lastly, the use of face masks may lead to discomfort, skin acne, headaches, respiratory difficulties, communication challenges (particularly for the deaf or hard of hearing), and reduced non-verbal communication. Moreover, some individuals may neglect other essential precautions such as hand washing and social distancing. Additionally, compared to surgical masks, the reuse of cotton masks, moisture retention, and inadequate filtration may increase the risk of respiratory virus transmission [10].

The study revealed that *Bacillus* was sensitive to the drugs administered for Gram-positive bacteria, while *Micrococcus* was resistant. Also, the Gram-positive bacteria showed high sensitivity to septrin and erythromycin but showed



resistance to zinnacef. A significant difference exists between the mean of drug administered and the microorganism (on Gram-positive bacteria), the microorganism reacted differently to each drug administered. *Proteus* showed high sensitivity to the antibiotics administered for Gram-negative bacteria, while *Yersinia* was resistant. The Gram-negative bacteria showed high sensitivity to septrin but were resistant to ciprofloxacin and tarvid. Antibiotics were more effective against Gram-positive bacteria than Gram-negative bacteria. There was no significant difference between the drug's mean and the microorganism (on Gram-negative bacteria). Also, the result exhibited that *N. sitophila* was resistant to administered antifungal drugs, while *M. mucedo* showed high susceptibility to the antifungal drugs. The drugs reacted equally on each organism they were administered to, but there was a significant difference between the drug administered and the microorganism (fungi).

### Conclusion

This study showed that harmful bacteria were frequently present on face masks included bacteria like, *Bacillus* species (24%), *Yersinia pseudotuberculosis* (19%), *Neisseria* specie (14%), *Staphylococcus aureus* (14%), *Micrococcus varians* (9%), *Pseudomonas aeruginosa* (5%), *Proteus mirabilis* (5%), *Streptococcus* species (5%), and *Vibrio* sp. (5%) and fungi, *Aspergillus niger* (50%), *Neurospora sitophila* (30%), *Aspergillus flavus* (10%), *Mucor mucedo* (5%), *Rhizopus stolonifer* (5%). This can have a major impact on the spread of many diseases. The presence of microorganisms on the nose mask sample indicates the presence of microorganisms on the skin, mouth, nose, and ear, as well as opportunistic pathogens due to the poor hygiene level of the person wearing them; microorganisms can also be present in the air, soil, and environment. Therefore, nose masks should be properly disposed of after use or sterilized before reusing to prevent disease transmission. It was observed that most of the microorganisms isolated were susceptible to the drugs administered to them. The result revealed that bacteria showed 70% sensitivity to the drug administered (for both sensitivity and intermediate result) and 30% resistance. This indicated that the microorganism can be treated with a wide range of drugs without posing a severe threat to public health.

### Authors' contribution

'AKO designed and supervised the study. 'KEA developed the methodology and Literature, conducted the study, acquired, analyzed and interpreted the data obtained. All the authors have read and approved the manuscript.

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### Competing interest

The authors declares that they have no competing interest

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