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MICROBIAL LOAD AND BACTERIAL DIVERSITY ON FACEMASKS USED BY STUDENTS OF CALEB UNIVERSITY, IMOTA, LAGOS STATE, NIGERIA

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ABSTRACT

Facemasks are essential in limiting bacterial shedding from the mouth and nose, thereby reducing the spread of infectious agents. This study aimed to isolate and identify bacterial species present on used facemasks worn by students of Caleb University, Imota, Lagos State. Forty (40) used facemasks were collected and analyzed microbiologically using phenotypic and biochemical methods. Results indicated that the majority of isolates were gram-negative rods. Identified bacteria included *Klebsiella pneumonia*, *Yersinia pestis*, *Salmonella enterica*, *Citrobacter werkmanii*, *Escherichia albertii*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Staphylococcus saprophyticus*, and *Yersinia pseudotuberculosis*, *Citrobacter werkmanii* (14%), while several species including *Klebsiella pneumoniae* and *Salmonella typhi* were frequent (4% each). A higher bacterial load was observed on facemasks used by female students compared to males. The primary source of contamination was identified as the students' own body surfaces, including the face, nose, mouth, and hands. These findings underscore the importance of proper facemask hygiene and it is strongly recommended that students replace masks daily after each use to reduce microbial contamination and the risk of infection.

Keywords: Facemask, Gram-negative, Student, *Escherichia albertii*, Species

INTRODUCTION

The use of face masks as a protective health measure dates back to the late 19th and early 20th centuries, with early medical use grounded in germ theory. Surgeons like Jan Mikulicz-Radecki and Paul Berger pioneered mask usage in the operating theatre, and Wu Lien-teh notably improved mask designs during the 1910 Manchurian plague to protect both wearer and others. Over time, facemasks evolved into essential personal protective equipment (PPE) in healthcare settings. During the COVID-19 pandemic, the use of masks expanded into communities worldwide, becoming a critical tool in controlling airborne infections (Howard *et al*., 2021).

Facemasks are now widely used in schools, public spaces, and other crowded environments where airborne disease transmission risk is high. However, while masks serve as barriers to respiratory pathogens, they also accumulate microorganisms from both the user and the environment over time. Moisture, prolonged use, and improper disposal can transform used facemasks into reservoirs of potentially infectious agents, facilitating cross-contamination and reducing protective efficacy **(**Chua *et al*., 2020**)**.

Settings like university campuses with frequent interpersonal interactions and high facemask usage promote microbial exchange. Students are especially vulnerable due to long durations of mask wearing under less-than-optimal hygienic conditions. Although masks prevent inhalation of aerosols containing pathogens, they can also become contaminated through exhaled breath, speech, and environmental exposure, serving as secondary sources of infection (Nightingale *et al*., 2023).

Recent studies among healthcare and general populations have demonstrated significant bacterial colonization of facemasks. For instance, a point-prevalence study of nursing-home staff found *Staphylococcus aureus* on 15.9 % of facemasks and gram-negative bacteria on 31.9 % of facemasks (Nightingale *et al*., 2023).

MATERIALS AND METHODS

Study Area

Caleb University is a prominent private institution located in Imota, Lagos State, in the southwestern region of Nigeria. Established in 2007, it is known for its commitment to academic excellence, discipline, and moral values, attracting a diverse student population from across Nigeria and beyond.

Collection of Samples

A cross-sectional study was conducted to evaluate bacterial contamination on forty used facemasks worn by students of Caleb University, Imota, Lagos State. The study population included both male and female students who voluntarily submitted their used masks. Each facemask was carefully collected using sterile zip-lock bags to prevent external contamination during transport. Samples were promptly transferred to the Microbiology Laboratory, Caleb University, for microbial analysis to ensure sample integrity and accurate identification of bacterial contaminants.

Bacterial Isolation

A 1 cm^2 section was aseptically excised from both the internal and external surfaces of each facemask near the nose bridge. Each section was inoculated into nutrient broth, prepared according to the manufacturer's instructions and sterilized at 121°C for 15 minutes. The inoculated broths were incubated at 37°C for 24 hours, after which turbidity indicated bacterial growth. Cultures were serially diluted with sterile distilled water and pour plated on nutrient agar. The plates were incubated at 37°C for 24 hours, and distinct colonies were counted and sub-cultured to obtain pure isolates. The pure cultures were preserved in glycerol broth for further analysis and subjected to Gram staining and biochemical tests for bacterial identification (Cheesbrough, 2006; Cappuccino and Sherman, 2014).

Selective and Differential Media

Selective and differential media were used to identify specific bacterial groups. MacConkey agar was used for Gram-negative rods, containing bile salts and crystal violet to inhibit Gram-positive bacteria; lactose fermentation was indicated by pink colonies. Mannitol salt agar was employed for the isolation of *Staphylococcus aureus*; mannitol fermentation produced yellow zones around colonies. Media were prepared according to the manufacturer's guidelines, sterilized, poured into Petri dishes, and inoculated with pure isolates before incubation at 37°C for 24 hours (Holt et al., 1994; Brooks et al., 2013).

Gram Staining

Gram staining was carried out to differentiate bacteria based on cell wall characteristics. Heat-fixed smears were stained sequentially with crystal violet for 60 seconds, Gram's iodine, decolorized with 70% ethanol, and counterstained with safranin. Slides were air-dried and examined under a light microscope to classify isolates as Gram-positive or Gram-negative (Gram, 1884; Cappuccino and Sherman, 2014).

Biochemical Identification Tests

i. ***Urease Test:*** Conducted to detect urease activity using urea agar. A colour change from yellow to pink after incubation at 37°C for 24 hours indicated a positive result.

ii. ***Indole Test:*** Performed in peptone water to assess tryptophan degradation. The addition of Kovac's reagent producing a cherry-red layer indicated a positive result.

iii. ***Catalase Test:*** A drop of 3% hydrogen peroxide was added to bacterial cultures on a sterile slide; bubble formation confirmed catalase activity.

iv. ***Citrate Utilization Test:*** Conducted using Simmon's citrate agar to test the ability to utilize citrate and ammonium salts. A colour change from green to blue after 48 hours indicated a positive result.

v. ***Sugar Fermentation Test:*** Isolates were tested for fermentation of lactose, glucose, maltose, and sucrose in phenol red broth. Yellow coloration indicated acid production, while gas bubbles in Durham tubes denoted gas production.

All tests were carried out according to standard bacteriological procedures (Cheesbrough, 2006; Cappuccino and Sherman, 2014; MacFaddin, 2000).

RESULTS AND DISCUSSION

The selective and differential media on MacConkey agar and gram reaction test outcome is shown in Table 1 indicating that Isolates 6^1B, 22^2, 20G, 3B, 21G, 231G, 14G, 15G, 4B, 13^2G were lactose fermenters, while 22^1B, 9B, 8^1B, 8^3B, 5B, 23^2G, 23^1G, 21^2B, 21^3B, 21^3G, 23^3B, 23^4G, 24^2G, 18B, 13G, 18^2G. 21^2B, 21^3G, 23^4G, 23^3B, 24G, 18B had cocci shape and gram + ve while others were rod and gram -ve.

Table 1: Selective and Differential Media on MacConkey Agar and Gram Reaction Test Outcome

Isolates	Lactose Fermenters	Non-Lactose Fermenters	Shape	Gram +ve	Gram -ve
6^1B	+		Rod		-
22^1B		+	Rod		-
22^2B	+		Rod		-
9B		+	Rod		-
20G	+		Rod		-
8^1B		+	Rod		-
3B	+		Rod		-
21G	+		Rod		-
8^3B		+	Rod		-
5B		+	Rod		-
23^2G		+	Rod		-
23^1G	+		Rod		-
21^2B		+	Cocci	+	
21^3G		+	Cocci	+	
23^3B		+	Cocci	+	
23^4G		+	Cocci	+	
24G		+	Cocci	+	
18B		+	Cocci	+	
13G		+	Rod		-
18^2G		+	Rod		-
14G	+		Rod		-
15G	+		Rod		-
4B	+		Rod		-
13^2G	+		Rod		-
7B			Rod		-
20^1G			Rod		-
18^3B			Rod		-

Isolates	Lactose Fermenters	Non-Lactose Fermenters	Shape	Gram +ve	Gram -ve
14B			Rod		-
18^4G			Rod		-
131G			Rod		-

Tables 2 revealed pigmented colonies indicative of lactose fermenters and mannitol fermenters. Biochemical profiling. Table 3 aided in confirming the identities of the isolates, while distribution data (Table 4) highlighted that *Proteus mirabilis* had the highest occurrence (21%), followed by *Citrobacter werkmanii* (14%). Other isolates such as *Klebsiella pneumoniae*, *Yersinia pestis*, *Providencia rettgeri*, *Escherichia albertii*, *Staphylococcus aureus*, *Staphylococcus saprophyticus*, and *Salmonella typhi* each showed lower occurrence rates (4%).

Table 2: Selective and Differential Media on Mannitol Salt Agar

Isolates	Pigment colonies	Non- pigment colonies
21^2B		+
21^3G		+
23^3B		+
23^4G	+	
24G	+	
18B	+	

Table 3: Biochemical Characteristics of Isolates obtained from Nose Masks

Isolates	Coagulase	Catalase	Citrate	H_2S	Indole	Motility	Urease	Glucose	Lactose	Maltose	Sucrose	Fructose	Hydrolysis	Haemolysis	Probable Organisms
6^1B	+	+	-	-	-	+	A+/G+	+	+	+	-				*Klebsiella pneumoniae*
22^1B	+	-	-	-	-	-	A+/G-	-	+	-	-				*Yersinia pestis*
22^2B	+	+	+	-	+	-	A+/G-	-	+	-	-				*Salmonella enterica*
9B	+	-	+	-	+	+	A+/G+	-	-	-	-				*Proteus mirabilis*
20G	+	-	+	-	+	+	A+/G+	+	+	-	-				*Citrobacter werkmanii*
8^1B	+	+	-	+	+	+	A+/G-	-	-	+					*Providencia rettgeri*
3B	+	+	+	-	+	+	A+/G-	-	+	-	-				*Salmonella enterica*
21G	+	-	-	-	+	-	A+/G+	-	-	-					*Escherichia albertis*
8^{13}B	+	-	+	-	+	+	A+/G+	-	-	-	-				*Proteus mirabilis*
5B	+	-	+	-	+	+	A+/G+	-	-	-	-				*Proteus mirabilis*
23^2G	+	-	+	-	+	+	A+/G+	-	-	-	-				*Proteus mirabilis*
23^1G	+	-	+	-	+	+	A+/G+	+	+	-	-				*Citrobacter werkmanii*
21^2 B -	+	-	+	-	-	+	A+/G+	+	+	+	+	-	-		*Staphylococcus saprophyticus*
13G	+	-	-	-	-	-	A-/G-	-	-	-					*Shigella dysenteriae*
18G	+	-	+	-	+	+	A-/G+	-	-	-					*Proteus mirabilis*
14G	+	+	-	-	+	+	A+/G+		-	-	+	-			*Pseudomonas aeruginosa*
15G	+	-	+	-	-	-	A+/G-	-	+	-	-				*Salmonella typhii*
21 ^{3}G	+	-	+	-	-	+	A+/G+	+	+	+	+	-	-		*Staphylococcus saprophyticus*
23 ^{3}G	+	-	+	-	-	+	A+/G+	+	+	+	+	-	-		*Staphylococcus saprophyticus*
23 ^{4}G	+	+	+	-	-	+	A+/G-	+	+	+	+	-	+		*Staphylococcus aureus*
4B	+	-	-	-	-	-	A-/G-	-	-	-					*Shigella dysenteriae*
13^2G	+	-	-	-	-	-	A-/G-	-	-	-					*Shigella dysenteriae*
7B	+	-	-	-	-	-	A+/G-	-	+	-					*Yersinia psudotuberculosis*
20^1G	+	-	+	-	+	+	A+/G+	-	-	-	-				*Proteus mirabilis*
18^2G	+	-	+	-	+	+	A+/G+	+	+	-					*Citrobacter werkmanii*

Coagulase	Catalase	Citrate	H_2S	Indole	Motility	Urease	Glucose	Lactose	Maltose	Sucrose	Fructose	Hydrolysis	Haemolysis	
14B	+						A+/G+							*Pseudomonas aeruginosa*
		+	-	-	+	+			-	-	+	-	+	
18[1]G	+						A+/G-							*Yersinia pseudotuberculosis*
		-	-	-	-	-		-	+	-				
13[1]G	+	-	+	-	+	+	A+/G+	+	+	-				*Citrobacter werkmanii*
24G	+	+	-	-	-	+	A+/G-	+	+	+	+	-	+	*Staphylococcus aureus*
18B		+	-	-	-	+	A+/G-	+	+	+	+	-	+	*Staphylococcus aureus*

Discussion

This study investigated the bacterial contamination of used face masks worn by selected students of Caleb University. Bacteriological analyses of samples from face masks revealed a diverse range of bacterial species. The findings suggest a general non-compliance with recommended mask usage and hygiene protocols among students. Improper mask handling such as storing masks in lab coat pockets, on desks, or inside bags, along with frequent touching and reusing without washing likely contributed to the contamination observed. Similar conclusions were drawn by Banu *et al*. (2012), who found significant bacterial presence in lab coat pockets, and by Varghese and Patel (1999), who reported that white coat pockets and sleeves often harbour pathogenic bacteria. Additionally, some students reportedly reused masks for extended periods especially black-coloured ones without cleaning, which may facilitate microbial accumulation and increase infection risk. Special-purpose media confirmed the presence of potentially pathogenic organisms. On MacConkey agar, organisms such as *Klebsiella spp.* and *Pseudomonas spp.* were identified. The presence of *Pseudomonas spp.*, known for their opportunistic pathogenicity, is of concern. These organisms are associated with urinary tract infections, respiratory infections, dermatitis, and septicemia (Monalisa *et al*., 2017). *Klebsiella spp*. are also significant, linked to pneumonia, bloodstream infections, and surgical site infections (Monalisa *et al*., 2017). On Mannitol Salt Agar, *Staphylococcus aureus* and *Staphylococcus saprophyticus* were isolated. *S. aureus* is known to cause serious infections including bloodstream infections, pneumonia, cellulitis, and osteomyelitis (Minnesota Department of Health, 2010). *S. saprophyticus* is recognized as a leading cause of urinary tract infections in young sexually active women (Natsis *and* Cohen, 2018). The detection of these organisms on face masks strongly suggests potential routes for infection transmission if hygienic practices are neglected. It also underlines the importance of environmental contamination and personal behavior in microbial colonization of personal protective equipment.

CONCLUSION

The presence of potentially pathogenic microorganisms on students' face masks emphasizes the critical need for proper usage, storage, and disposal practices. The findings highlight a significant gap in hygiene compliance, increasing the risk of opportunistic and nosocomial infections.

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