











Research Article

## Bacteria Contamination and Antibiotic Resistance Profile on Worn Reusable Facemasks in Githurai Market, Kenya

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### Abstract

Facemasks have been widely used in the theater to prevent surgical site infections as well as in hospital wards to prevent infectious infections. Also masks have been recommended to be used by the public during pandemics of respiratory infectious diseases. However, the prolonged use of these masks may have inadvertently harbored unseen dangers by serving as reservoirs for bacteria contamination and antibiotic resistant bacteria when in use. The objective of this study was to quantify bacteria as well as isolate, characterize and determine the antibiotic profiles of bacteria from worn reusable facemasks. Before masks were worn, the participants' mouth, nose and facial skin were swabbed with sterile transport media. Thereafter, the masks were worn at two-, four- and six-hour intervals. Afterwards, bacteria on worn masks, mouth, nose and skin were identified via morphological, biochemical and molecular methods. Antibacterial susceptibility was determined via the Kirby–Bauer method. The results of this study revealed bacterial colony forming units were significantly higher in the polycotton masks ( $4.30 \times 10^2$  CFUs) than in the cotton masks ( $3.38 \times 10^2$  CFUs). Colony forming units increased with extended mask use from two, four, and six hours respectively ( $7.61 \times 10^1$  CFUs,  $1.28 \times 10^2$  CFUs,  $4.26 \times 10^2$  CFUs). Also, inside part of the masks had significantly high colony forming units than outside of the masks. The isolated bacteria were from different genera including *Bacillus*, *Staphylococcus*, *Enterococcus*, *Pseudomonas*, *Stenotrophomonas*, *Acinetobacter*, *Neisseria*, *Proteus*, *Klebsiella* and *Enterobacter*. From the antibiotic resistance profiles, with 85.2% of isolates classified as resistant, Ampicillin had the highest resistance rate. Spectinomycin and Amoxicillin both showed 66.7% resistance, whereas Cefotaxime showed 63.0% resistance. Streptomycin showed the highest susceptibility (48.1%), suggesting comparatively improved efficiency against the isolates. The study findings provides bacteriological insights risks associated with facemask upon use as well as antibiotic resistance.

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## Keywords

Bacteria, Reusable Mask (RM), Cotton Mask (CM), Polycotton Mask (PM), Antibiotic Resistance

## 1. Introduction

The impact and strategy of wearing reusable face masks in the workplace to control infectious infections have not been well investigated in Kenya. These masks can store a variety of pathogens, including harmful bacteria, because they are manufactured from locally available textiles and reused repeatedly without adequate sanitation. Facemasks have been used in specific settings, such as in theatre to prevent surgical site infections and in hospital wards to prevent the spread of infectious diseases such as COVID-19, Tuberculosis, Ebola, the Spanish flu, and Influenza [1]. However, during the COVID-19 pandemic all persons, especially those in public places, were urged to use facemasks as a strategy of preventing the spread of SARS-CoV-2. As a result, there was overconsumption of market-available masks [2] and the World Health Organization (WHO) recommended use of reusable facemasks to curb costly and unavailable disposable masks [3]. However, the type of mask, the specific duration required to put on the mask before disposal, and the microbial contamination rate of the worn masks remain unclear [2]. When masks are used for lengthy periods, they might cause discomfort and occasion the development of facial skin lesions, irritating dermatitis, or worsening acne [4]. Acne and the accumulation of *Staphylococcus aureus* (*S.aureus*) have been associated with the use of face masks, [5] thus a need to investigate the presence of pathogenic bacteria on masks. Reusable masks mainly made from cotton, silk, and polyester are highly recommended [6]. However, cotton is a potential microbial growth substrate because of its ability to absorb moisture known to support the growth and multiplication of microbes [7]. The environment inside the mask naturally provides a conducive environment for the survival of bacteria due to moisture and nutrients from sweat. Also, the contact between the skin and the masks creates a naturally conducive temperature for bacterial survival [8].

According to [9], microbes from facemasks may be normal flora or pathogenic acquired from the environment. In a study by [10] cotton mask had  $1.46 \times 10^5$  CFU/mask and surgical masks  $1.32 \times 10^4$  CFU/mask after four hours of use. *Bacillus*, *Staphylococcus*, and *Acinetobacter spp.* were among the bacteria isolated from the masks. Mask wearing resulted in an increase in the number of *S.aureus* among respondents and the longer the time a mask was used the higher the number of colonies of bacteria in it. In a study on the amount of masks worn in the community as a measure of microbiological contamination, the amount of bacterial contamination on the utilized mask progressively rose as the number of days of use

increased. *Staphylococcus*, a harmful bacterium, was identified as the most common strain through the use of 16S rRNA PCR. Based on this study, it was concluded that wearing a cotton mask creates the ideal conditions for microorganisms associated with skin and respiratory system to thrive [11]. Bacterial colony numbers were reported to be greater on the face-side than the outer-side. Also, a longer mask usage did not significantly increase the bacterial colony numbers. Non-pathogenic microbes in humans including *S.epidermidis*, *S.aureus*, and *Cladosporium* as well as pathogenic microbes; *Bacillus cereus*, *S.saprophyticus*, *Aspergillus*, and *Microsporum* were isolated from the masks [12]. Despite the reported implications associated with facemask use, there is limited information on the type of mask, the specific duration required to put on the mask before disposal, and the microbial contamination rate in worn masks [13].

The accumulation of human saliva-based pathogens in the mask may lead to some biosafety problems [14]. As a result, shedding bacteria from saliva, expiration, and touching the skin mainly when worn for long periods, reused without sufficient or no disinfection, and improper handling after and before usage is a concern. Medical pathogens are present in the mouth during the acute phase of a non-oral infection, as well as in the saliva of healthy people [15] including *S.aureus*, *Pseudomonas aeruginosa*, *Candida albicans*, *Klebsiella pneumoniae*, and *Streptococcus* [16]. Therefore, microbes that are spread from the mouth when speaking, from the nose during exhalation, and during sweating on the skin area might accumulate on the mask with prolonged usage. According to [17] antibiotic resistance is a threat to global public health due to increased antibiotic resistance by bacteria. Addressing this threat requires better antibiotic use practices and prevention of the spread of antibiotic-resistant bacteria [18]. A serious threat to the community of long-time mask wearers is the presence of resistant microbial viability on face masks. Because of the increased strain on health care, it raises morbidity and mortality and is linked to significant economic expenses [17]. [10] In Belgium reported that after use, surgical and cotton masks develop an accumulation of pathogens and bacteria that are resistant to antibiotics. In a study to determine antibiotic sensitivity profiles of microorganisms isolated from used and unused nose masks, Gram-positive bacteria were resistant to zinnacef but very sensitive to erythromycin and septrin. *Yersinia* was resistant to the antibiotics used to treat Gram-negative bacteria, whereas *Proteus* showed high sensitivity to them. The Gram-negative bacteria were resistant to

tarvid and ciprofloxacin but extremely susceptible to septrin [19].

According to [20] it is important to consider that wearing a mask can have drawbacks. There is a need to report on what bacteria adhere on masks and the quantity of bacteria for different wearing time in Kenya. Awareness of the potential bacterial contamination on masks will be of importance, especially in combating self-contamination and secondary infections among immunocompromised persons. In addition, it's a necessity to understand how long a mask should be worn before change to overcome underlying side effects. In Kenya, no studies have focused on the bacterial load, identity and antibiotic resistance profiles of bacteria on reusable masks manufactured from cotton (100%) and poly-cotton (45% cotton, 55% polyester) fabrics in informal markets. This research documents data on the quantity, identity and antibiotic profile of bacterial contamination on worn reusable cotton and polycotton face masks with use together with identity of bacteria in the mouth, nose and skin before wearing a mask. From this study, mask users learn on the potential of bacterial contamination on masks during use as well as how frequent they should change fabric mask within a day. Therefore, this study aimed to determine the CFU levels resulting from the prolonged use of facemasks, isolate and characterize bacteria on worn reusable facemasks, evaluate the antibiotic susceptibility of the isolated bacteria to selected antibiotics as well as isolate and characterize bacteria in the mouth, nose and skin before masks were worn. This study hypothesized that there were no significant differences in bacterial CFUs from reusable masks worn at different time intervals, there are no viable bacteria contamination on worn masks, the isolated bacteria were not resistant to selected antibiotics and there are no bacteria in the mouth, nose and skin before wearing a mask.

## 2. Materials and Methods

### 2.1. Study Area

The study was carried out in a densely populated peri-urban open market, Githurai 45, which is found in Kiambu County, Kenya [21]. The market is located twelve kilometers from Nairobi city and lies at latitude 1°12'11" S and longitude 36°55'02"E. This area was chosen because of its high population density, and traders work in market work for more than six hours a day and hence could participate in wearing masks for the required study time.

### 2.2. Sampling and Collection of Samples from Participants

The Kenyatta University Ethics Review Committee reviewed and approved this research under the approval number PKU/2595/11722. In addition, National Commission for Science, Technology and Innovation provided a research

permit.

A purposive sampling technique was used to select fourteen respondents who voluntarily agreed to participate and worked in the market for at least six hours daily, willing to wear reusable face masks manufactured from cotton and polyester fabrics, and without a history of preexisting health conditions.

Sterile swabs were used to collect samples from the respondents' mouths, noses, and skins before masks were worn. Seven respondents wore 100% cotton masks (CMs), whereas seven wore 70% poly-cotton masks (PMs) for a specified duration. On day one, they wore masks for two hours; on day two, they wore them for four hours; and on the third day, they wore them for six hours. The worn mask from each participant was carefully removed, placed in a sterile zip lock bag, kept in a cool box, and transported to the Kenyatta University laboratory for bacterial quantification, isolation, and characterization.

### 2.3. Enumeration of Bacteria on Masks

In the laboratory, the worn reusable facemasks were cut and separated into two parts; the outside and the inside, soaked in 50 ml of normal saline solution separately and then shaken thoroughly to wash the mask. A total of 0.1 ml of saline was then pipetted from 50 ml of normal saline and spread on nutrient agar to quantify the bacteria via the viable plate count method [22]. For the samples collected at six hours, serial dilution was performed, and 0.1 ml of the serially diluted normal saline (3-1 dilution) was subsequently plated on nutrient agar for quantification. The plates were further incubated for 24 hours, and CFUs were counted.

### 2.4. Isolation of Bacteria from Masks, Nose, Skin and Mouth

One (1 ml) of 50 ml of normal saline was inoculated in broth and incubated for 24 hours. After 24 hours, turbidity was observed, and a streak made from each sample was plated on blood agar for the isolation of bacteria. After streaking, the samples were incubated at 37°C for 24 hours and observed for differences in hemolysis and morphology on blood agar before being subcultured on nutrient agar. Distinct colonies were subcultured on nutrient agar to obtain pure cultures. Thereafter, the pure cultures were stored in the refrigerator until further analysis.

Additionally, the swabs from mouth, nose and skin were cut separately, placed in broth and incubated at 37°C for 24 hours, after which the turbidity was checked. A streak from each sample was made on blood agar and incubated at 37°C for 24 hours. The samples were observed for morphological differences and hemolysis before subculturing. Distinct colonies were subcultured on nutrient agar. Pure colonies were placed in broth and stored in a refrigerator for subsequent morphological, biochemical, and molecular characterization.

## 2.5. Morphology and Biochemical Identification and Characterization of the Bacteria

The pure cultures were identified and characterized via routine morphological characteristics (color, Gram stain, hemolysis, and opacity) and biochemical tests (triple sugar iron (TSI) catalase, coagulase, and methyl red (MR), Voges-Proskauer (VP), and carbohydrate fermentation).

## 2.6. Genomic DNA Extraction and Polymerase Chain Reaction (PCR)

Genomic DNA was extracted using standard protocol described by [23]. Pure cultures were inoculated in nutrient broth and incubated overnight. One (1 ml) sample was placed in an Eppendorf tube and centrifuged at 13000 rpm for 5 minutes, after which the pellet was used for DNA extraction. 800 µl of CTAB buffer, 30 µl of proteinase K, and 30 µl of lysozyme were added to the pellet in the tube, vortexed and then suspended in a water bath at 65°C for 45 minutes. Afterwards, the tube was centrifuged at 10000 rpm for 5 minutes, and the supernatant was transferred to a new tube. An equal volume of chloroform-isoamyl alcohol (24:1) was added, and the mixture was gently flipped several times. The mixture was then centrifuged at 12000 rpm for 8 minutes at 4°C and thereafter transferred into a new tube. Then, 100 µl of 3 M sodium acetate was added to the tube, which was gently flipped, and an equal volume of cold (-20°C) isopropanol was added and stored at -20°C for two hours. The sample was subsequently centrifuged at 14000 rpm for 10 minutes at 4°C, and the supernatant was discarded. Then, 500 µl of 70% ethanol was added to the pellet, which was subsequently washed by centrifuging at 10000 rpm at 4°C for 5 minutes. The supernatant was discarded again, and 500 µl of absolute ethanol was added to the pellet, which was subsequently washed by centrifuging at 10000 rpm at 4°C for 5 minutes. After centrifugation, the supernatant was discarded, and the pellet was dried on a sterile paper towel at room temperature for 5 minutes. Then, 50 µl of PCR mixture was added to the pellet, which was subjected to gel electrophoresis to confirm the presence of genomic DNA in the sample. A 0.8% agarose gel with 1 µL of ethidium bromide and 100 mL of TBE buffer was prepared. One microliter of 6X dye was mixed with 4 µl of the sample and loaded in the gel in a gel tank with 1X TBE buffer. The gel was then allowed to run for 30 minutes at 80 W, then visualized over a UV trans illuminator and documented by photographing.

The extracted DNA was amplified via the [20] protocol. The target segment was amplified via the universal primers 27 F (5'AGAGTTTGATCMTGGCTCAG 3') and 1492 R (5'TACGGHTACCTTGTTACGAC 3') for the V1-V9 region of the 16S rRNA gene. A total of 25 µl of PCR amplification mixture, which contained 1 µl of DNA template, 1 µl of forward primer, 1 µl of reverse primer, 12.5 µl of master mixture and 9.5 µl of PCR mixture, was used. Amplification was

performed in a thermal cycler (BIOBASE) with initial denaturation for 2 minutes at 94°C, followed by 35 cycles of initial denaturation for 30 seconds at 94°C, annealing for 30 seconds at 55°C and extension for 1 minute at 68°C. The final extension was performed for 5 minutes at 68°C, after which the samples were stored at 4°C. To confirm that the PCR process was successful, 3 µl of the PCR product was loaded on a 1% agarose gel containing ethidium bromide and subjected to gel electrophoresis. One microliter of 6X dye was mixed with 3 µl of the PCR product and loaded in a gel tank with 1X TBE buffer. The gel was then allowed to run for 30 minutes at 80 W, then visualized over a UV trans illuminator and documented by photographing.

## 2.7. DNA Sequencing

The obtained PCR product was purified and subjected to Sanger sequencing via an automated genome sequence analyzer machine at Inqaba Biotech African's Genomics Company in South Africa. The 27F and 1492R primers were utilized for sequencing.

## 2.8. Antibiotic Susceptibility Testing

Ampicillin, Streptomycin, Spectinomycin, Cefotaxime, Amoxicillin and Sulfan are the antibiotics that were tested. The Kirby-Bauer disk diffusion method was employed to test the antibiotic susceptibility of the bacteria isolated [24]. Pure culture isolates were inoculated into sterile 5 ml distilled water. Then, 1 µl of the dilution was spread plated on Mueller-Hinton agar, and the antibiotic disc was inoculated on the medium. Discs containing distilled water were used as negative controls. The plates were incubated and, after 24 hours, observed for the formation of zones of inhibition. Zones of inhibition were measured by placing a ruler from the antibiotic disk's center to a location on the zone's perimeter where a noticeable edge was visible then the diameter of the zone of inhibition multiplied the value by two. No zone at all was recorded as 6mm. The reaction to antibiotics by the isolates was termed as resistant (R) ( $\leq 14$ mm), intermediate (I) (15-19mm) and susceptible (S) ( $\geq 20$ mm) according to the Clinical and Laboratory Standards Institute (CLSI) standards (M100 30th edition).

## 2.9. Data Analysis

Significant differences in bacterial quantity between masks and time were generated via two-way ANOVA at  $p < 0.05$ , and Tukey's post hoc test was used to separate the means via SAS version 9.0. Relationships between the isolated bacteria and hypothesized controls on the basis of morphology and biochemical test were analyzed, and heatmaps were generated via Python. For the molecular data, sequences obtained from sequencing were used to create a consensus and cleaned via BioEdit and BLASTED on the NCBI database, and matching organisms were obtained.



Multiple sequences were aligned, and a phylogenetic tree was produced via MEGA-X software. The significant differences in the mean zones of inhibition of the bacteria against antibiotics were determined via one-way ANOVA at  $P < 0.05$  [25], whereas Tukey's honest significance difference (HSD) test was used to separate the means [26]. The means were then interpreted using CLSI standards and percentages calculated for each antibiotic category R, S, and I.

$$\text{Percentage (\%)} = \frac{\text{Number of isolates in category}}{\text{Total number of isolates}} \times 100$$

### 3. Results

#### 3.1. Bacterial Quantity on CM and PM Worn for 2 Hours, 4 Hours, and 6 Hours

There was a significant difference in the number of bacterial CFUs between the mask types at  $p = 0.0001$  (Table 1). The highest number of bacteria was reported on PM, with  $4.30 \times 10^2$  CFUs, followed by CM,  $3.38 \times 10^2$  CFUs and the controls ( $4.2 \times 10^1$  and  $3.11 \times 10^1$  CFUs, respectively). In addition, the CFUs differed significantly between the time points ( $p = 0.0001$ ). The highest CFUs were recorded in masks worn for six hours ( $4.26 \times 10^2$  CFUs), followed by those worn for 4 hours ( $1.28 \times 10^2$  CFUs), whereas the lowest CFUs were recorded in masks worn for two hours ( $7.61 \times 10^1$  CFUs) (Table 1). A comparison based on the mask location revealed that the CFUs differed significantly ( $p = 0.0013$ ) between inside and outside of masks. The highest number of CFUs were detected inside the masks, with a mean of  $2.40 \times 10^2$  CFUs, whereas  $1.80 \times 10^2$  CFUs were detected outside the masks. Similarly, there were significant interactions between mask type and location ( $p = 0.0009$ ), between location and time ( $p = 0.0319$ ), and between mask type and time ( $p < 0.0001$ ) (Table 1). However, there was no significant interaction between mask type, time, and location.

**Table 1.** Bacterial quantity on CM and PM worn for 2 hours, 4 hours, and 6 hours.

Mask Types	Colony Forming Units (CFUs)/0.1 ml of 50 ml wash
Cotton	338.73±32.68 <sup>b</sup>
Poly-cotton	430.28±47.75 <sup>a</sup>
Cotton control	31.11±0.09 <sup>c</sup>
Poly-cotton control	42±1.07 <sup>c</sup>
Time	
2 hours	76.12 ±5.33 <sup>c</sup>

Mask Types	Colony Forming Units (CFUs)/0.1 ml of 50 ml wash
4 hours	128.56±9.09 <sup>b</sup>
6 hours	426.92± 43.88 <sup>a</sup>
Location	
Inside (face side)	240.98± 26.39 <sup>a</sup>
Outside	180.08±20.49 <sup>b</sup>
P values of the main effect and their interaction	
Mask Type	<.0001
Time	<.0001
Location	0.0013
Mask type*location	0.0009
Location*Time	0.0319
Mask type*Time	<.0001
Mask type*Location*Time	0.1269

Means with the same letters within the same column are not statistically significant at  $p < 0.05$  according to Tukey's post hoc test.

#### 3.2. Morphological Identification of the Bacterial Isolates

Morphological characteristics, color, margin, size, Gram stain, opacity, hemolysis and shape clustered into two clades: Clade 1 and Clade 2 (Figure 1). Clade 1 clustered color, whereas Clade 2 clustered margin, size, Gram stain opacity, hemolysis and shape. The isolated bacteria clustered into two major clades, Clades A and B (Figure 1), on the basis of their closeness and similarity with reference bacteria. Clade A included isolates E59, E29, E45, E49, E20, E11 and E44, whose morphological features are closely similar to those of *B. cereus*, *Streptococcus pneumoniae*, *K. pneumoniae*, and *Acinetobacter spp.* Clade B included isolates E30, E55, E28, E34 and E40, which are closely related to *P. aeruginosa*, *S. aureus*, and *Proteus mirabilis* (Figure 1).

The key characteristics are as follows: 1- (white color, irregular margin, large size, opaque, gamma, gram-positive, rod-shaped), 2- (cream color, smooth margin, medium size, translucent, beta-hemolytic, gram-negative, coccus shaped), 3- (cream white color, small size), 4- (yellow color), 5- (pale white color), 6- (grayish color), 7- (orange color), and 8- (green color).

#### 3.3. Biochemical Identification of the Bacterial Isolates

On the basis of their biochemical characteristics, the bacterial isolates clustered into two clades, clade 1 and clade 2. In Clade 1, the bacterial isolates clustered on the basis of nitrite,

motility, oxidase, indole, and hydrogen sulfide production results, whereas in Clade 2, they clustered on the basis of citrate, methyl red, bile esculin, urease, TSI and catalase tests. Among the reference isolates, the bacterial isolates clustered into two clades, clade A and clade B. Clade A included iso-

lates of E46, E49, E20, E28, E51, E11, and E30, which were closely related to *B. cereus*, *S. pneumoniae*, *S. aureus*, and *Enterobacter cloacae*. Clade B included isolates E29, E52, E40, E34, E44 and E55, which were closely related to *P. aeruginosa*, *Bacillus subtilis*, and *P. mirabilis* (Figure 2).

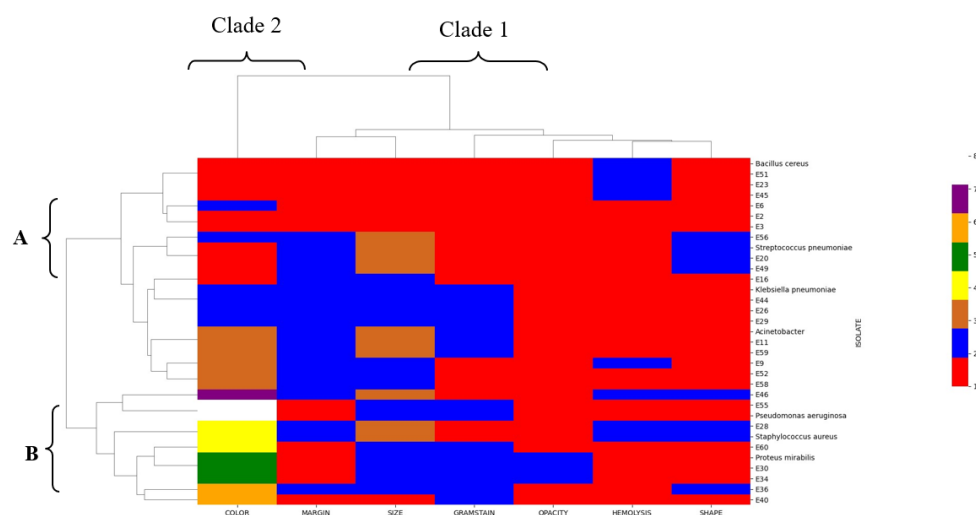


Figure 1. Morphology heatmap of bacterial isolates.

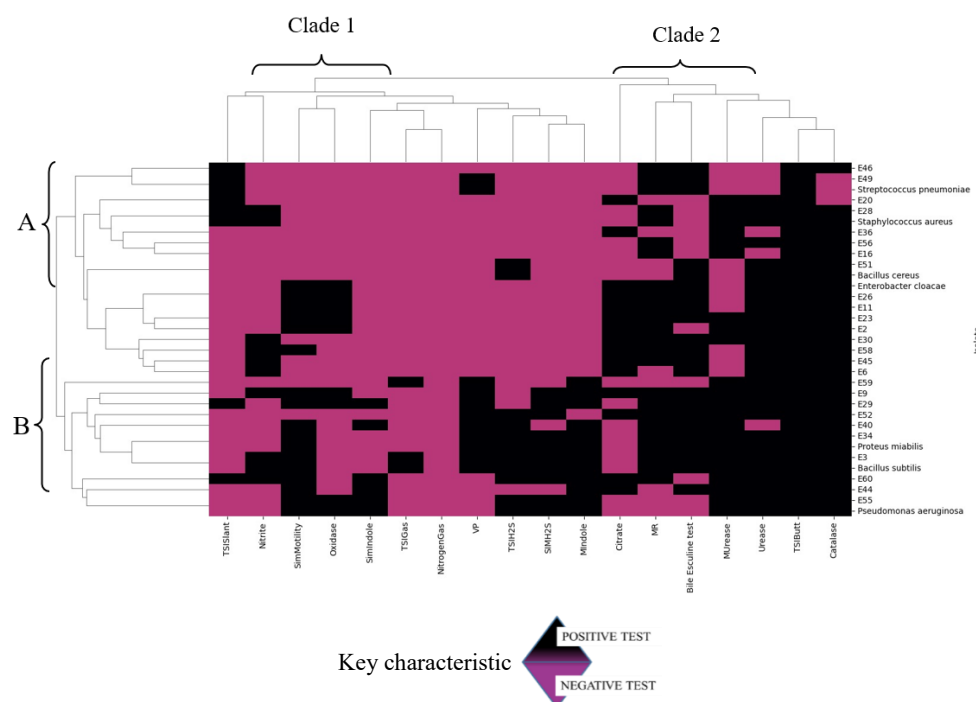


Figure 2. Heatmap of the biochemical characterization of isolates.

### 3.4. Carbohydrate Fermentation of Isolated Bacteria

Carbohydrate fermentation tests of glucose, sorbitol,

arabinose, mannose, lactose, maltose, raffinose, dulcitol, galactose, sucrose, mannitol, and rhamnose were also used to cluster the isolates (Figure 3). The tests clustered into two clades: clade 1 and clade 2. Clade 1 represented gas production, and clade 2 represented acid production by bacterial

isolates. In carbohydrate fermentation, the bacteria clustered into two clades: clade A and clade B (Figure 3). Most bacteria in clade A were positive for acid production and negative for gas production in the fermentation tests. These bacteria included E28, E2, E56 and E3. However, clade A clustered further into two subclades, A1 and A2. Clade A1 had the most bacteria that were positive for acid and negative for gas production in the tests (Figure 3). The isolates included E26, E28, E2, E56, E45 and E36. These bacteria are closely related to *S. pneumoniae*, *P. mirabilis*, *B. subtilis*, *S.aureus* and *E.cloacae*

in terms of sugar fermentation. Clade A2 comprised most bacteria that were negative for both gas and acid production in most of the tests. The isolates included E20, E51, E55 and E44, which were similar to *B. cereus* and *P. aeruginosa*.

Clade B contained isolates that were positive for both gas and acid production in the tests. The isolates included E6, E58 and E59. However, clade B isolates did not show similarity with any of the controls in the carbohydrate fermentation tests (Figure 3).

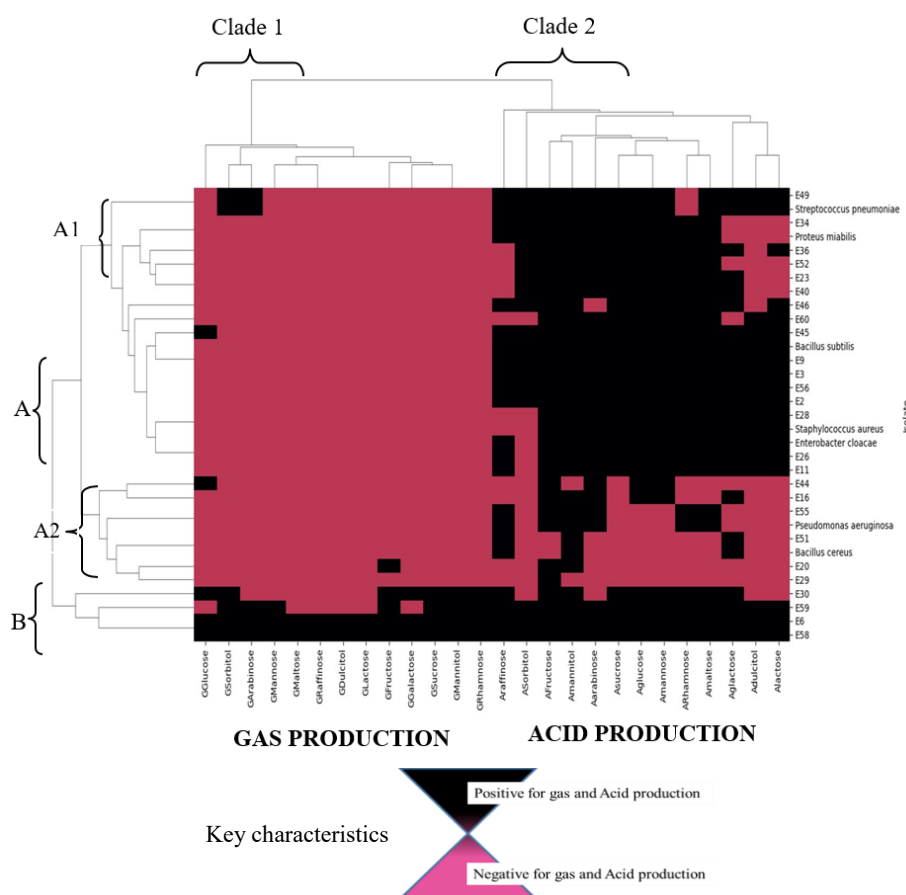


Figure 3. Carbohydrate fermentation of bacteria isolates.

### 3.5. Molecular Identification of the Isolates

#### Phylogenetic Tree of the Sequenced Bacteria.

As a result of the morphological and biochemical identification of the isolates, 26 isolates were subjected to 16S rRNA gene-based Sanger sequencing for molecular identification. Phylogenetic analysis of successfully sequenced bacterial

isolates revealed that the isolates belonged to eleven taxonomic genera, including *Bacillus*, *Staphylococcus*, *Mammaliococcus*, *Enterococcus*, *Stutzerimonas*, *Pseudomonas*, *Stenotrophomonas*, *Acinetobacter*, *Alcaligenes*, *Neisseria*, *Proteus*, *Klebsiella* and *Enterobacter*. *Bacillus* had the highest number of bacterial isolates, accounting for 40% of the isolates (Figure 4).

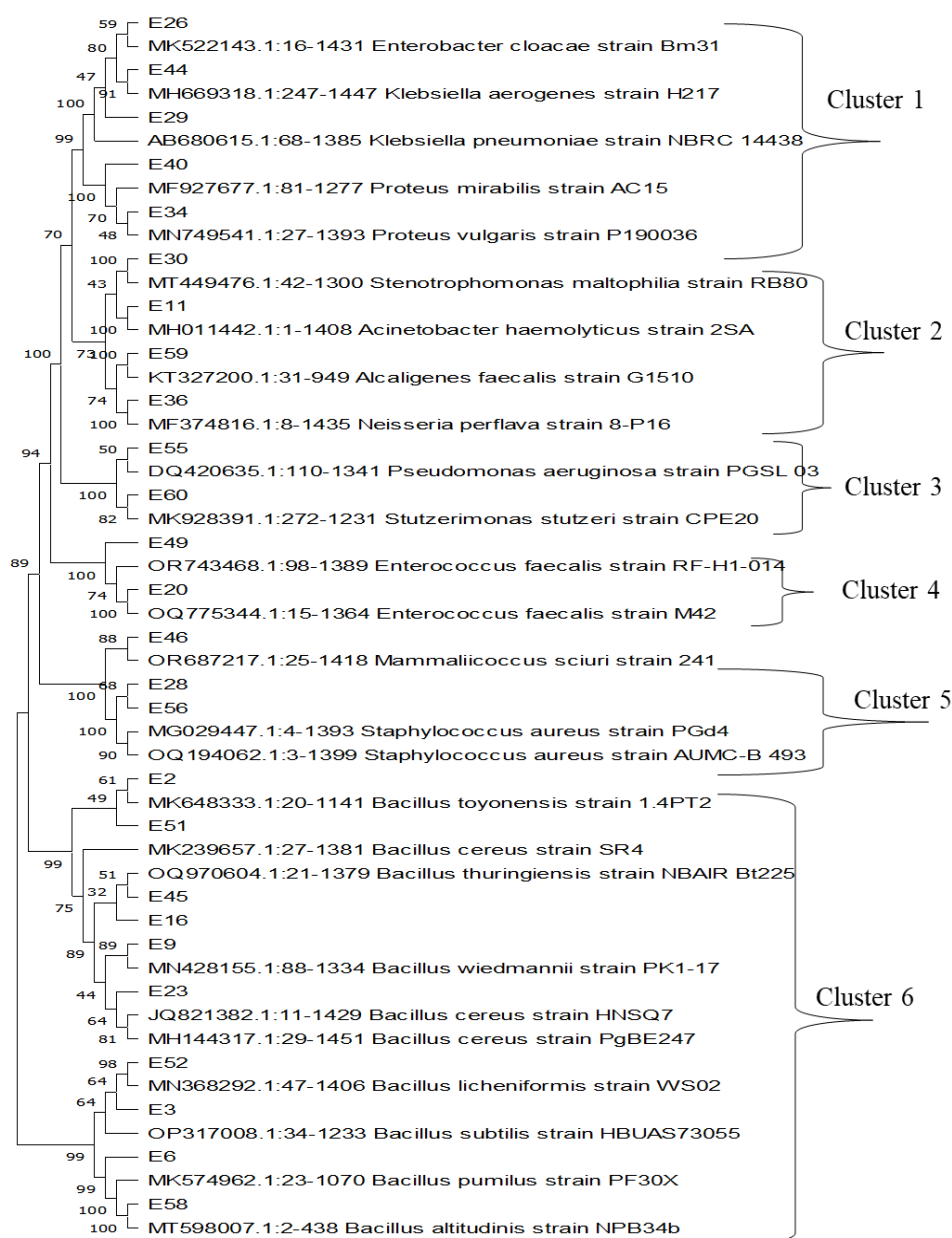


Figure 4. Phylogenetic tree of the sequenced bacteria.

### 3.6. Bacterial Identity and Isolation Source

Sequencing revealed 26 bacterial isolates from CM and PM, nose, mouth, and skin (Table A1). 80% of the bacteria were isolated from inside the masks, with *Bacillus* being the most dominant genus. *Bacillus wiedmannii*, *Enterobacter cloacae*, *P. mirabilis*, and *Alcaligenes faecalis* were only isolated inside parts of the masks, whereas *Proteus vulgaris* and *Stutzerimonas stutzeri* were isolated from outside parts of the masks. In addition, *B. wiedmannii*, *Acinetobacter hemolyticus*, and *K. pneumoniae* were bacteria only isolated from CM, whereas *Alcaligenes faecalis*, *Stutzerimonas stutzeri*, and *P. mirabilis* were isolated from PM. *Klebsiella aerogenes* was isolated from the mouth, whereas *Stenotrophomonas malto-*

*philia* was isolated from the nose. However, *S. aureus* and *B. cereus* were among the bacteria isolated from all of the study sources: masks, nose, mouth, and skin. *S. aureus* was isolated from inside both masks and from the mouth, skin, and nose. Generally, the isolated bacteria were diverse and from 13 different genera (*Bacillus*, *Mammaliococcus*, *Staphylococcus*, *Enterococcus*, *Stutzerimonas*, *Pseudomonas*, *Stenotrophomonas*, *Acinetobacter*, *Alcaligenes*, *Neisseria*, *Proteus*, *Klebsiella* and *Enterobacter*). Compared with CM, PM resulted in a greater number of bacterial genera. For instance, *A. hemolyticus* and *P. aeruginosa* were isolated both inside masks and from the mouth, indicating that the bacteria might have been from the mouth to the mask during mask use.



### 3.7. Antibiotic Susceptibility of the Isolated Bacteria to Selected Antibiotics

Statistically significant differences were recorded among isolates for all antibiotics at P values < 0.0001 (Table A2). Also the means were interpreted according to the CLSI standards in (Table A3). In Table 2, Ampicillin exhibited the lowest efficacy, with only 14.8% of isolates classified as susceptible, and a striking 85.2% resistance. This high resistance rate suggests that Ampicillin is largely ineffective against the majority of isolates recovered from the masks. Streptomycin demonstrated a relatively balanced response, with 48.1% of isolates susceptible and another 48.1% resistant, indicating moderate effectiveness and highlighting variability in bacterial response to this antibiotic. Spectinomycin, Cefotaxime, and Amoxicillin all showed similar patterns, with high resistance rates (66.7%, 63.0%, and 66.7%, respectively) and low susceptibility rates (22.2% for each), suggesting limited therapeutic potential for bacteria in this setting. Sulfan showed slightly better performance compared to the others, with 29.6% of isolates susceptible. However, 63.0% of isolates were still resistant, reinforcing the prevalence of multi-drug resistance among the sampled bacteria. Very few antibiotics showed notable intermediate responses, except for Cefotaxime.

**Table 2.** Antibiotic susceptibility patterns based on the percentage of bacterial isolates that are classified as Susceptible (S), Intermediate (I), or Resistant (R) to different antibiotics.

Antibiotic	% Susceptible (S)	% Intermediate (I)	% Resistant (R)
Ampicillin	14.8%	0.0%	85.2%
Streptomycin	48.1%	3.7%	48.1%
Spectinomycin	22.2%	11.1%	66.7%
Cefotaxime	22.2%	14.8%	63.0%
Amoxicillin	22.2%	11.1%	66.7%
Sulfan	29.6%	7.4%	63.0%

## 4. Discussion

*Bacterial quantity on masks, inside and outside masks and wearing time.*

Compared with CM, PM resulted in a significantly greater number of bacterial CFUs. This could be a result of the water retention capacity of the polycotton fabric. For example, cotton absorbs moisture better than do polyester fabrics, which repel moisture and are less absorbent and thus hydrophobic [27]. Additionally, CM is comfortable in terms of breathing and quickly releases perspiration and other bodily moisture by absorbing it, resulting in a low quantity of bac-

teria with mask use time in this study. In addition, polyester fibers are less permeable than cotton fibers and tend to retain heat and moisture near the skin instead of allowing them to escape, providing a conducive environment for bacterial survival [28] and thus high bacterial counts on the PM. Microorganisms have varying temperature and pH requirements for growth; therefore, the microclimate created by polycotton fabric, particularly when it traps moisture, may provide conditions that are favorable for certain microorganisms to thrive. This study is in line with the findings of [10], who reported that the number of bacterial colonies on surgical masks was significantly lower than that on cotton masks because of the differences in temperature and high degree of ventilation between surgical masks and cotton masks.

There were significantly greater bacterial CFUs on the face side than on the outer side of the masks. This may be due to the different environments inside and outside the mask. The high number of bacteria on the inner layers of the masks may be due to the conducive environment, high humidity, and moisture inside the mask caused by contact between the mask and the skin. Inside a worn mask, there is a favorable environment for shed bacteria through exhalation, saliva droplets, and perspiration [29]. Additionally, the nutrients present in perspiration and saliva help bacteria survive and multiply [30]. Furthermore, the pH and temperature of the skin support bacterial survival [31].

The prolonged use of masks significantly increased the number of bacterial colonies in this study. The longer the mask is worn, the greater the provision of nutrients from saliva and sweat [30] as well as moisture retention and temperature in the mask, thus increasing bacterial survival [8]. Therefore, the increase in bacterial counts with increasing mask use time in this study could be a result of the environment created during mask use, which increases the survival of bacteria on the mask. This finding is supported by research by [32], who reported that prolonged wearing of surgical masks significantly increased bacterial CFUs. Moreover, after two and four hours, the number of bacterial colonies generated via respirators and surgical masks increased significantly [33]. Additionally, [10] reported that masks contained  $1.46 \times 10^5$  CFU/mask and that surgical masks contained  $1.32 \times 10^4$  CFU/mask after four hours of use.

*Bacteria isolated from cotton and poly-cotton masks.*

The isolation of bacteria from masks has resulted in the detection of specific strains belonging to the *Staphylococcus* spp., *Bacillus* spp., and *Acinetobacter* spp. genera known to be associated with skin and respiratory tract infections [10]. In this study, the bacterial strains isolated from the masks included *Bacillus* spp., *Staphylococcus* spp., *Pseudomonas* spp., *Klebsiella* spp., and *Proteus* spp., some of which are known as commensals but are pathogenic under extreme conditions, whereas others are naturally pathogenic. *B.cereus* and *S.saprophyticus* have been isolated from face masks [12]. In addition, [34] isolated *Staphylococcus* spp., *Escherichia coli*, *Peptococcus* spp., and *P.aeruginosa* from constantly used face

masks. Moreover, *Acinetobacter*, *Pseudomonas*, and coagulase-negative *Staphylococcus* were the most common strains, whereas *Klebsiella* and *Enterococcus* were the least common species isolated from facemasks of hospitalized patients in a COVID-19 center [6]. These findings are similar to our findings; however, our study was in a public market setting, and the most isolated genus was *Bacillus*. CMs have been reported to be potential substrates of bacteria since they become moist when worn, providing a conducive environment for bacterial multiplication [7]. As bacteria multiply, they increase in number and may result in secondary infections, especially in immunocompromised personnel. Additionally, *Neisseria subflava*, *S. epidermidis*, and *S. aureus* were isolated from the outside part of a medical mask of a dentist after attending to a dental patient, an indication that masks become contaminated with bacteria when in use [35]. Therefore, bacteria from *Bacillus*, *Proteus*, *Enterococcus*, and *Stutzerimonas* were found on the outside of the mask, and this contamination could be from the external market environment. Similarly, [36] reported that bacterial contamination in masks may be caused by mask users and the external environment.

#### *Bacteria isolated from the mouth, skin and nose*

Pathogenic bacteria, including *S. aureus*, *P. aeruginosa*, *Neisseria meningitis*, *Streptococcus* sp. and *K. pneumoniae*, have been found in healthy human saliva [16, 37]. Therefore, saliva harbors pathogenic bacteria and can serve as a primary source of contamination on worn cotton masks through droplets; thus, more bacteria are isolated from the mouth than from the nose or skin. In 60% to 70% of healthy adults and children, the nasopharynx contains several harmful bacteria, such as *S. pneumoniae* [38]. In addition, airborne pathogens are a broad category of microorganisms that can enter the respiratory system through dust, aerosols, or droplets. They can significantly increase morbidity and mortality rates, particularly in persons with weakened immune systems [38]. Coagulase-negative staphylococci, especially *S. epidermidis*, anaerobic *Cutibacterium acnes*, *Corynebacterium*, *Micrococcus*, *Streptococcus*, and *Acinetobacter*, are the dominant species in the skin microbiome [39], while some are commensals or allergens [40].

#### *Clinical Significance and Microbiological Risks of Isolated Bacteria.*

Several clinically significant pathogens were identified in the study, including *S. aureus*, *P. aeruginosa*, and *E. faecalis*. *S. aureus* is a major nosocomial human pathogen that can cause a wide range of illnesses, including bacteremia, skin abscesses, bone infections, pneumonia, respiratory tract infections, prosthetic joint infections, surgical site infections, cardiovascular infections, and, occasionally, multiresistant infections. *S. aureus* has been the main cause of secondary bacterial infections in previous viral pandemics, which has led to a marked increase in patient fatality rates [41]. *E. faecalis* causes serious sickness in people whose immune systems are weakened and are responsible for bacteremia, urinary tract infections (UTIs), endocarditis, wound infections, meningitis, intraabdominal and pelvic infec-

tions, and nosocomial and iatrogenic infections [42]. A common cause of nosocomial infections, including pneumonia, infections in immunocompromised hosts, and infections in people with structural lung diseases such as cystic fibrosis, is the pathogen *P. aeruginosa* [43]. *A. hemolyticus* has been isolated from sputum samples of respiratory infections and soil [44], confirming the findings of this study. In addition, *A. faecalis* causes opportunistic infections in humans, although some strains have been isolated from poultry. This bacterium causes bloodstream infections, endocarditis, meningitis, appendicitis, abscesses, and postoperative endophthalmitis. In a few cases, it has been linked to fatal pneumonia and death [45]. *N. perflava* was isolated inside both the fabric of the mask, the mouth, and the skin in this study and is generally considered part of the normal flora of the human respiratory tract and mucous membranes [46]. *P. mirabilis* is an environmental pathogen that can infect the skin, respiratory system, wounds, and urinary tract [47]. *K. pneumoniae* is well known as an opportunistic pathogen that can cause invasive human infections such as bacteremia. *Proteus vulgaris* has been isolated from UTI patients and is among the resistant microbes. *K. aerogenes* and *E. cloacae* are present in the normal flora of the human gastrointestinal tract and are also widely encountered in the environment. These bacteria are found in soil, sewage, and water and are considered phytopathogens for several plant species.

*Bacillus* was the most isolated genus in this study. Among them, *B. cereus* and *B. thuringiensis* are known for their pathogenic potential. Although *B. cereus* strains are generally known for their ability to cause food poisoning, they cause systemic illness, localized infections of the eyes and wounds [48-50]. *B. licheniformis* is a potential human pathogen because it can cause illnesses in individuals who are unable to establish typical immune responses. It was found to be the cause of unilateral maxillary sinusitis in a patient with a functioning immune system [51].

#### *Antibiotic effects of isolated bacteria.*

The antibiotic susceptibility patterns observed in this study reveal a concerning prevalence of resistance among bacterial isolates obtained from worn reusable facemasks. Notably, Ampicillin exhibited the highest resistance rate, with 85.2% of isolates categorized as resistant, and followed by Spectinomycin and Amoxicillin, each showing 66.7% resistance, and Cefotaxime with 63.0% resistance. These findings suggest that a significant proportion of bacteria isolated from the facemasks possess mechanisms to evade the action of commonly used antibiotics, potentially complicating treatment if such bacteria were to cause infection. In contrast, Streptomycin demonstrated the highest susceptibility (48.1%), indicating relatively better efficacy against the isolates, though still reflecting considerable resistance. The presence of intermediate resistance patterns, especially in drugs like Cefotaxime (14.8%) and Sulfan (7.4%), further points to emerging resistance and the risk of reduced therapeutic effectiveness. These results align with global concerns over rising antimicrobial resistance (AMR), particularly among environmental and opportunistic pathogens. The findings highlight the

critical role of routine antimicrobial surveillance and stress the importance of promoting responsible antibiotic use, alongside public health messaging on mask hygiene, to curb the spread of resistant bacteria within the community. Respiratory tract pathogens are increasing in prevalence and are becoming increasingly difficult to treat [14]. In this study, the most resistance genera were *Pseudomonas*, *Enterococcus* and *Enterobacter*. Some ESKAPE pathogens are global health concerns. In the United States, multidrug-resistant *P. aeruginosa* and drug-resistant *S. pneumoniae* are among the serious threats to healthcare. Owing to the many virulence characteristics of *E. faecalis*, this opportunistic pathogen is linked to secondary apical illnesses and antibiotic evasion mechanisms. Current medications are ineffective against developing strains of *S. aureus* [52].

## 5. Conclusion

Cotton and polycotton masks are contaminated with bacteria when worn, and the quantity of bacterial contamination varies with time. The level of contamination increases with increasing time. In addition, these bacteria remain viable for more than six hours of use. The rate of bacterial contamination on masks significantly depends on the mask type. In addition, worn cotton and polycotton masks harbor bacteria including *Bacillus*, *Staphylococcus* and *Pseudomonas* among others with varying degrees of resistance to commonly used antibiotics. Also the mouth, nose and skin harbor bacteria before masks are worn. The presence of pathogenic bacteria on used masks establish a risk to the health of the wearer. This study provides insights into factors to be considered when choosing reusable mask types, especially those worn for prolonged durations. It also highlights bacteriological contamination during mask use thus the need to provide awareness to the public before mask type recommendation. Worn masks contain antibiotic resistant bacteria thus the need to encourage proper mask disposal after use and proper sterilization procedures before reuse. However, the study had several limitations. The sample size and geographic scope were limited, which may restrict the generalizability of the findings. In addition, the range of antibiotics tested was not exhaustive, and molecular methods were not employed to confirm resistance mechanisms. Future research should include a broader selection of antibiotics, screening of resistance genes, and long-term monitoring across different environments to enhance the validity and applicability of the results.

## Abbreviations

COVID-19	Coronavirus Disease 2019
SARS-CoV 2	Severe Acute Respiratory Syndrome Coronavirus 2
WHO	World Health Organization
CFU	Colony Forming Unit
RM	Reusable mask

CM	Cotton mask
PM	Polycotton mask
TSI	Tripple Sugar Iron
SIM	Sulfide Indole Motility
MIU	Motility Indole Urease
MR	Methyl Red
VP	Voges-Proskauer
DNA	Deoxyribonucleic acid
CTAB	Cetrimonium bromide
TBE	Tris-borate-EDTA
UV	Ultraviolet radiation
ANOVA	Analysis of Variance
SAS	Statistical Analysis Software
BLAST	Basic Local Alignment Search Tool
NCBI	National Council of Biotechnology Institute
MEGA-X	Molecular Evolutionary Genetics Analysis across Computing Platforms.
HSD	Tukey's Honest Significance Difference
PCR	Polymerase Chain Reaction

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## Ethical Approval

The Kenyatta University Ethics Review Committee ap-

proved this research project under the approval number PKU/2595/11722.

## Data Availability Statement

The accession numbers for the sequenced data is accessible in the National Centre for Biotechnology Information. <https://submit.ncbi.nlm.nih.gov/subs/?search=SUB14274966>

<https://submit.ncbi.nlm.nih.gov/subs/?search=SUB14275714>.

More data is available from the corresponding author upon request

## Conflicts of Interest

Authors declare no conflict of interest.

## Appendix

**Table A1.** Bacterial identity and isolation source.

Isolate	Source	Identity	Percentage Identity	Accession Number
E2	CO, PI	<i>Bacillus toyonensis</i>	94.39	PP408994
E3	CO, M, CI	<i>Bacillus subtilis</i>	96.26	PP408970
E6	PI, CI	<i>Bacillus pumillus</i>	95.90	PP408991
E9	CI	<i>Bacillus wiedmannii</i>	92.36	PP408978
E11	CI, M	<i>Acinetobacter hemolyticus</i>	98.23	PP408988
E16	PO, CI	<i>Bacillus thuringiensis</i>	98.24	PP408972
E20	PI, CI, M	<i>Enterococcus faecalis</i>	99.11	PP408973
E23	PI, CI, S, N	<i>Bacillus cereus</i>	99.51	PP408976
E26	PI	<i>Enterobacter cloacae</i>	97.16	PP408989
E28	CI, CO, PI, PO, S, M, N	<i>Staphylococcus aureus</i>	99.21	PP408975
E29	CI, M	<i>Klebsiella pneumoniae</i>	98.79	PP408979
E30	N	<i>Stenotrophomonas maltophilia</i>	98.73	PP408974
E34	PI	<i>Proteus mirabilis</i>	98.25	PP408971
E36	PI, CI, S, M	<i>Neisseria perflava</i>	92.33	PP408987
E40	CO	<i>Proteus vulgaris</i>	90.43	PP408980
E44	M	<i>Klebsiella aerogenes</i>	90.43	PP408990
E45	PI, CI, S, M, N	<i>Bacillus cereus</i>	99.51	PP408981
E46	PI, CI, M, N	<i>Mammliococcus sciurii</i>	95.63	PP408982
E49	PI, CO, S, M	<i>Enterococcus faecalis</i>	99.69	PP408986
E51	CO	<i>Bacillus cereus</i>	96.25	PP408977
E52	PI, CI, S, N	<i>Bacillus licheniformis</i>	94.64	PP408985
E55	PI, M	<i>Pseudomonas aeruginosa</i>	86.79	PP408984
E56	PI, CI, S, M, N	<i>Staphylococcus aureus</i>	96.14	PP408983
E58	CO, S, N	<i>Bacillus altitudinis</i>	100	PP437268
E59	PI	<i>Alcaligenes faecalis</i>	100	PP408993
E60	PO	<i>Stutzerimonas stutzeri</i>	100	PP408992

Keywords: CO—Cotton mask outside, CI—Cotton mask inside, PO—Polycotton mask outside, PI—Polycotton mask inside, M—Mouth, N—nose, S—skin.

**Table A2.** Antibiotic susceptibility means of the isolated bacteria to selected antibiotics.

ISOLATE	ZONES OF INHIBITION(Millimeter) mm					
	Ampicillin	Streptomycin	Spectinomycin	Cefotaxime	Amoxicillin	Sulfan
E2	6.00±0.00 <sup>g</sup>	24.00±0.58 <sup>cde</sup>	25.67±0.33 <sup>b</sup>	6.00±0.00 <sup>i</sup>	14.00±0.00 <sup>fgh</sup>	6.00±0.00 <sup>e</sup>
E3	9.00±0.00 <sup>e</sup>	10.00±0.00 <sup>ij</sup>	14.00±0.00 <sup>fgh</sup>	8.00±0.00 <sup>ij</sup>	35.67±2.60 <sup>a</sup>	37.00±2.89 <sup>a</sup>
E6	9.00±0.00 <sup>e</sup>	28.67±0.88 <sup>a</sup>	8.00±0.00 <sup>jk</sup>	19.00±0.58 <sup>d</sup>	22.00±1.15 <sup>de</sup>	13.00±0.58 <sup>d</sup>
E9	6.00±0.00 <sup>g</sup>	28.00±1.15 <sup>ab</sup>	24.00±0.57 <sup>bc</sup>	11.00±0.00 <sup>gh</sup>	13.67±1.45 <sup>fgh</sup>	6.00±0.00 <sup>e</sup>
E11	13.33±0.67 <sup>d</sup>	8.00±0.00 <sup>jk</sup>	10.00±0.00 <sup>ij</sup>	22.00±0.00 <sup>c</sup>	12.00±0.00 <sup>ghi</sup>	18.00±0.00 <sup>c</sup>
E16	6.00±0.00 <sup>g</sup>	6.00±0.00 <sup>k</sup>	10.33±0.88 <sup>i</sup>	29.67±0.33 <sup>b</sup>	14.00±0.00 <sup>fgh</sup>	6.00±0.00 <sup>e</sup>
E20	7.00±0.00 <sup>fg</sup>	8.00±0.00 <sup>jk</sup>	6.00±0.00 <sup>k</sup>	16.00±1.15 <sup>e</sup>	6.00±0.00 <sup>k</sup>	8.00±0.00 <sup>e</sup>
E23	7.00±0.00 <sup>fg</sup>	6.00±0.00 <sup>k</sup>	6.00±0.00 <sup>k</sup>	8.00±0.00 <sup>ij</sup>	6.00±0.00 <sup>k</sup>	6.00±0.00 <sup>e</sup>
E26	6.00±0.00 <sup>g</sup>	6.00±0.00 <sup>k</sup>	8.00±0.00 <sup>jk</sup>	11.00±0.00 <sup>gh</sup>	7.00±0.00 <sup>jk</sup>	12.00±0.00 <sup>d</sup>
E28	6.00±0.00 <sup>g</sup>	25.00±0.00 <sup>cd</sup>	30.00±0.00 <sup>a</sup>	6.00±0.00 <sup>i</sup>	18.00±0.00 <sup>ef</sup>	24.00±0.00 <sup>b</sup>
E29	14.00±0.00 <sup>d</sup>	12.00±0.00 <sup>i</sup>	22.00±0.00 <sup>cd</sup>	10.00±0.00 <sup>ghi</sup>	12.00±0.00 <sup>ghi</sup>	18.00±0.00 <sup>c</sup>
E30	8.00±0.00 <sup>ef</sup>	28.00±0.00 <sup>ab</sup>	6.00±0.00 <sup>k</sup>	14.00±0.00 <sup>ef</sup>	8.00±0.00 <sup>ijk</sup>	14.00±0.00 <sup>d</sup>
E34	6.00±0.00 <sup>g</sup>	30.00±0.00 <sup>a</sup>	15.67±0.33 <sup>efg</sup>	32.00±1.15 <sup>b</sup>	11.00±0.00 <sup>hij</sup>	6.00±0.00 <sup>e</sup>
E36	8.67±0.33 <sup>e</sup>	6.00±0.00 <sup>k</sup>	13.67±0.88 <sup>gh</sup>	6.00±0.00 <sup>i</sup>	6.00±0.00 <sup>k</sup>	23.00±0.58 <sup>b</sup>
E40	6.00±0.00 <sup>g</sup>	19.67±0.33 <sup>g</sup>	17.00±0.00 <sup>e</sup>	20.33±0.88 <sup>cd</sup>	12.67±0.33 <sup>gh</sup>	8.00±0.00 <sup>e</sup>
E44	6.00±0.00 <sup>g</sup>	16.00±0.00 <sup>h</sup>	10.00±0.00 <sup>ij</sup>	6.00±0.00 <sup>i</sup>	16.00±0.00 <sup>fg</sup>	14.00±0.00 <sup>d</sup>
E45	6.00±0.00 <sup>g</sup>	7.00±0.00 <sup>k</sup>	6.00±0.00 <sup>k</sup>	9.00±0.00 <sup>hi</sup>	6.00±0.00 <sup>k</sup>	6.00±0.00 <sup>e</sup>
E46	20.00±0.00 <sup>c</sup>	20.00±0.00 <sup>ef</sup>	11.00±0.58 <sup>i</sup>	22.00±1.15 <sup>c</sup>	29.00±0.58 <sup>bc</sup>	26.00±0.00 <sup>b</sup>
E49	8.00±0.00 <sup>ef</sup>	6.00±0.00 <sup>k</sup>	6.00±0.00 <sup>k</sup>	11.67±0.33 <sup>fg</sup>	7.00±0.00 <sup>jk</sup>	6.00±0.00 <sup>e</sup>
E51	6.00±0.00 <sup>g</sup>	23.67±0.88 <sup>de</sup>	12.00±0.00 <sup>hi</sup>	6.00±0.00 <sup>i</sup>	24.00±0.88 <sup>cd</sup>	14.67±0.88 <sup>cd</sup>
E52	6.00±0.00 <sup>g</sup>	6.00±0.00 <sup>k</sup>	20.00±0.00 <sup>d</sup>	6.00±0.00 <sup>i</sup>	6.00±0.00 <sup>k</sup>	6.00±0.00 <sup>e</sup>
E55	6.00±0.00 <sup>g</sup>	10.00±0.00 <sup>ij</sup>	6.00±0.00 <sup>k</sup>	18.67±0.33 <sup>d</sup>	6.00±0.00 <sup>k</sup>	6.00±0.00 <sup>e</sup>
E56	23.00±0.00 <sup>b</sup>	22.00±0.58 <sup>cd</sup>	6.00±0.00 <sup>k</sup>	8.00±0.00 <sup>ij</sup>	18.00±0.00 <sup>ef</sup>	35.00±0.00 <sup>a</sup>
E58	32.00±0.58 <sup>a</sup>	25.00±0.58 <sup>cd</sup>	28.00±1.15 <sup>a</sup>	6.00±0.00 <sup>i</sup>	36.00±2.31 <sup>a</sup>	38.00±0.00 <sup>a</sup>
E59	6.00±0.00 <sup>g</sup>	26.00±0.58 <sup>bc</sup>	6.00±0.00 <sup>k</sup>	15.00±0.00 <sup>e</sup>	6.00±0.00 <sup>k</sup>	6.00±0.00 <sup>e</sup>
E60	20.00±0.00 <sup>c</sup>	23.00±0.00 <sup>de</sup>	16.00±0.58 <sup>ef</sup>	35.00±0.00 <sup>a</sup>	33.00±0.58 <sup>ab</sup>	23.00±0.58 <sup>b</sup>
Controls	6.00±0.00 <sup>g</sup>	6.00±0.00 <sup>k</sup>	6.00±0.00 <sup>k</sup>	6.00±0.00 <sup>i</sup>	6.00±0.00 <sup>k</sup>	6.00±0.00 <sup>e</sup>
P values	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001

Keywords: Values with the same superscript within the columns are not significantly different from each other according to Tukey's honestly significant difference (HSD) at P<0.05.

**Table A3.** Antibiotic susceptibility interpretative categories according to CLSI

Isolate	Ampicillin	Streptomycin	Spectinomycin	Cefotaxime	Amoxicillin	Sulfan
E2	R	S	S	R	R	R
E3	R	R	R	R	S	S



Isolate	Ampicillin	Streptomycin	Spectinomycin	Cefotaxime	Amoxicillin	Sulfan
E6	R	S	R	I	S	R
E9	R	S	S	R	R	R
E11	R	R	R	S	R	I
E16	R	R	R	S	R	R
E20	R	R	R	I	R	R
E23	R	R	R	R	R	R
E26	R	R	R	R	R	R
E28	R	S	S	R	I	S
E29	R	R	S	R	R	I
E30	R	S	R	R	R	R
E34	R	S	I	S	R	R
E36	R	R	R	R	R	S
E40	R	S	I	S	R	R
E44	R	I	R	R	I	R
E45	R	R	R	R	R	R
E46	S	S	R	S	S	S
E49	R	R	R	R	R	R
E51	R	S	R	R	S	S
E52	R	R	S	R	R	R
E55	R	R	R	I	R	R
E56	S	S	R	R	I	S
E58	S	S	S	R	S	S
E59	R	S	R	I	R	R
E60	S	S	I	S	S	S
Controls	R	R	R	R	R	R

Key: S = Susceptible ( $\geq 20$  mm), I = Intermediate (15–19 mm), R = Resistant ( $\leq 14$  mm)

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