Original Research Article

Gram Negative Bacteria (GNB) Isolated From Home-made and Surgical Nose Mask Used by Local Residents, A Threat to Life and False Sense of Protection against SARS-CoV-2 (COVID-19)

ABSTRACT

Nose/Face masks are physical barriers to respiratory droplets that may enter through the nose and mouth to cause infections in the respiratory tract. The study was carried out to assess the presence of gram-negative bacteria in Nose mask of residents of Akungba-Akoko Ondo State and to determine the antimicrobial susceptibility and resistant profile of the isolated bacteria to eight (8) different antimicrobial agents. The antimicrobial analysis was done using standard microbiological and biochemical methods. Antimicrobial Susceptibility test of all identified isolates to antimicrobial agents was determined using the standard Kirby-Bauer disk diffusion method. The Gram-negative bacteria that were detected from the nose mask in this study include: Haemophilus influenza, Proteus mirabilis, Escherichia coli, Pseudomonas aeruginosa and Klebsiella pneumonia. It was recorded in this study that all the Gram-negative bacteria isolates were resistant to Ciproflox in both homemade and surgical nose masks whereas, all the isolates were also resistant to Amplicin, Augmenting, Septrin and Streptomycin. In this study, Escherichia coli was the most commonly isolated organism, as for the Homemade nose mask, Escherichia coli was resistant to Augmentin, Tarivid, Ciproflox, Gentamycin, and Reflaxine as for Surgical nose mask. Escherichia coli were resistant to Tarivid, Ciproflox, Gentamycin, and Reflaxine. The second most isolated Gram-negative bacteria were Pseudomonas aeruginosa which was resistant to Tarivid, Ciproflox, and Nalidixic acid as for surgical mask and resistant to Augmenting and Gentamycin for Homemade nose mask. Klebsiella pneumonia was resistant to streptomycin, Septrin, Amplicon, and Gentamicin, Proteus mirabilis was resistant to Ciproflox and Haemophilus influenza was resistant to Amplicon, Septrin, Streptomycin, and Augmentin. Addition of antibiotics to the organisms at the 48thhours speeds up the resistance of the organisms isolated from the Nose mask. The result of this study validates the potency at which microorganisms invade both surgical and homemade nose masks, thereby causing infections, and stringent measures needed to be implemented to halt and combat this situation.

Keywords: Gram Negative Bacteria, Homemade Nose Mask Surgical Nose Mask

INTRODUCTION

Micro-organisms can spread easily, and the air itself (Szymańska and Sitkowska 2012). Bioaerosols formed from specific equipment usages, These are invisible to the naked eye and can remain in the environment as aerosols for long periods of time. These aerosols may be inhaled into the lungs to reach the alveoli or may come in contact with the skin or mucous membranes (Shivakumar and Prashant *et al.,* 2007). Aerosol that are 100 micrometer or more in diameter are thought to be too large to be inhaled; however, they may still come into contact with the skin, eyes, and mucous membranes or may settle down on the exposed hair and clothing. Thus, diseases like pneumonia, influenza, hepatitis, may be transmitted with skin and eye . (Shivakumar and Prashant *et al.,* 2007). Since masks protect the mucous membranes of the nose and mouth, they must be worn wherever there is a potential for splashing, saliva or body fluids, or where there is a probability of the inhalation of aerosols with a potential for transmission of airborne pathogens.

Nose mask is an essential infection control barrier is an important subject in the prevention of infectious diseases.. Surgical masks are fluid- repellent paper filter masks and are suitable for both surgical and non-surgical individuals procedures that generate aerosols.. This three-ply material is made up from a melt blown material placed between non-woven fabrics. The melt-blown material acts as the filter that stops microbes from entering or exiting the mask. Most surgical amd home made masks feature pleats/folds commonly three pleated are used allowing the user to expand the mask so it covers from the nose and under the chin (Baratam *et al.,* 2014). According to the CDC guidelines, surgical nose and home made mask is a personal protective barrier (CDC guidelines, 2003). The use of surgical nose masks is synonymous with the use by the public and is so deeply ingrained that to question it would have been unheard of until recently (Lipp, 2003). Unlike the white coats, the filtration abilities of a mask begins to decline after approximately 20 minutes with exposure to moisture and the external surface of a mask gets contaminated by the aerosols present in the environment and becomes a source of cross contamination and thus requires proper disposal.

A surgical nose mask is a single-use device designed to retain infective agents present in the exhaled breath. Surgical masks are often referred to as face masks, but not all commercially

available face masks are regulated as surgical masks a very good example is the home made nose mask. Surgical masks are made to act as barrier to droplets or aerosols while surgical respirators are made to filter out airborne particles including viruses and bacteria. Surgical masks and surgical respirators are marked as medical devices. For example, N95 means that the mask provides the intended effectiveness of filtering 95% of particles with a mass median diameter of 0.3 micrometers.

Surgical masks have a multi-layered structure, where generally a layer of textile is covered on both sides by non-woven bonded fabric. Non-woven fabric has better bacteria filtration efficiency and air permeability, while remaining less slippery than the woven cloth (Henneberry, 2020). It is most commonly made of polypropylene, or, in combination with polyethylene of PET polyester. The filtration level of a mask will therefore depend on the types of the non-woven fabrics used for its manufacture and these will vary according to the application. According to the standards surgical masks are made to be effective at filtering out particles such as bacteria above 1 micron.

Fig. 1; Homemade Nose mask(HNM) [Matuschek et *al.*, 2020].

Fig. 2.; Surgical Nose mask(SNM) [Matuschek et *al.*, 2020].

The home made nose mask are nose mask that is hand weaving or swing machine made nose mask, made from different fabric of layered cloth, a, mechanical barrier for inhalation of Bioaerosols. Both Home-made Nose mask(HNM) and Surgical Nose mask(SNM) are effective

in preventing the transmission of infectious diseases like influenza virus and Corona virus . (Johnson *et al.,* 2009; Cowling *et al*., 2010).

The level of protection of masks against infectious diseases depends on multiple factors such as the appropriate usage and fit of the mask, level of exposure, compliance, complementary interventions (such as hands washing), early use [Makison Booth *et al.,* 2013], as well as the type of mask[Macintyre and Chughtai, 2015].A recent study indicated that surgical face masks could, in a real-life situation, prevent the transmission of common cold and corona viruses from symptomatic individuals (Leung *et al*., 2020, Greenhalgh *et al.,* 2020).

The WHO recommends that PPE masks should be used based on the risk of exposure (e.g., type of activity) and the transmission dynamics of the pathogen (e.g., contact, droplet, or aerosol). The use of masks may give users a false sense of protection, thus encouraging risk-taking. Although the effectiveness of reusable face masks is unclear, a response from [Macintyre *et al.,* 2015] on the shortage of single-use masks states that reusable masks do offer some form of protection. However, protocols on how to use reusable masks alongside complementary interventions should be developed to increase their effectivity in protecting against infection.. (Davies *et al.,* 2013) studied the effectiveness of homemade mask in blocking transmission of the microorganisms in healthy volunteers. Generally, the effectiveness of a cloth masks would depend on the fit, fineness of the cloth and the number of layers indicating that there is potential to design more effective cloth masks. Most single-use face masks have an inbuilt filter. Allowing for the insertion of a filter in a cloth mask, may increase their filtration capacities .There are concerns that use of masks may give general public a false sense of protection, thus encouraging risk-taking. Protocols should be developed on how to use and clean reusable masks alongside complementary interventions frequent to increase their affectivity in protecting against infection

MATERIALS AND METHODS

Media and Reagents used

The culture media routinely used for the study include Nutrient agar, Tryptic soy agar, Mueller Hinton and MacConkey agar. Also reagent used includes; Gram staining reagents and Hydrogen peroxide. All media for this study was prepared according to the manufacturer's description and then homogenized, sterilized in an autoclave at the temperature of 121ºC for 15minutes. In

addition, the distilled water that was used for serial dilution was sterilized for 15minutes at temperature of 121ºC. The work bench was also disinfected with 95% alcohol.

Source of test organism

The test organism were isolated from Nose mask samples of both male and female residing in, Akungba Akoko, Ondo state, Nigeria.

Collection of Nose **mask sample**

Research and ethics committee and from the people of Adekunle Ajasin University Akungba Akoko Ondo State. A questionnaire designed for collection of their individual data was completed by the people e.g. Age, time of collection, including date of collection, gender, duration of wear, frequency of changing, location differentiation of mask, occupation.Fifty(50) Nose mask sample were aseptically collected from student and staff (25) Home-made and (25) Surgical Nose Mask in Akungba Akoko Ondo State, Nigeria. From 12th of August 2021 to 16th of August 2021. The samples were collected into a clean neat and new nylon and were then transported to the laboratory and processed within an hour of collection.

Preparation of Nose **mask sample for culturing**

Nine (9)ml of distilled water was dispensed into 6 test tubes and the mouth was corked with cotton-wool wrapped with aluminum foil and then sterilized at 121^{0}C for 15 minutes using an autoclave. After sterilization, the water as allowed to cool for few minutes, each test tube was then labeled as 10^{-1}- 10^{-6}respectively. 1 ml of the soaked Nose mask sample was dispensed into 9 ml of sterile test tube and 1ml of the sample was then transferred to 9ml of sterile distilled water in a test tube and serial diluted in an aliquot manner up to the sixth diluents.

Bacteriological analysis of Nose mask isolates

Using the pour plate method of inoculation, 0.5ml of the six-fold dilution 10^{-6} of the face mask (inoculum) was aliquoted into sterile petri dishes. MacConkey medium was prepared by dissolving 12.1 grams of the Agar, into 220 liter of distilled water in a sterile conical flask, corked with cotton and aluminum foil and then homogenized to dissolve. It was sterilized in an Autoclave at a temperature of 121^{0}C for 15 minutes. After the sterilization, the media was

allowed to cool but not solidify. 20ml amount of the prepared media was then poured into different sterile Petri dishes containing the 0.5ml of the inoculum was allowed to set, properly labelled using paper tape and inverted. Then the plates were incubated at 37^{o}C for 48hrs. After 48hrs, the cultural characteristics on the plates were studied and recorded. Resultant colonies were sub-cultured on fresh Nutrient agar and then incubated for 24hrs. pure isolates were preserved on a double strength tryptic soy agar slant for further studies.

Microscopic examination and biochemical characteristics of Nose mask isolates

Cultural and microscopic examination was done to identify the pure isolate. Identification of the isolates was based on the cellular morphology characteristics which have an undulate, entire, lobate and filamentous margin, round, spindle, punctiform, filamentous and irregular shape, convex, pulvinate, umbonate and flat in elevation, translucent, mucoid, moist, and dry texture and opaque, milky, white, pink and brown colour. In addition various biochemical tests which includes: Catalase, Indole, Citrate, Methyl red, Motility, Gram staining, Fermentation of sugars (Sucrose, Lactose, Dextrose), Urease, Hydrogen sulfide, Gas production, Glucose, Vogue's Proskauer, Manitol, Sorbitol and oxidase tests were done for conventional identification of the isolates.

Gram staining technique used for Nose **mask isolates**

A sterile inoculating loop was used to make a smear of the culture on a clean grease free slide labeled with each isolate code and heat fixed. The smear was flooded with crystal violet (primary stain) for 60 seconds and rinsed with water, after that, Gram's Iodine which is a mordant was used to flood the slide and allowed to stay for 1 minute, rinsed with water and allowed to stay for 30 seconds. The smear was decolorized with 70% ethanol for only 15 seconds and immediately rinsed off in gently running tap water to remove the ethanol effect. The slide was counterstained with safranin for 60 seconds after which it was rinsed with water and then blot dried. The slides were viewed under the microscope using oil immersion (×100). Gram positive cells are purple, since they are not decolorized with alcohol and retain the purple color of the primary stain (crystal violet) while Gram negative cells are pink because alcohol removes the crystal violet-iodine complex (Fawole and Oso, 2007).

Biochemical characteristics of Nose mask isolates

Catalase Test

This test is used to differentiate organism that have enzyme catalase, capable of decomposing hydrogen peroxide (H_2O_2). Two drops of hydrogen peroxide solution was dropped on a clean grease free glass slide, with an applicator stick, a colony from the stock isolate was picked and rocked on the slide with hydrogen peroxide solution, colonies that produced oxygen bubbles were recorded as being catalase positive while those that did not produce bubbles were recorded as catalase negative (Fawole and Oso, 2007). The result was then recorded for each isolate.

Indole test

Indole and broth was prepared, 10ml of the broth was dispensed into clean test tubes and autoclaved at 121^0C for 15 minutes, it was then allowed to cool. A distinct colony was picked from the subculture plate seeded with the test organism and inoculated into the broth which was incubated for 24hours at 37^0C. After the incubation period, Kovac's reagent was added to the incubated broth culture, shaken gently and allowed to stand for 20 minutes and color change was observed. A red color change indicate positive result, while those that retained the color of the reagent indicated a negative result. (Tankeshwar, 2012).

Motility test

Motility, indole and urease broth was prepared, 10ml of the broth was dispensed into clean test tubes and autoclaved at 121^0C for 15 minutes, it was then allowed to cool. A distinct colony was picked from the subculture plate seede with the test organism and inoculated into the broth which was incubated for 24hours at 37^0C. After the incubation period, a diffuse zone of growth flaring from the line of inoculation indicates a positive result and a restricted along the stab line indicates a negative result (Tankeshwar, 2012)

Oxidase test

Nutrient broth was prepared and autoclaved at 121^0C for 15 minutes, it was then allowed to cool. A distinct colony was picked from the subcultured plate seeded with the test organisms and inoculated into the broth which was incubated for 24hours at 37^0C, after incubation, kovac's

oxidase reagent was added to the culture, the colour change to purple indicates an oxidase positive and no colour change indicates an oxidase negative (Cheesbrough, 2002).

Urease test

Motility, indole and urease broth was prepared, 10ml of the broth was dispensed into clean test tubes and autoclaved at 121^0C for 15 minutes, it was then allowed to cool. A distinct colony was picked from the subcultured plate seeded with the test organism and inoculated into the broth which was incubated for 24hours at 37^0C. Urea solution was prepared and then added to the culture before incubation. After the incubation period, a colour change from yellow-orange to pink red indicates a positive result and no colour indicates a negative reaction (Tankeshwar, 2012)

Fermentation of other sugars (Dextrose, Lactose and sucrose) and Hydrogen sulfide, Gas production on Nose mask isolates

Triple sugar iron agar was prepared and autoclaved at 121^0C for 15 minutes, it was then allowed to cool. A distinct colony was picked from the subcultured plate seeded with the test organism and inoculated into the broth which was incubated for 24hours at 37^0C. After incubation, an alkaline or acid (red slant/yellow butt) reaction indicates dextrose fermentation only, an acid/acid(yellow slant/yellow butt) reaction indicates the fermentation of dextrose, lactose and sucrose, an alkaline/alkaline(red slant/ red butt) reaction indicates absence of carbohydrate fermentation results, blackening of the medium occurs in the presence of H_2, bubbles or cracks in the agar indicates the production of gas (formation of CO_2 and H_2) (Huston, *et al.*, 2008).

Methyl red Test

This test was used to detect which of the isolates could produce and maintain sufficiently a stable acid product from glucose fermentation. The test is usually used as an acid in the identification and differentiation of the Enterobacteriaceae [Mathai and Neu, 2011]. Using a Pasteur's pipette, 10 drops of methyl red pH indicator was added to each tube, and tube was swirled gently to mix them into the broth. Each tube was examined for color change. Bacteria that produce many acids from the breakdown of dextrose (glucose) in the MR-VP medium case the pH drop to 4.2. At this pH, methyl red becomes red. A red color represents a positive. Bacteria that produce fewer

acids from the breakdown of glucose drop the pH to 6.0. At this pH methyl red is yellow and this represents a negative test (Olutiola, *et al.,* 2000).

Citrate utilization Test

(Simmons Citrate Agar) This test was used to identify which of the isolates can utilize citrate as the sole source of carbon for metabolism. The test is usually used as an acid in the differentiation of organisms of Enterobacteriacae and most other genera [Forbes *et al.,* 2016]. The Simmons citrate agar was prepared by weighing 9.1g of the agar and dissolved in 250ml of distilled water. A 9ml each of the solution was inoculated into the test tubes and sterilized by autoclaving at 121○C for 15 minutes at 15psi. These test tubes were slanted and allowed to cool to 45○C on the bench. The isolates were inoculated into each of the test tubes and incubated at 37○C for 24hrs. Change in color from Green to blue coloration indicated positive citrate test [Forbes *et al.,* 2016].

Voges-Proskauer Test

This test was best carried out by inoculating MRVP medium and incubating at 30oC for 5days or 37oC for 2days. Test with methyl red and then added 0.6ml of alpha napthol solution [about 15drops] and 0.2ml of 40% KOH [about 10drops]. Shaked and examined for the red color of a positive reaction after 15minutes and 1hour a positive reaction is the development of a red color after 15-60minutes under alkaline conditions and in the presence of oxygen, acetyl-methyl-carbinol was oxidized to diacethyl which reacts with creatine to give a red color, creatine is present in peptone [Olutiola *et al.,* 2000]

Antibiotic susceptibility test (Antibiogram) Nose mask isolates

Antimicrobial susceptibility tests were performed using Kirby-Bauer's disc diffusion method on Muller-Hinton agar (Bauer *et al.,* 1996).This test was performed to determine the phenotypic resistant traits of the bacteria isolate to the commonly used antibiotic. This was carried out following Kirby-Bauer method of the NICLS (2005). Muller-Hinton agar plate was prepared and standardized inoculums of each isolate were inoculated on each of the Muller Hinton agar plates respectively. An overnight culture of the test bacteria grown in nutrient broth was adjusted to 0.5 McFarland turbidity standards. 0.5 McFarland equivalent standard of the test organisms was inoculated on the surface of the Muller-Hinton (MH) agar plates using a swab stick. The following antibiotics discs and their

concentration which are tarivid 10mcg[OFX], , reflacine 10mcg[PEF], ciprofloxacin 10mcg[CPX], augmentin30mcg[AU], gentamycin10mcg[CN], streptomycin30mcg[S], ceporex 10mcg [CEP], nalidixic acid 30mcg[NA],septrin 30mcg[SXT], amplicin 30mcg[PN] as impregnated aseptically on the plate. Following the manufacturer's description by Optun laboratories Nig. Ltd. predetermined commercial [0xoid] antibiotics discs were applied to the surface of the inoculated agar plates using a pair of sterile forceps. Gram Negative antibiotics susceptibility discs was used because all the isolates were Gram Negative. The antimicrobial diffused from the disc to the medium and the growth of the organism was inhibited at a distance from the disc that is associated to the sensitivity of the organism. A strain that was sensitive to the antimicrobial was inhibited at a distance from the disc where as resistant strains had smaller zones of inhibition. The zones of inhibition was measured with meter rule and compared with clinical and laboratory standard institute (CLSI) guidelines [Pinson, Hooton, 2006]. The isolates were scored as either sensitive or resistant depending on the diameter of the zone of inhibition [Babalola and Balogun, 2013]. The results were interpreted according to the Clinical Laboratory Standard Institute (CLSI, 2016) guidelines.

RESULT

In this study, five species of Gram Negative bacteria were isolated from [50] fifty(25) Home-made and (25) Surgical Nose Mask. The organisms isolated include *Haemophilus influenza, Pseudomonas aeruginosa, Klebsiella pneumoniae, Proteus mirabilis,* and *Escherichia coli.*

Table 1: shows the questionnaire about the collection of samples which were recorded under the following headings: variables and result percentage (%). The variables consist of the age, gender, time, date, type of mask, duration of wearing the mask, removal of the mask, storage of the Nose mask and disposal of the mask. The percentage of the age limit of 20-27 is 90% while that of 37 is 10%. It shows that the sample was collected from both male and female gender, the percentage of face mask collected from male was 30% while that of female was 70%. The time at which the samples were collected was between 7:00am-7:49am at 50%, 6:45am at 10%, and 8:15am-8:35am at 40%. The type of Nose mask collected were classified into two basically: Cotton Nose mask and medicated Nose mask and the percentage of cotton mask collected was 60% whereas, that of medicated face mask collected was 40%. The date at which the samples were collected was between 12/08/2021 at 30%, 15/08/2021at 40% and 16/08/2021at 30%. Duration of wearing the mask, about 80% have the practice of putting on their face mask inside the school

laboratory, about 10% of the participants wear their face mask all the time and also 10%wear the mask at the school health center. Although a large number of participants believed that face mask can cause cross-contamination when touched, About 70% have the habit of removing their face mask after hand washing, 20% remove their mask before hand washing and 10%remove their mask at crowded area. It was also recorded that 30% participants store their face mask inside their school bag, 30% store their face mask inside hand bag, 20% store their face mask inside wardrobe, 10% store their mask inside nylon and 10%participants store their face mask inside the laboratory drawer. 60% of participants indicated that a face mask can be disposed alongside with normal household waste, 30% disposed their face mask any where and 10% participants disposed their face mask inside school incinerator.

Table 1.Cross section of Questionnaire of Nose Mask Samples Used By Local Residents,

Variables	Variables	Result%
Age	20-27	90%
	37	10%
Gender	Male	30%
	Female	70%
Time	7:00am-7:49am	50%
	6:45am	10%
	8:15am-8:35am	40%
Date	12/08/2021	30%
	15/08/2021	40%
	16/08/2021	30%
Type of mask	Cotton	60%
	Medicated	40%
Duration of wearing the mask	All the time	10%
	School health center	10%
	Inside the school laboratory	80%

Removal of mask	Before hand washing	20%
	After hand washing	70%
	Crowded area	10 %
Storage	Inside school bag	30%
	Inside hand bag	3o%
	Inside wardrobe	20%
	Inside nylon	10%
	Inside laboratory	10%
Disposal	Household refuse	60%
	Anywhere	30%
	School incinerator	10%

Table 2a & 2b. Shows the cultural characteristics and macroscopic morphology of Gram negative bacteria isolated from used face mask. It was observed that the PC2 and FB2 isolates has undulate margin, GC1, FM1, MM1, MM2, MM3, and FB1 has entire margin, FM2 has lobate margin and FM3 has filamentous margin. PC2, GC1, MM1, MM2, and FB1 has convex elevation, FM1 and FM3 has pulvinate elevation, FM2 has umbonate elevation; MM3 and FB2 has flat elevation, PC2, FMC and GC2 has translucent texture, FM3 has moist texture, FM1, MM1 and FB1 has mucoid texture;MM2, MM3 and FB2 has dry texture. PC2 and MM1 has opaque colour, GC1 has milky colour, FM1 has white colour, FM2, MM2, MM3 and FB1 has pink colour;FB2 and FM3 has brown colour. PC2 has irregular shape, GC1 and FB2 has round shape, FM2 has punctiform shape, FM3 and MM2 has filamentous shape; FM1, MM1, MM3 and FB2 has spindle shape.

Table 3a & 3b.; Shows Gram negative bacteria reaction, the microscopic and arrangements of the isolate from used nose mask. It was observed in this table that all the isolates were Gram negative when viewed under the microscope at x40 and all the isolates appeared pink in colour, PC1, GC1, FM3, FB1, and FB2 were arranged in cluster, FM1, FM2, MM1, MM2 and MM3 were arranged in chains and all the isolates were rod in shape.

Table. 2a. Cultural Characteristics and Macroscopic Morphology of Gram Negative Bacteria from Used Nose **Mask (Home Made Mask)**

Isolates	Margin	Elevation	Texture	Colour	Shapes
PC2	Undulate	Convex	Translucent	Opaque	Irregular
GC1	Entire	Convex	Translucent	Milky	Round
FM1	Entire	Pulvinate	Mucoid	White	Spindle
FM2	Lobate	Umbonate	Translucent	Pink	Punctiform
FM3	Filamentous	Pulvinate	Moist	Brown	Filamentous
MM1	Entire	Convex	Mucoid	Opaque	Spindle
MM2	Entire	Convex	Dry	Pink	Filamentous
MM3	Entire	Flat	Dry	Pink	Spindle
FB1	Entire	Convex	Mucoid	Pink	Spindle
FB2	Undulate	Flat	Dry	Brown	Round

Table.2b. Cultural Characteristics and Macroscopic Morphology of Gram Negative Bacteria from Used Nose Mask (Surgical Face Mask)

Isolates	Margin	Elevation	Texture	Colour	Shapes
PC2	Entire	Convex	Mucoid	Opaque	Irregular
GC1	Undulate	Convex	Translucent	Milky	Round
FM1	Entire	Pulvinate	Mucoid	White	Spindle
FM2	Lobate	Umbonate	Translucent	Pink	Punctiform
FM3	Filamentous	Pulvinate	Moist	Brown	Filamentous
MM1	Entire	Convex	Mucoid	Opaque	Spindle
MM2	Undulate	Convex	Dry	Pink	Filamentous
MM3	Entire	Flat	Dry	Pink	Spindle
FB1	Entire	Convex	Mucoid	Pink	Spindle

FB2	Undulate	Convex	Mucoid	Brown	Spindle

Table. 3a. Gram Negative Bacteria Reaction, Microscopic And Arrangements Of Isolate From Used Nose **Mask (Home Made Mask)**

Isolates	Gram Reaction	Colour	Arrangement	Shapes
PC2	Negative	Pink	Clusters	Rods
GC1	Negative	Pink	Clusters	Rods
FM1	Negative	Pink	Chains	Rods
FM2	Negative	Pink	Chains	Rods
FM3	Negative	Pink	Clusters	Rods
MM1	Negative	Pink	Chains	Rods
MM2	Negative	Pink	Chains	Rods
MM3	Negative	Pink	Chains	Rods
FB1	Negative	Pink	Clusters	Rods
FB2	Negative	Pink	Clusters	Rods

Table .4. Shows the biochemical characteristics and probable identities of the bacteria isolates. This table shows that all the isolates were catalase positive. PC2, MM1, MM3 and FM3 were citrate positive while, GC1, MM2, FB2, PC, FM2 and FB1 were citrate negative, all the isolates were gas positive. PC2, MM1, MM2, FB2, MM3, PC1, FM3 and FM2 were hydrogen sulfide positive whereas, GC1, and FB1 were hydrogen sulfide negative. PC2, GC1, MM1, MM3, FM3 and FB1 were indole negative while, MM2, FB2, PC1 and FM2 were indole positive. PC2, MM1, MM3, and FM3 were motility positive while, GC1, MM2, FB2, PC1, FM2 and FB1 were motility negative. PC2, MM1, FB2, MM3, PC1, FM3 and FM2 were methyl red positive while, GC1, MM2 and FB1 were methyl red negative. PC2, GC1, MM1, FB2, MM3, PC1, FM2 and FB2 were oxidase negative while, MM2 was oxidase positive. PC2, GC1, MM1, MM2, MM3,

FM3 and FB1 were urease positive while, FB2, PC1 and FM2, were urease negative. GC1, MM1 and FB1 were Vogue's Proskauer positive while, PC2, MM2, FB2, MM3, PC1, FM3 and FM2 were Vogue's Proskauer negative. All the isolates were glucose positive. PC2, MM2, MM3 and FM3 were lactose negative while, GC1, MM1, FB2, PC1, FM2 and FB1 were lactose positive. PC2, MM1, MM3 and FM3 were manitol negative while, GC1, MM2, FB2, PC1, FM2 and FB1 were manitol positive. PC2, MM1, MM3, FM3 were sorbitol negative while, GC1, MM2, FB2, PC1, FM2 and FB1 were sorbitol positive. PC2, MM2, MM3 and FM3 were sucrose negative while, GC1, MM1, FB2, PC1, FM2 and FB1 were sucrose positive. The probable organisms that were isolated includes: *Pseudomonas aeruginosa* which was present in three different isolates such as PC2, MM3 and FM3, *Escherichia Coli* was also present in three different isolates such as FB2, PC1 and FM2, *Proteus mirabilis* appeared once in MM1 isolates; *Haemophilus influenzae* appeared once in MM2 Isolate and *Klebsiella pneumoniae* also appeared once in GC1isolate.

Table 3b. Gram Negative Bacteria Reaction, Microscopic and Arrangements of Isolate from Used Nose Mask (Surgical Nose Mask)

Isolates	Gram Reaction	Colour	Arrangement	Shapes
PC2	Negative	Pink	Chains	Rods
GC1	Positive	Pink	Clusters	Rods
FM1	Negative	Pink	Chains	Rods
FM2	Negative	Pink	Chains	Rods
FM3	Negative	Pink	Clusters	Rods
MM1	Positive	Pink	Chains	Rods
MM2	Negative	Pink	Chains	Rods
MM3	Negative	Pink	Chains	Rods
FB1	Negative	Pink	Clusters	Rods
FB2	Positive	Pink	Chains	Rods

Table. 4. Biochemical Characteristics and Probable Identity of Bacteria Isolated From Used Nose Mask

Isolates	Catalase	Citrate	Gas productio	Hydroge n	Indole	Motility	Methyl red	Oxidase	Urease	Vogues Proskaue	Glucose	Lactose	Manitol	Sorsbitol	Sucros	Probable Organis ms
PC2	+	+	+	+	-	+	+	-	+	-	+	-	-	-	-	***Pseudomonas aeruginosa***
GC1	+	-	+	-	-	-	-	-	+	+	+	+	+	+	+	***Klebsiella pneumoniae***
MM1	+	+	+	+	-	+	+	-	+	+	+	+	-	-	+	***Proteus mirabilis***
MM2	+	-	+	+	+	-	-	+	+	-	+	-	+	+	-	***Haemophilus influenzae***
FB2	+	-	+	+	+	-	+	-	-	-	+	+	+	+	+	***Escherichia coli***
MM3	+	+	+	+	-	+	+	-	+	-	+	-	-	-	-	***Pseudomonas aeruginosa***
PC1	+	-	+	+	+	-	+	-	-	-	+	+	+	+	+	***Escherichia coli***
FM3	+	+	+	+	-	+	+	-	+	-	+	-	-	-	-	***Pseudomonas aeruginosa***
FM2	+	-	+	+	+	-	+	-	-	-	+	+	+	+	+	***Escherichia coli***
FB	+	-	+	-	-	-	-	-	+	+	+	+	+	+	+	***Klebsiella pneumoniae***

KEY: + indicates positive signs. -indicates negative signs.

Fig 3a; Resistant Antibiotic Susceptibility Pattern of Gram Negative Bacteria (GNB) Isolated From Used Nose (Surgical Nose Mask).

Fig 3b; Resistant synergy of Antibiotic Susceptibility (Modified Antibiotics) Pattern of Gram Negative Bacteria (GNB) Isolated From Used Nose (Surgical Nose Mask).

Fig 4a; Resistant Antibiotic Susceptibility Pattern of Gram Negative Bacteria (GNB) Isolated From Used Nose (Surgical Nose Mask).

Fig 4b; Resistant synergy Antibiotic Susceptibility (Modified Antibiotics) Pattern of Gram Negative Bacteria(GNB) Isolated From Used Nose (Home-made Nose Mask

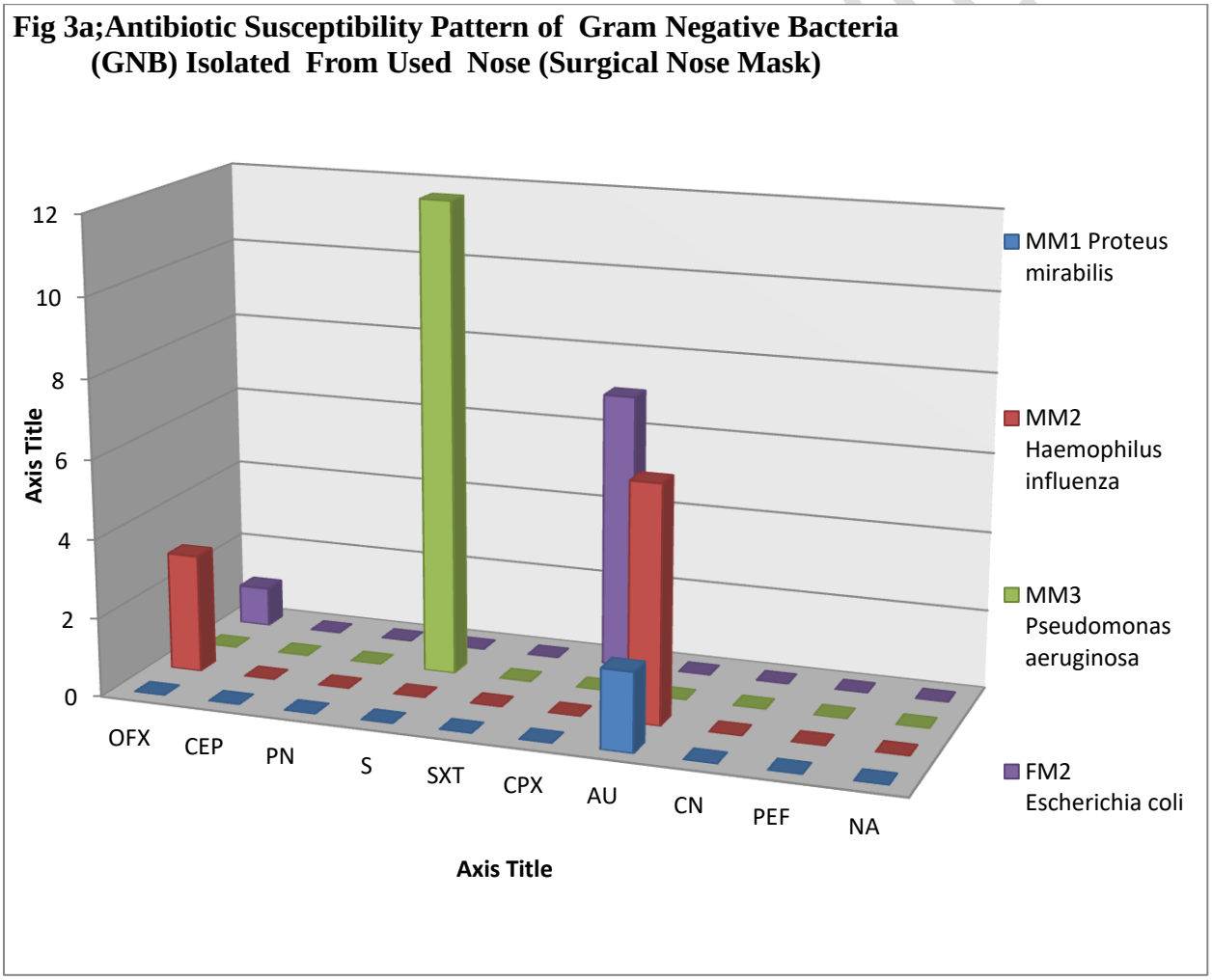
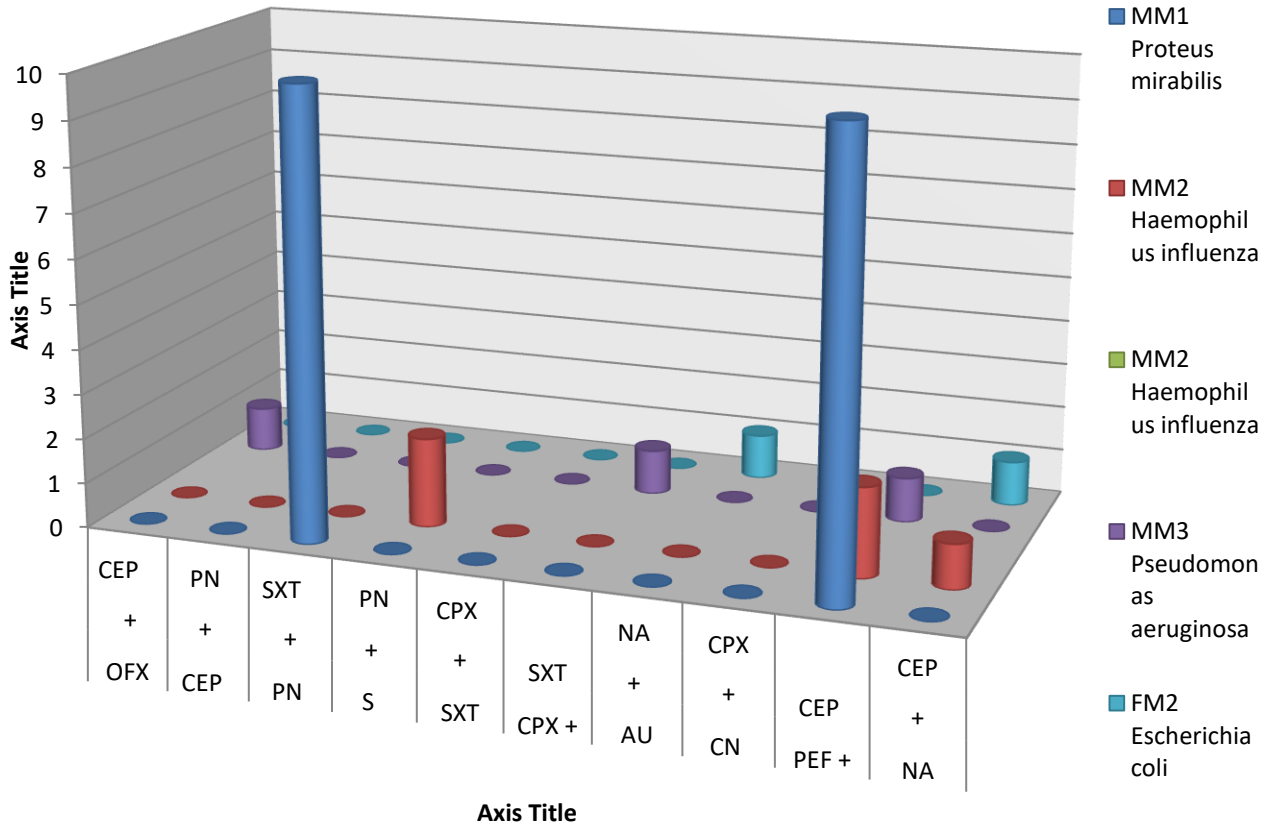


Fig 3b;Resistant synergy of Antibiotic Susceptibility(Modified Antibiotics) Pattern of Gram Negative Bacteria(GNB) Isolated From Used Nose (Surgical Nose Mask)



Key: OFX= Tarivid [10mcg], **CEP=** Ceporex [10mcg], **PN=** Amplicin [30mcg], **S=** Streptomycin [30mcg], **SXT=** Septrin [30mcg], **CPX=** Ciproflox[10mcg], **AU=** Augmentin [30mcy], **CN=** Gentamycin [10mcg], **PEF=** Reflaxine [10mcg], **NA**= Nalidixic Acid [30mcg].

Fig 4a;Antibiotic Susceptibility Pattern of Gram Negative Bacteria (GNB)Isolated From Used Nose (Surgical Nose Mask)

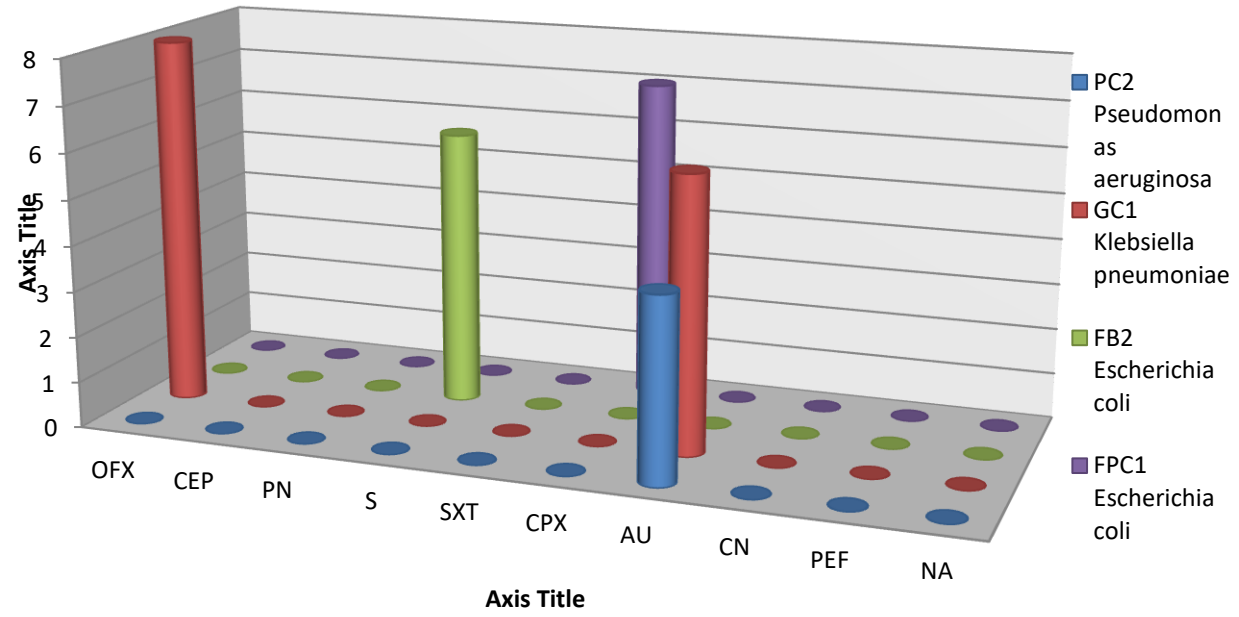
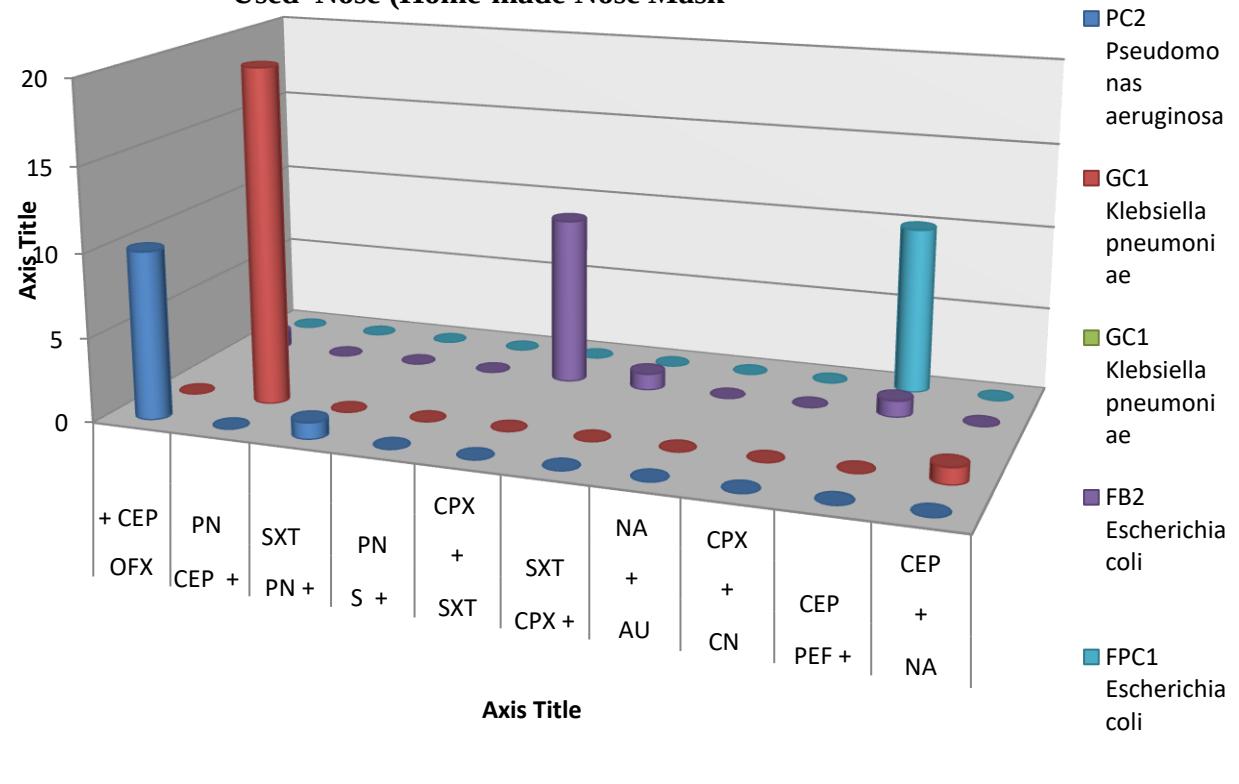


Fig 4b;Resistant synergy Antibiotic Susceptibility (Modified Antibiotics) Pattern of Gram Negative Bacteria(GNB) Isolated From Used Nose (Home-made Nose Mask



DISCUSSION

The purpose of this research work is to isolate, identify and characterized the Gram negative bacteria isolated from homemade and surgical nose mask used by local residents in Akungba Akoko, Ondo state, Nigeria. The World Health Organization (2020) stated that, in dire need of nose mask for respite to the intimidating negative impact on humanity. The mask is intended to be worn in public, when contact with infected or uninfected person, i.e with a person's that are coughing, talking and sneezing (Baratam, 2014) which will aid to prevent the scourge of COVID 19 worldwide. But come to think of it, the nose mask has become another threat to life even more that the proposed prevention it meant to be even more than Covid 19 pandemic.

Nose Mask made to stop infections in the public, by preventing organisms released in respiratory liquid droplets and aerosols (Bennette, 2006). From wearers mouth and nose. People touch their eyes 15 to 20 times per hour on average, due to itchy, sweating or poorly fitted mask. More so, wearing a face mask allows the exhaled air move into the eye, generates an impulse or feeling to touch the eye, thereby infecting your hands (WHO, 2020).

Nose masks are physical barriers to respiratory droplets that may enter through the nose and mouth and to the expulsion of mucosalivary droplets from infected individuals (Leung and Cheng *et al.,* 2020), but with this study, the nose mask pose a threat to the populate for the fact that it is false level of protection to Gram negative organisms scourge and a positive level of protection against SARS-CoV-2 (COVID-19) Osuntokun *et al*.(2021).

Another study by (Macintyre *et al.,* 2015) indicates that home made nose masks (two layers, made of cotton) have poorer filtration capacities than surgical masks, and due to higher moisture retention, the reuse of cloth mask may increase the risk of infection. In this study, five (5) species of gram negative bacteria were isolated from 50(Fifty different nose masks (twenty five medicated and twenty five Home-made) and was collected from both male and female. Questionnaires were used to collect the information of the participants as tabled in the results obtained. The organisms that were isolated includes: *Proteus mirabilis, Escherichia coli, Haemophilus influenza, Klebsiella pneumonia* and *Pseudomonas aeruginosa*.

From this research work, Most commonly isolated Gram negative organisms were *Escherichia coli* and was observed to be resistant to Ciproflox, Augmentin, Tarivid**,** Gentamycin and

Reflaxine for home- made nose mask and Reflaxine, Rentamicin, Augmentin and Tarivid for Surgical nose masks. The Gram negative isolate from Surgical nose mask were resistant to Ampicilin. Augmentin, Septrin and Nalidixic Acid but resistant to Tarivid , Ceporex, Streptomycin, Ciproflox, Gentamycin and Reflaxine, while in home-made nose mask, the organisms were resistant to Ceporex, Amplicin, Septrin and Nalidixic Acid. The resistant were clearly seen in the Gram negative organism, which is in accordance to literature that Gram-negative bacteria are more intrinsically resistant to antibiotics - they don't absorb the toxin into their insides (Fig 3b and 4b).

The ability of Gram negative bacteria to resist traditional antibiotics makes them more dangerous in hospital and residential settings, where patients are weaker and bacteria are stronger(Michelle *et al*,.2017) that is the reason a modified antibiotic becomes very inperative to the activity of antibiotic on Gram negative bacteria. In addition, Gram negative bacteria have a distinct structure which enables the organism to attach, grow and invade the respiratory tract and if not treated with immediate effect may lead to high risk of infection (Leung, 2020). The second most commonly isolated organisms were *Pseudomonas aeruginosa* which was observed to be susceptible to Tarivid, Ciproflox and Nalidixic acid as for medicated and susceptible to Augmentin and Gentamycin for home made nose mask

Pseudomonas aeruginosa for example is an opportunistic pathogen to humans and can invade any immune-deficient tissues and cause infections mostly in the respiratory system leading to respiratory failure, shock and death (Gender, 2003). Gram-negative bacteria (GNB) are among the most significant public health problems in the world due to the high resistance to antibiotics. These microorganisms have great clinical importance in hospitals because they put patients in the intensive care unit (ICU) at high risk and lead to high morbidity and mortality but howadays, Gram negative bacteria were randomly isolated in the nose mask, this may be a greater health threat to the Nigeria populace and the world at large.

Commom Symptoms of Gram-negative bacteria include confusion.,high fever, sweats, and/or chills, lack of interest in eating or drinking, nausea, seizures, sensitivity to light, severe headache, and sleepiness. Then the nose mask become a false prevention to the said infection of Covid 19 than a frequent case of self distruction of human health(Michelle *et al*,.2017).The effort to find new antibiotics to combat these pathogens has failed again and again simply because almost all

new drugs are unable to penetrate the gram-negative bacterial cell wall,but with this new research of combining two antibiotics, the solution is immanent.

The increasing prevalence of infectious disease in recent decades has posed threat to public health. Routes of transmission differs, but the respiratory droplets or airborne route has the greatest potential to disrupt social intercourse, while being amenable to prevention by the humble nose mask. Different types of mask such as surgical and home-made mask offer different level of protection to users (Leung, Chu and Shiu *et al.*, 2020). This study entails the types of mask worn by residents of Akungba and identity of probable organisms.

CONCLUSION

This study has proved that, the inherent pathological properties expressed by the isolated organisms, are similitude of the mild and severe clinical manifestations exhibited by Gram negative bacteria on the residents of Akungba ondo state Nigeria . Therefore, there is need to embark on personal hygiene practices, as outlined by the World Health Organization (WHO) to stop the spread of infections devastating effects on mankind. Most of the organisms isolated in this study from the Nose masks were potentially pathogenic. Stringent measures needs to be implemented to halt and combat this alarming situation. Strict adherence to the infection control protocol, use of personal protective wear and its disposal must be followed especially by all those who work in the laboratory environment and in public. Cotton masks should be the last resort to prevent droplet transmission from infected individuals, they would be better than no or false protection.

.**RECOMMENDATION**

From the analysis observed from the antibiotic activity to the isolated organisms as studied in this research work. I hereby recommend that nose mask should not be worn for too long and exchanged due to the presence of invisible opportunistic and pathogenic organisms that may be inhaled during the process of exchange.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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