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Research Article

Antimicrobial Drug Resistance of Bacteria Isolated From Different Used and Unused Nose Mask

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ABSTRACT

Since the wide spread of severe acute respiratory syndrome of coronavirus 2 (SARS-CoV-2) disease in 2003, the use of face masks has become omnipresent all over the world. Nose masks are believed to contribute to an adequate protection against respiratory infections spread through micro-droplets from the infected persons to non-infected others. The present study investigated the microbial profile and the antibiogram of bacteria isolated from used and unused face/nose mask used in the prevention of corona virus. The analysis was carried out using standard microbiological procedures. The results revealed that both the used and unused mask harbor different bacterial species which included, *Bacillus* sp., *Staphylococcus* sp., *E. coli, Micrococcus* sp., *Streptococcus* sp., *Pseudomonas* sp., and *Enterobacter* sp. The presence of these isolates on the mask is of public health concern as it can lead to the spread of disease. The antibiogram profile of the isolate revealed that some of the organisms were resistant to more than one antibiotics indicating multi drug resistance. The isolates were mostly resistant to Nalidixic acid (NA), Septrin (S) and Augmentin (AU). The study proves that nose/mouth mask is a major source of contamination and spread of multi drug resistant organisms, thus good personal hygiene and following a proper infection control protocol is necessary to reduce the spread of the virus and other organisms.

Keywords: Coronavirus 2 (SARS-CoV-2), nose masks, antibiotics, *Streptococcus, Pseudomonas*, multi drug resistance.

Introduction

Respiratory infections are not new; they are far from being a worry of the past. From seasonal flu to catastrophic outbreaks, respiratory pathogens continue to plague us to this day. People in several countries died after being infected by the severe acute respiratory syndrome (SARS) coronavirus in 2003, and more suffered from the subsequent H5N1 and H1N1 outbreaks (WHO, 2013). In June 2012, a novel coronavirus causing SARS-like disease – human betacoronavirus 2c EMC/2012 (HCoV-EMC) – was discovered in the Middle East (Burgess and Horii, 2012).

Globally, lower respiratory tract infections are the third most common cause of death and the most common infectious cause of death (3).

In rural area, acute upper respiratory tract infection, which includes influenza, is the third most common reason for attendances at public primary care clinics; it accounted for 11.1% of the 4.3 million attendances in 2010 (Aiello *et al*., 2012). In other words, even if respiratory infections are non-epidemic, they strain healthcare systems substantially (Jefferson *et al*., 2008). Pharmaceutical and non-pharmaceutical measures against respiratory infections are available. Pharmaceuticals such as vaccines and antiviral medications are highly effective in eradicating respiratory infections, as evidenced in the case of smallpox. However, as vaccines and antiviral medications take time to develop and are limited in supply, they are unable to sufficiently contain an outbreak caused by new pathogens, especially in the early stages of the outbreak (Kiliç and Gray, 2007).

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Non-pharmaceutical interventions, on the other hand, are not only able to aid in the control of the early stages of a new outbreak, but are also useful in everyday disease prevention in the general population (Aledort *et al*., 2007). Measures such as frequent hand washing have been proven to be effective in preventing the transmission of viral infections (odds ratio [OR] 0.45) (Luby *et al*., 2005). Non-pharmaceutical interventions are a cheap and noninvasive method to reduce mortality and morbidity from respiratory infections.

The World Health Organization (WHO) is constantly updating the number of COVID-19 cases around the world. The number of deaths and the overall data is alarming. WHO informs that research and development efforts are advancing quickly around the world (WHO, 2013) The Head of WHO reiterates the need for seeking possibly alternative actions and strategies to reduce the impacts of the pandemic, mainly among underserved populations and the general community (Maclntyre *et al*., 2015). In the face of the pandemic, there is a relentless search for strategies that may guide and minimize contamination levels within the population and allow the rational use of personal protective equipment by frontline providers. Minimizing the spread of the pandemic within the community is paramount. Considering the conditions caused by the coronavirus (i.e., severe acute respiratory syndrome, SARS, and Middle East respiratory syndrome, MERS) and the containment and prevention experiences observed so far, evidence points out to the transmission person-to-person of COVID-19 by droplets and contact (To *et al*., 2020). The indications for the use of face masks by the population are based on previous experiences in dealing with respiratory syndromes.

However, this measure must be combined with hygiene measures, especially hand washing, disinfecting of frequently touched surfaces, cough etiquette, and avoiding touching face.

The use of face masks by the general population has been seen to potentially hinder the spread of influenza during a pandemic outbreak and reduce infection attack rate, therefore, allowing the reducing of transmission enough to stop the contamination (Brienen *et al*., 2010). Due to the increased risk of contamination in a pandemic such as COVID-19, and also considering the reach of saliva droplets and the worldwide limited supplying of personal protective equipment, it is crucial to identify strategies that may provide barriers against droplets, and minimize the risk of respiratory infections in the general population. The use of cloth and disposable face masks is especially significant to symptomatic patients at home, caregivers, and those living with multiple people, skilled nursing facilities, and crowded spaces, such as public transportation.

The present study seeks to investigate the microbial profile and the antibiogram of bacteria isolated from used and unused face/nose mask used in the prevention of corona virus.

Materials and Methods

Nose Mask Collection

Different samples of used and unused nose masks were aseptically collected from individuals and sellers within Eziama in Aba North LGA of Abia State. The samples were immediately transported to the Microbiology Laboratory, Abia State Polytechnic, Aba in sterile bags packed in insulated cool containers. Analyses were carried out within 4 hours after sampling. All experimental determinations were made in duplicate.

Bacterial Detection

In order to isolate the bacteria, each mask was immersed in a sterile physiological serum separately for 20 minutes. Then 1 ml of supernatant was added to 9 ml of Tryptic Soy Broth (TSB) culture medium and incubated at 37^0C for 4 hours to enhance bacterial growth. The resulting suspension (physiological serum and bacteria in TSB) was thereafter cultured on Nutrient agar, Eosin Methylene Blue (EMB), and blood agar and incubated at 37^0C over- night. After this period, in case of bacterial growth and colony formation, a suspension was prepared from each colony separately and each bacterial isolate was identified by microscopic or morphological and biochemical methods.

Antimicrobial Susceptibility Testing

The antibiogram process was investigated by the Kirby-Bauer disc diffusion method. For each isolated sample, a suspension with a turbidity equivalent to 0.5 McFarland standards (density of a bacterial

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suspension with a 1.5×10^8 cfu/ml) was prepared and transferred to Mueller Hinton agar medium.

The antibiotic disks were placed on the plate and incubated at 37^0C. After 24 hours, the growth inhibition diameter was measured. According to the Clinical and Laboratory Standards Institute (CLSI) guidelines (Monalisa *et al*., 2017), microorganism susceptibility pattern for each antibiotic is classified as sensitive, intermediate and resistant.

Results

The result of the mean values of the various groups of microbial population (count) of Unused Face mask and used Face mask is as shown in the Table 1 below. While the occurrence (%) of bacterial isolated from the used and unused face mask and percentage sensitivity of each antibiotic of the test isolates are presented in Table 2 and Figure 1 respectively.

Table 1: Mean values of the various groups of microbial population of unused face mask and used face mask

Nose Mask Sample	Total Aerobic Bacteria Count (TAB)		Total Coliform Count (TCC)	
	Unused face mask	Used face mask	Unused face mask	Used face mask
A	$1.5x10^4$	$6.0x10^5$	$1.0x10^4$	$2.2x10^4$
B	$2.3x10^4$	$2.1x10^4$	$1.3x10^4$	$2.0x10^4$
C	$1.4x10^4$	$5.0x10^5$	-	$1.0x10^5$
D	$2.0x10^4$	$5.5x10^5$	$1.0x10^4$	$2.2x10^5$
E	$1.0x10^4$	$3.7x10^5$	-	$1.9x10^5$

Table 2: Occurrence (%) of bacterial isolate in used and unused face mask in the study

Bacterial Isolate	Unused face mask					Used face mask				
	A	B	C	D	E	A	B	C	D	E
Bacillus sp	+	-	+	-	-	+	-	-	+	+
Staphylococcus aureus	+	+	+	+	+	+	+	+	+	+
Escherichia coli	-	-	+	-	-	-	-	+	+	-
Micrococcus	+	+	-	-	-	-	-	+	+	+
Streptococcus	-	+	-	-	+	+	+	+	+	+
Pseudomonas sp	-	-	-	-	-	+	-	-	+	-
Enterobacter sp	-	+	-	-	-	+	+	-	-	+
Occurrence (%)	42.8	57.1	42.8	14.2	28.5	71.4	42.8	57.1	85.7	71.4

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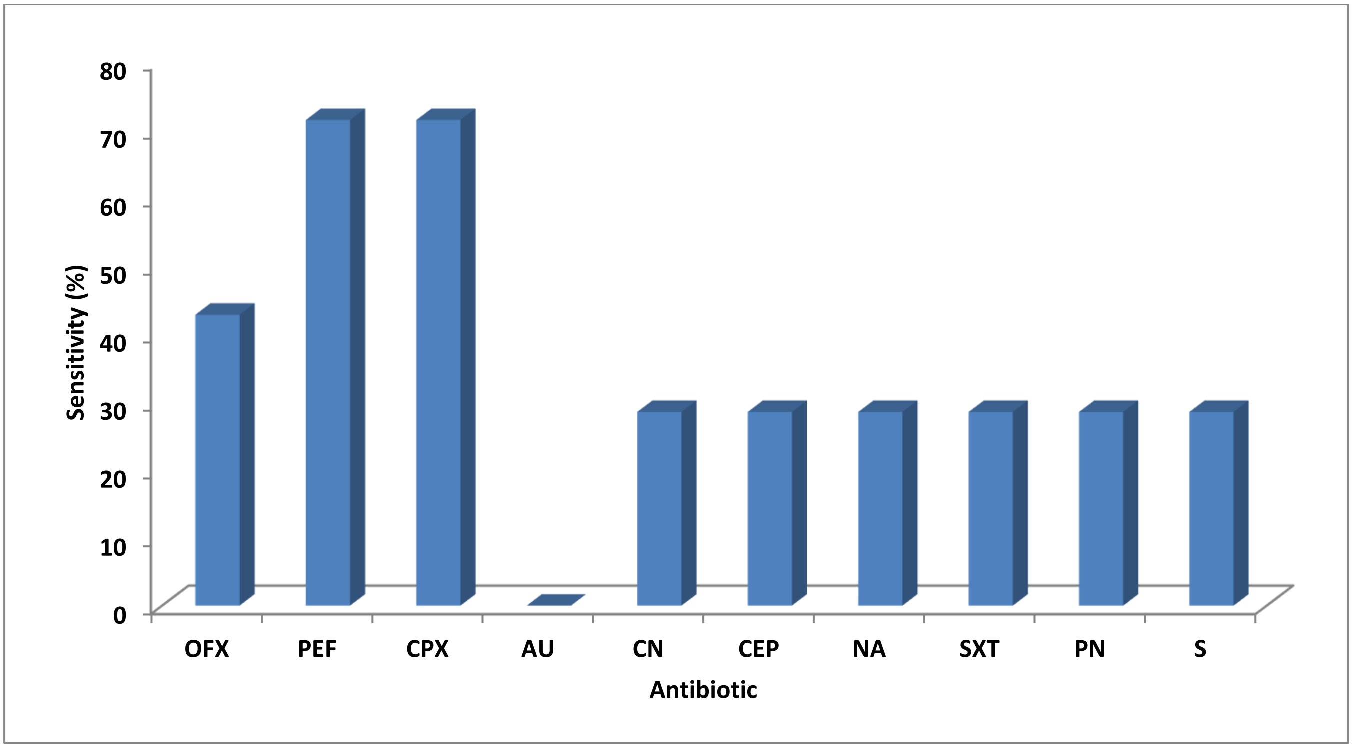

Fig. 1: Percentage sensitivity of each antibiotic of the test isolates

Discussion

The demand for face masks during the coronavirus disease 2019 (Covid-19) pandemic keeps increasing. In some countries, medical workers have to deal with a shortage of face masks. Currently, there are many types of masks fabricated from household or cloth material to substitute for a regular disposable surgical mask. Face masks are being used by individuals who are not medically diagnosed with COVID-19 as a means to limit the spread of COVID-19 in several countries around the world. So far, only limited studies discuss the safety and efficacy of these face masks as relate to its use.

The present study investigated the microbiological quality of some used and unused face mask used in the prevention of Corona-virus disease. The study revealed some microbial contamination in both the used and unused face mask (Table 4.3) these included, *Bacillus, Staphylococcus, E. coli, Micrococcus, Streptococcus, Pseudomonas, Enterobacter*.

Although no work have been conducted on the microbial quality of free mask used during the Covid-19 pandemic, Luksamijarulkul *et al.* (2014), Monalisa *et al.* (2017) and Delanghe *et al.* (2021) and have previously reported the microbial contamination of surgical face mask within hospital setting and as a source of nosocomial infection and/spread at diseases.

The results of the study showed that these contamination on the used masks have been related to the hygiene practice and handling by the users. Findings show that individuals often poorly handle and use the face/nose mask, exposing it to different environmental conditions which serves as a source of contamination. Some of the isolates are of human origin and can be trace to the human body.

The presence of these isolates reported on the cleansed mask can be related to the poor handling by sellers who carry them around with bare hands and exposed to the environment in displayed openly for prospective buyers.

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The presence of these organisms such as *E. coli* and *Pseudomonas* sp needs attention since they are opportunistic pathogens and have been implicated to cause nosocomial infections. *E. coli* and *Pseudomonas* sp are known to cause urinary tract infections, dermatitis, soft tissue infections, bacteremia, bone and joint infections, diarrhea, upper respiratory tract infections, osteomyelitis etc. *Enterobacter* sp. are known to cause, bloodstream infections, surgical site infections, endocarditis and intra-abdominal and pelvis infections, lower respiratory tract infections, septic arthritis, CNS and ophthalmic infections. *S. aureus* and *Micrococcus* sp are known to cause skin infections, cellulitis, abscess, pneumonia, recurrent bacteremia, nosocomial infections, septic shock, septic arthritis, endocarditis, meningitis, cavitating pneumonia, osteomyelitis, toxic shock syndrome, parotitis, mucositis, angular cheilitis, etc.

Also, *Enterococcus* and *Streptococci* are known to cause bacteremia, endocarditis hematogenous osteomyelitis, septic arthritis, pneumonia, urinary tract infection, meningitis, neonatal sepsis, bacterial peritonitis, vertebral osteomyelitis etc. The commonly encountered bacterial pathogens like *Escherichia coli*, *Staphylococcus aureus* indicate that clinically significant contaminants are harboring the mouth mask. This finding is similar to the findings of Baratam *et al.* (2014) where most dominant pathogens isolated were *E. coli* in 53% of the total samples and *S. aureus* in 23% samples. This finding of the present study is of public health importance due to the high demand and use of face mask for the prevention of corona virus.

In conclusion, this present study revealed that the presence of bacterial contamination on the used and unused mask can be related to the personal hygiene of the users and sellers of the masks which might lead to the spread of disease among the users. To reduce the spread of microbial contamination on the used and unused masks, the personal hygiene, handling and packaging of the mask need to be improved. In the present day, the risk of face/nose masks associated infections and its transmission not only involves the health workers who often use them but the general public as well considering the level of the spread of respiratory disease. Most of the organisms isolated in this study from the mouth-masks are potential pathogens. Stringent measures need to be implemented to halt and combat this alarming situation. Strict adherence to the infection control protocol, proper use and its disposal must be followed by the general public. Educating the public on the risk and consequences of using contaminated face/nose masks and advocating simple preventive measures such as hand-washing, these simple preventive measures can be helpful in limiting the spread of infections via face/nose mask.

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