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Full Length Research

Study on bacterial contamination of cloth face mask among students of Modibbo Adama University Yola Adamawa State Nigeria

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ABSTRACT: Despite its potential to reduce respiratory droplet transmission and offer some level of protection, cloth masks have a number of disadvantages and potential problems when used. This research's objective is to determine the prevalence of pathogenic microorganisms associated with fabric face masks among students of Modibbo Adama University Yola. Streak plate method and biochemical test were used to isolate and identify microorganisms on Nutrient Agar. Antimicrobial susceptibility testing was done using the Kirby Bauer technique using Pefloxacin, Gentamycin, Ampiclox, Zinnacef, Amoxicillin, Rocephin, Ciprofloxacin, Septrin, and Erythromycin. Nine bacterial genera, seven gram-negative strains, and two gram-positive strains were found through bacterial analysis. *Bacillus spp*. *Staphylococcus. spp., E. coli*, *Pseudomonas spp.*, *Shigella spp.*, *Klebsiella spp.*, *Proteus spp*., *Enterobacter spp.* and *Salmonella spp*. were the bacteria isolated. The most frequent bacterium was *Escherichia coli* (25.6%), followed by *Bacillus species* (17.7%%), while Shigella species was the least frequent organism with a 2.2 percent rate of incidence. Only Shigella species are highly resistant (100%) to all tested antibiotics, with the exception of Ciprofloxacin, which had a resistance rate of 50%, according to the findings of isolates' susceptibility tests. Similar to this, *Pseudomonas species* have demonstrated significant rates of resistance to Pefloxacin, Gentamycin, Ampiclox, Amoxicillin and Rocephin. However, the many antibiotics examined have varied effects on the *Staphylococcus species, E. coli, Klebsiella species, Salmonella species and Proteus species*. It is inferable that applying cloth face masks without following the health protocols can be a source of contamination itself, which in turn leads to the spread of many multidrug resistant pathogens such as *Bacillus* and *Pseudomonas* in environments.

Keywords: Antibiotic resistance, contamination, fabric mask, pathogenic microorganism.

INTRODUCTION

A face mask is a protective covering that is put over the mouth and nose to assist stop the transmission of airborne particles, especially respiratory droplets that might have viruses or bacteria in them (CDC, 2021; WHO, 2020). Face masks are frequently worn in a variety of environments, such as offices, factories, and public locations. Face masks come in a variety of styles, each created with a specific use in mind, such as surgical masks (disposable masks that are frequently worn by medical personnel during operations), N95 Respirators (N95 respirators are specialized masks designed to filter out at least 95% of airborne particles, including very small particles and droplets), Loose-Fitting Masks (loose-fitting masks protect the wearer from large respiratory droplets and prevent the wearer's respiratory secretions from contaminating the environment) (Bourouiba, 2020).

The usage of face masks is advised by health officials as a precaution to lessen the transmission of respiratory infections, particularly during disease outbreaks like COVID-19. Depending on the circumstance and amount of protection required, a certain mask type may be appropriate (WHO 2020). Cloth masks have various drawbacks and possible issues with their usage, despite the fact that they can be successful in reducing the

transmission of respiratory droplets and providing some amount of protection. Comparatively to N95 respirators or surgical masks, cloth masks are often less effective in filtering out fine particles and offering a tight seal but they could not shield you as well from extremely tiny respiratory droplets or infectious aerosols (Van Doremalen *et al*., 2020).

Despite these drawbacks, cloth masks can still be an effective preventative strategy when worn appropriately, especially when paired with additional precautions like physical separation and excellent hand cleanliness (Pung *et al*., 2020). The particular circumstance and the degree of danger of exposure to infectious agents should be taken into account while choosing a mask. It is critical that people wearing cloth masks are aware of these possible concerns and take the necessary safety measures. To reduce health concerns connected to the usage of cloth masks, it is imperative to heed public health recommendations and guidelines (Van Doremalen *et al*., 2020).

Utilizing a cotton mask increases your risk of becoming sick more frequently depending on the type of fabric, quantity of layers, and degree of fit, a cloth mask may or may not be useful at avoiding infection (Leung *et al*., 2020; Pung *et al*., 2020). No matter the type of mask worn, infection risk is often higher in places with high transmission rates. The risk of infection can be decreased by consistently using the mask in environments where it is advised (such as crowded indoor settings and public transportation) and by using it properly (covering both the nose and mouth, avoiding touching the mask often) (Delanghe *et al*., 2021). In addition to wearing a mask, various preventative steps can affect how often infections occur. Physical separation, consistent hand washing, and immunization, as needed, are a few of these precautions (Delanghe *et al*., 2021).

When used in conjunction with other preventive measures, cloth masks can still provide some protection, even if they are not as effective as medical-grade masks like N95 respirators. The frequency of infection when using a cotton mask varies depending on these factors. In order to reduce the danger of infection in a given circumstance, it is imperative to abide by local public health recommendations and rules. The possibility of respiratory tract pathogens spreading through infectious droplets and aerosols, along with CDC (2019) guidelines suggesting face mask use to stop the spread of CoV disease 2019 (COVID-19), led us to assess the effectiveness of face masks in preventing the spread of infectious droplets and aerosols through the air.

MATERIALS AND METHODS

Study area

Modibbo Adama University Yola in Girei Local Government Area, Adamawa State, Nigeria, served as the study location with coordinates 9.3486°N, 12,5008°E. There are roughly 17,000 students total, including graduate and undergraduate ones. The institution has nine (9) halls of residence.

Sample collection

A total of forty (40) used cloth masks were collected. The sample was taken from four (4) different university dormitories: Abuja male hostel (A), Nana Asma'u female hostel (N), Kabiru Umar male hostel (K), and Chukwu female dormitory (C). Ten used cloth masks were gathered from each hostel. The samples were then separately placed into sterile bags and within 30 minutes, the samples were delivered to the Modibbo Adama University's Microbiology laboratory for examination.

Isolation and identification of bacteria

Each sampled mask's interior was swabbed with sterile moistened swab sticks before being inoculated onto Nutrient agar (NA) using the streak plate method. Agar plate was then incubated at 37°C for 24 hours. Following incubation, the colonies' physical and cultural traits were examined and noted. Further identification was done by Gram staining and biochemical test (coagulase, catalase, oxidase, indole and citrate tests) following standard microbiological techniques (Owuama, 2015).

Antimicrobial susceptibility test

Antimicrobial sensitivity test was carried by Kirby-Bauer disc diffusion method using Mueller-Hinton agar. A standard inoculum of each isolate was made after which a sterile swab was then used to inoculate the plates by dipping it in the suspension of the bacterial strains and streaked onto the agar. Antibiotic disc such as Pefloxacin, Gentamycin, Ampiclox, Zinnacef, Amoxicillin, Rocephin, Ciprofloxacin, Septrin and Erythromycin were used according to the recommendation of Clinical Laboratory Standard Institute (CLSI, 2020). Using sterile forceps, the discs were picked and placed on the cultured plate which was then incubated at 37°C for 24 hours. The zones of inhibition were measured to the nearest millimeter using a ruler and interpreted.

RESULTS

The result of this study showed that cloth mask harbored a lot of bacteria species. A total of 90 bacterial isolates belonging to nine (9) different genera were obtained. These includes be *Bacillus spp*. *Staphylococcus spp., E. coli*, *Pseudomonas spp.*, *Shigella spp.*, *Klebsiella spp.*,

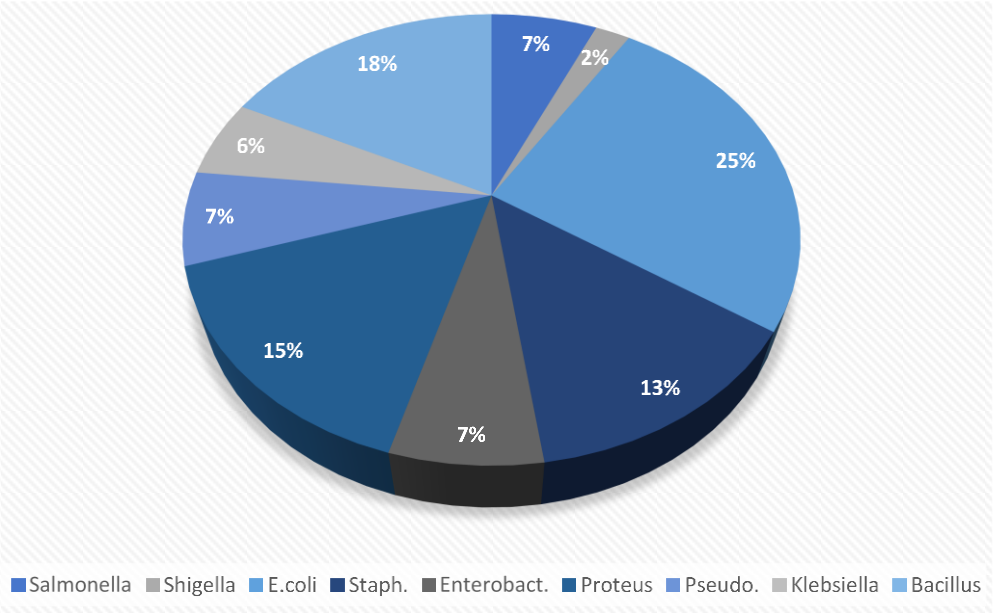

Figure 1. Frequency distribution of bacterial isolates.

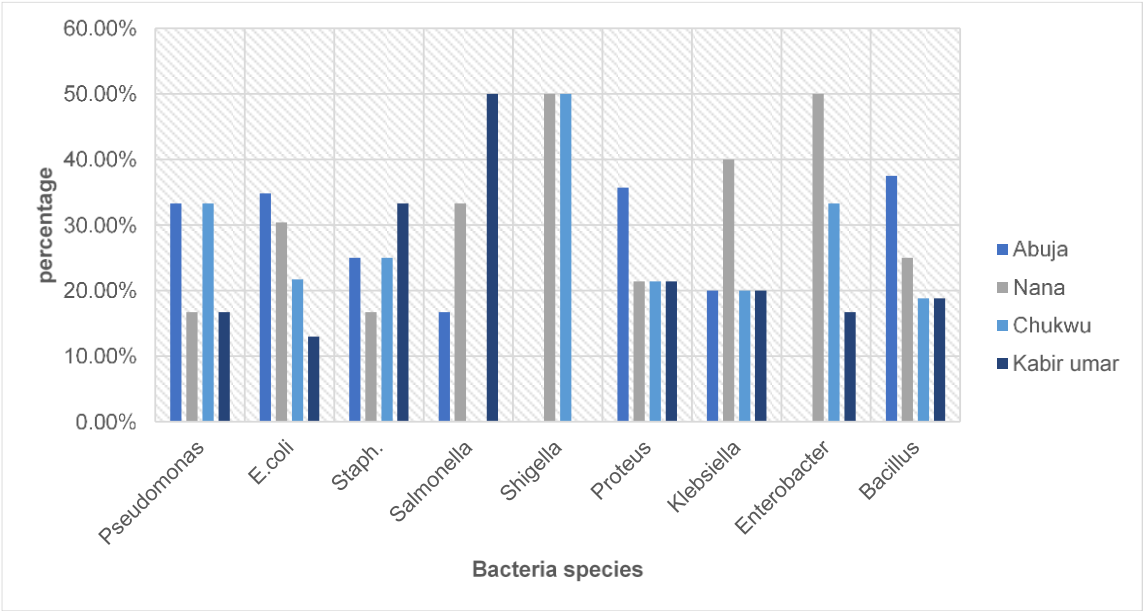

Figure 2. Occurrence rate of bacterial isolate with respect to sample site.

Proteus spp., *Enterobacter spp.* and *Salmonella spp*. Table 1 and Figure 1 show the morphological characteristics and frequency distribution of isolates respectively. *Escherichia coli* (25.6%) is the most predominant bacteria followed by *Bacillus species* (17.7%) while *Shigella species* is the least occurring organism with 2.2% rate of occurrence. The frequency of occurrence of each bacteria with respect to sample site is shown is in Figure 2. Pseudomonas species, staphylococcus species, *E. coli* species and Klebsiella species were found in all sampled site while Shigella species and Enterobacter species were only found in 2 and Three 3 respectively out of all sampled site. The results of susceptibility test of isolates have shown that only *Shigella* species are highly resistant (100%) to all antibiotic tested with exception to Ciprofloxacin which had a resistant rate of 50%. Similarly, *Pseudomonas species* have shown relatively high resistance to Pefloxacin, Gentamycin, Ampiclox, Amoxicillin

Table 1. Morphology and biochemical characteristics of bacterial isolates.

Parameters	*Proteus spp.*	*Pseudomonas spp.*	*Bacillus spp.*	*Salmonella Spp.*	*Klebsiella Spp.*	*Staphylococcus Spp.*	*Escherichia Coli*	*Shigella Spp.*	*Enterobacter Spp.*
Gram reaction	-ve	-ve	+ve	-ve	-ve	+ve	-ve	-ve	-ve
Cellular morphology	Rod swarm	Rod distinct	Rod irregular	Rod	Rod slimy	Cocci distinct	Rod distinct	Rod distinct	Rod distinct
Coagulase test	-	-	-	-	-	+	-	-	-
Catalase test	+	+	+	+	+	+	+	+	+
Oxidase test	-	+	-	-	-	-	-	-	-
Simon's citrate	+	+	+	+	+	+	-	+	+
Indole test	-	-	-	-	-	-	+	-	+

Key: + = positive; - = negative.

Table 2. Susceptibility profile of bacterial isolates in percentages

Bacteria	susceptibility in percentages (%)																	
	PEF		CN		APX		Z		AM		R		CPX		E		SXT	
	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R
Bacillus	50	50	69	31	25	75	38	62	56	44	50	50	56	44	75	25	25	75
E. coli	48	52	78	22	43	57	39	61	26	74	13	87	0	100	43	57	52	48
Enterobacter	67	33	50	50	83	17	17	83	100	0	0	100	0	100	33	67	33	67
Klebsiella	80	20	40	60	60	40	100	0	100	0	0	100	80	20	60	40	60	40
Staphylococcus	100	0	100	0	70	30	90	10	80	20	50	50	58	42	67	33	90	10
Salmonella	50	50	83	17	50	50	67	33	33	67	100	0	0	100	17	83	67	33
Shigella	0	100	0	100	0	100	100	0	0	100	0	100	50	50	100	0	100	0
Pseudomonas	100	0	80	20	0	100	80	20	60	40	20	80	40	60	0	100	20	80
Proteus	21	79	71	29	86	14	50	50	57	43	21	79	64	36	57	43	29	71

Note: PEF = Pefloxacin, CN = Gentamycin, APX = Ampiclox, Z= Zinnacef, AM=Amoxicillin, R = Rocephin, CPX = Ciprofloxacin, SXT = Septrin, E = Erythromycin, R = Resistant, S = Susceptible.

and Rocephin with 100% rates respectively. However*, Staphylococcus species, E. coli, Klebsiella species, Salmonella species* and *Proteus species* have varying response to the different antibiotic tested as shown in Table 2.

DISCUSSION

Assessment of the effectiveness of face masks in preventing the spread of infectious droplets and aerosols through the air was aimed in this study. Bacterial analysis detected 9 bacterial genera, 7 gram-negative and 2 gram-positive strains. Overall, most predominant species from cloth masks were *E. coli* and *Bacillus species* which were reported to be 25.6 and 17.7%, respectively which is proposed that burden bacterial colonization of cloth face mask. In contrast, *Shigella species* was found less (2.2%). It is important to clarify the reason if the high prevalence of *E. coli and Bacillus* which may be related to skin and hand contamination. Furthermore, the results of this study completely contradict with article reviews that reported *Coagulase Negative Staphylococcus* strains have the most frequency in mask associated with clinical cares (ECDC, 2020).

Another study also reported that *S. aureus* are the predominant bacterial species connected to device-associated infections (Maleki *et al*., 2019). On the other hand, *Staphylococcus* are a part of normal flora of skin, and can be easily transported

to the mask due to the direct contact of the mask with the face and skin, as well as moving the mask by hand, both surfaces of the mask become infected with this bacterium (Delanghe *et al*., 2021). Similarly, *Acinetobacter* was frequently detected from the mask samples in this study which can be related to the fact that the most common site for Acinetobacter infection is respiratory tract. Besides, Acinetobacter is one of the most prevalent isolates tending to attach to surfaces like medical devices in hospitals and easily transported to the staff PPE such as masks and gloves (Rebic *et al*., 2018).

The results indicate that the highest resistance among the strains were related to Rocephin rather than other antibiotics, in which *Shigella* and *Pseudomonas species* showed the highest resistance rate to Rocephin that were 100 and 80% respectively. This can be due to the intrinsic or acquired ability of the organism located in DNA is responsible for resistance to Rocephin. The high resistance rate to Rocephin can be due to the availability and also uses as empirical therapy makes the drug accessible and gradually cause resistance as it observed in a retrospective study in a teaching hospital in Ghana (Codjoe and Donkor, 2017). Therefore, increasing rate of resistance to antibiotics during the last decades can be a result of overuse and misuse of disinfectants, making an opportunity for microbial cells to have gene exchanges (Lineback *et al.,* 2018).

Furthermore, the results also showed high susceptibility of most strains to Zinnacef. This can be due to the release of free hydroxyl radicals which have detrimental effects on proteins and lipids of yeast and bacterial cell membrane and also disrupts the S-S ligands of bacterial DNA (Rozman *et al.,* 2021). As reported by Center for Disease Control and Prevention (CDC, 2019), Zinnacef has a strong antimicrobial efficacy on a wide range of microorganisms including bacteria, fungi, and viruses and also spores.

Another finding of this study is the high susceptibility of *Staphylococcus species* to all antibiotics, while *Enterococcus* was barely inhibited by the effect of Rocephin and Ciprofloxacin with the highest resistant rate (100%). This agrees with the reports by Lineback *et al*. (2018) indicating high susceptibility of *Staphylococcus* to common antibiotics. *Pseudomonas* was among the resistant isolate against Ampiclox, Rocephin and Erythromycin with the rates of 100, 80, 100%. respectively. Moreover, *Pseudomonas* is a frequent isolate in clinical setting which shows high degree of tolerance to many antibiotics and disinfectant due to the ability of bacterium to live in biofilms and also overexpression of genes boosting resistance to antibiotics as confirmed by that *Pseudomonas species* can tolerate a range of biocide (Goodarzi *et al.,* 2021). It is inferable that applying cloth face masks without following the health protocols can be a source of contamination itself, which in turn leads to spread of many MDR pathogens such as *Bacillus* and *Pseudomonas* in environments.

Conclusion

In conclusion, this research has depicted that cloth facemask are prone to contamination by both gram negative and gram-positive bacteria. *E. coli* and *Bacillus species* are the most frequently occurring bacteria, while most strains are relatively susceptible to commonly used antibiotics *Shigella* and *Pseudomonas* has shown a very high resistance to antibiotics. The precise link between the components of droplets/aerosols and the protective efficacy of fabric masks will need to be further thoroughly analyzed.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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