

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

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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 determined and assess the presence of Gram-negative bacteria in used home-made and surgical nose mask by 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 were performed using standard microbiological and biochemical methods. Antimicrobial Susceptibility test of all identified isolates to antimicrobial agents were determined using the standard Kirby-Bauer disk diffusion method. The Gram-negative bacteria that were detected from the used home-made and surgical nose mask in this study include: *Haemophilus influenza*, *Proteus mirabilis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumonia*. During this study, all the Gram-negative bacteria isolates were resistant to Ciproflox in both used home-made and surgical nose mask. All isolates were also resistant to Ampicilin, Augmentin, Septrin and Streptomycin. In this study, *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* were isolated organism from used home-made nose mask, it was observed that *Escherichia coli* were resistant to Augmentin, Tarivid, Ciproflox, Gentamycin, and Reflaxine, and *Pseudomonas aeruginosa* were resistant to Tarivid, Ciproflox, and Nalidixic acid between 20mm and 24mm zones of inhibition respectively. *Haemophilus influenza*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Proteus mirabilis* were isolated organism from used surgical nose mask. It was observed that all isolated organisms from the used surgical nose/face mask were resistant to Augmentin and Gentamycin between 20 and 24 mm zones of inhibition respectively. *Klebsiella pneumoniae* were isolated from both used home-made and surgical nose/face mask and were found to be resistant to Streptomycin, Septrin,

Ampicilin, and Gentamicin between 20 to 22 mm zones of inhibition respectively. *Proteus mirabilis* were isolated from used surgical nose/face mask, they were found to be resistant to Ciproflox at 21mm zones of inhibition. *Haemophilus influenza* were resistant to Ampicilin, Septrin, Streptomycin, and Augmentin at 23mm zones of inhibition. Isolates from used both home-made and surgical nose/face mask were subjected to modified and synergized antibiotics, it was observed that the isolates from both used home-made and surgical nose mask were resistant to all modified and synergized antibiotics between 20 and 25 mm zones of inhibition respectively. The result of this study validates the potency of Gram negative bacteria isolated from used both home-made and surgical nose/face mask and the degree of invasion and evasiveness, thereby causing various degrees of infections and a false sense of protection against SARS-CoV-2 (COVID-19). Finding from this research recommends a stringent measures were needed to be implemented, to halt and combat this revenging situation especially in the new era of mutating SARS-CoV-2 Virus not only in Nigeria, worldwide at large

Keywords: Gram Negative Bacteria, Used Homemade and Surgical Nose/ face Mask

INTRODUCTION

Micro-organisms can spread easily, and the air itself (1). 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 migrate to the alveoli or may come in contact with the skin or mucous membranes (1). 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 (1). 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, a very 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 and 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 (2). According to the CDC guidelines, surgical nose and home made mask is a personal protective barrier (3). 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 (4). 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, but unfortunately, local resident of Akungba Akoko, Ondo state Nigeria dispose the used nose/ face mask indiscriminately and reused the nose mask, this create a false sense of protection against various diseases causative agent. This research work is an eye opener to the inherent danger of reused nose/face mask.

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.

Non-woven fabric i.e Home made mask, has better bacteria filtration efficiency and air permeability, while remaining less slippery than the woven cloth (5). It is most commonly made of polypropylene, or, in combination with polyethylene or 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) [5].



Fig. 2.; Surgical Nose mask(SNM) [5].

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(6;7) .

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 [8], as well as the type of mask [9].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 (10, 11).

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 (10).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, this is one of the reason that necessitate this research work, a response from [9] on the short age of single-use masks states that reusable masks do offer some form of protection(25).

However, protocols on how to use reusable masks alongside complementary interventions should be developed to increase their affectivity in protecting against infection.(12) studied the

effectiveness of homemade mask in blocking transmission of the microorganisms in healthy volunteers. Generally, the effectiveness of a cloth(Home-made) masks would depend on the fit, fineness of the cloth and the number of layers indicating that there is potential to design more effective fabrics (Home-made)masks. Most single-use face masks have an inbuilt filter,allowing for the insertion of a filter in a fabrics (Home-made), 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

Source of test organism

The test organism were isolated from used home-made and surgical nose/face mask samples of both male and female residing in, Akungba Akoko, Ondo state, Nigeria.

Collection of Used Home-made and Surgical 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 were completed by the people e.g. Age, Time of collection, including Date of collection, Gender, Duration of mask wearing, Frequency of Nose mask changing, Location differentiation of mask, Occupation. Fifty (50) used home-made and surgical nose/face 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 (13).

Preparation of Culturing (Used Home-Made and Surgical Nose/Face Mask Sample)

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 121⁰C for 15 minutes using an autoclave. After sterilization, the water is allowed to cool, for few minutes, each test tube was then labeled as 10⁻¹- 10⁻⁶r respectively. 1 ml of the soaked used home-made and surgical

nose/face 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 serially diluted in an aliquot manner up to the sixth diluents(13).

Bacteriological Analysis for Isolation of Used Home-Made and Surgical Nose/Face Mask

Isolates

Using the pour plate method of inoculation, 0.5ml of the six-fold dilution 10^{-6} of the **used home-made and surgical nose/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°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 labeled using paper tape and inverted. Then the plates were incubated at 37°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 (14).

Microscopic Examination for Identification of Used Home-Made and Surgical Nose/Face

Mask Isolates

Cultural and microscopic examination were done to identify the pure isolate. Identification of the isolates was based on the cellular morphology characteristics which **has 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, Vogues Proskauer, Mannitol, Sorbitol and oxidase tests were done for conventional identification of the isolates(14).

Gram staining technique for identification of Used Home-made and Surgical Nose/Face

Mask isolates

A sterile inoculating loop were 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 ($\times 100$). 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 (14). (15).

Biochemical characteristics for identification of Used Home-made and Surgical Nose /Face 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 (13). The result was then recorded for each isolate (14).

Indole test

Indole and broth was prepared, 10ml of the broth was dispensed into clean test tubes and autoclaved at $121^{\circ}C$ 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 $37^{\circ}C$. 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. 14). (16).

Motility test

Motility, indole and urease broth was prepared, 10ml of the broth was dispensed into clean test tubes and autoclaved at 121⁰C 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 37⁰C. 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(16).

Oxidase test

Nutrient broth was prepared and autoclaved at 121⁰C 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 37⁰C, 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 (17).

Urease test

Motility, indole and urease broth was prepared, 10ml of the broth was dispensed into clean test tubes and autoclaved at 121⁰C 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 37⁰C. 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 (16)

Fermentation of Sugars (Dextrose, Lactose,Sucrose)Hydrogen Sulfide and Gas Production

For Identification of Used Home-Made and Surgical Nose/Face Mask Isolates

Triple sugar iron agar was prepared and autoclaved at 121⁰C 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 37⁰C. 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 H₂, bubbles or cracks in the agar indicates the production of gas (formation of CO₂ and H₂) (14).

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. 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 cause the pH to 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 (18).

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 Enterobacteriaceae and most other genera [19]. 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 [19].

Voges-Proskauer Test

This test was best carried out by inoculating MRVP medium and incubating at 30°C for 5 days or 37°C for 2 days. Test with methyl red and then added 0.6ml of alpha naphthol solution [about 15 drops] and 0.2ml of 40% KOH [about 10 drops]. Shake and examined for the red color of a positive reaction after 15 minutes and 1 hour a positive reaction is the development of a red color after 15-60 minutes under alkaline conditions and in the presence of oxygen, acetyl-methyl-

carbinol was oxidized to diacetyl which reacts with creatine to give a red color, creatine is present in peptone[18].

Antibiotic Susceptibility Test (Antibiogram) of Used Home-Made and Surgical Nose/Face Mask Isolates

Antimicrobial susceptibility tests were performed using Kirby-Bauer's disc diffusion method on Muller-Hinton agar. 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. 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], Augmentin 30mcg[AU], Gentamycin 10mcg[CN], Streptomycin 30mcg[S], Ceporex 10mcg [CEP], Nalidixic acid 30 mcg [NA], Septrin 30mcg[SXT], Ampicilin 30mcg[PN] were impregnated aseptically on the plate. Following the manufacturer's description by Optun laboratories Nig. Ltd. predetermined commercial [Oxoid] 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 [20]. The isolates were scored as either sensitive or resistant depending on the diameter of the zone of inhibition. The results were interpreted according to the Clinical Laboratory Standard Institute (21) guidelines.

RESULT

In this study, five species of bacteria were isolated from [50] fifty (25) used home-made and (25) surgical nose/face 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 years was 10%. It shows the samples were collected from both male and female gender, the percentage of used home-made and surgical nose 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: used home-made and surgical nose mask and the percentage of used home-made collected were 60% whereas, that of used surgical nose mask collected were 40%. The date at which the samples were collected was between 12/08/2021 at 30%, 15/08/2021 at 40% and 16/08/2021 at 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% wears the mask at the school health center. About 70% has the habit of removing their face mask after hand washing, 20% remove their mask before hand washing and 10% remove their mask in crowded area. It was also recorded that 30% participants store their used face mask inside their school bag, 30% store their face mask inside hand bag, 20% store their used face mask inside wardrobe, 10% store their used nmask inside nylon and 10% participants store their used face mask inside the laboratory drawer. 60% of participants indicated that a used face mask can be disposed alongside with normal household waste, 30% disposed their used face mask anywhere and 10% participants disposed their used face mask inside school incinerator.

Table 1. Cross section of Questionnaire of Used Home-made and Surgical Nose/Face Mask Used by Local Residents of Akungba Akoko, Ondo State, Nigeria.

Variables	Variables	Result%
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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	30%
	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 examination/morphology of Gram negative bacteria isolated from **used home-made and surgical nose 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 home-made and surgical nose/ face 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 Examination of Isolated Bacteria from Used Home-Made Nose/Face Mask

Isolates Code	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 Examination of Isolated Bacteria from Used Surgical Nose/Face Mask

Isolates Code	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 Reaction, Microscopic Examination of Isolated Bacteria from Used Home-Made Nose/Face Mask

Isolates Code	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 production 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 Vogues Proskauer positive while, PC2, MM2, FB2, MM3, PC1, FM3 and FM2 were Vogues 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 Mannitol negative while, GC1, MM2, FB2, PC1, FM2 and FB1 were Mannitol 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 were present in three different isolates such as PC2, MM3 and FM3, *Escherichia Coli* were also present in three different isolates such as FB2, PC1 and FM2, *Proteus mirabilis* present in MM1 isolates; *Haemophilus influenzae* present in MM2 Isolate and *Klebsiella pneumoniae* also present in GC1 isolate.

Table 3b. Gram Reaction, Microscopic of Isolated Bacteria from Used Surgical Nose/ Face Mask

Isolates Code	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 of Isolated Bacteria from Used Home-made and Surgical Nose/Face Mask

Isolates Code	Catalase	Citrate	Gas production	Hydrogen Sulfide	Indole	Motility	Methyl red	Oxidase	Urease	Voges Proskau	Glucose	Lactose	Manitol	Sorbitol	Sucros	Probable Organisms
PC2	+	+	+	+	-	+	+	-	+	-	+	-	-	-	-	<i>Pseudomonas aeruginosa</i>
GC1	+	-	+	-	-	-	-	-	+	+	+	+	+	+	+	<i>Klebsiella pneumoniae</i>
MM1	+	+	+	+	-	+	+	-	+	+	+	+	-	-	+	<i>Proteus mirabilis</i>
MM2	+	-	+	+	+	-	-	+	+	-	+	-	+	+	-	<i>Haemophilus influenzae</i>
FB2	+	-	+	+	+	-	+	-	-	-	+	+	+	+	+	<i>Escherichia coli</i>
MM3	+	+	+	+	-	+	+	-	+	-	+	-	-	-	-	<i>Pseudomonas aeruginosa</i>
PC1	+	-	+	+	+	-	+	-	-	-	+	+	+	+	+	<i>Escherichia coli</i>
FM3	+	+	+	+	-	+	+	-	+	-	+	-	-	-	-	<i>Pseudomonas aeruginosa</i>
FM2	+	-	+	+	+	-	+	-	-	-	+	+	+	+	+	<i>Escherichia coli</i>
FB	+	-	+	-	-	-	-	-	+	+	+	+	+	+	+	<i>Klebsiella pneumoniae</i>

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

Table 5. Probable Identity of Gram Negative Bacteria Isolated From Used Home-made and Surgical Nose/Face Mask

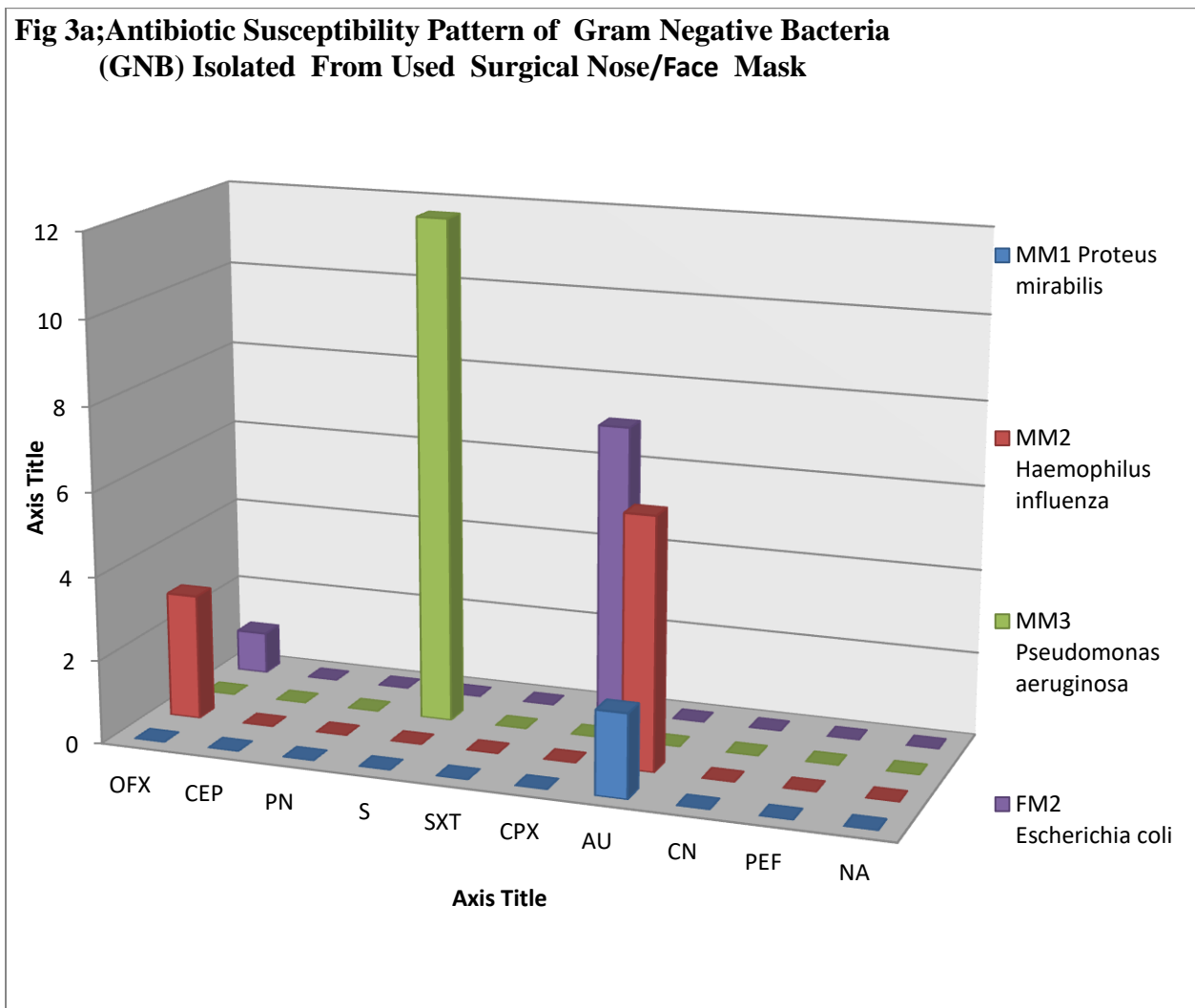
Probable Identity of Gram Negative Bacteria Isolated From Used Home-made Nose/Face Mask		
S/N	Isolates Code	Probable Organisms
1.	PC2	<i>Pseudomonas aeruginosa</i>
2.	GC1	<i>Klebsiella pneumoniae</i>
3.	PC1	<i>Escherichia coli</i>
4.	FM3	<i>Pseudomonas aeruginosa</i>
5.	FB	<i>Klebsiella pneumoniae</i>
Probable Identity of Gram Negative Bacteria Isolated From Used Surgical Nose/Face Mask		
6.	MM1	<i>Proteus mirabilis</i>
7.	MM2	<i>Haemophilus influenzae</i>
8.	FB2	<i>Escherichia coli</i>
9.	MM3	<i>Pseudomonas aeruginosa</i>
10.	FM2	<i>Escherichia coli</i>

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

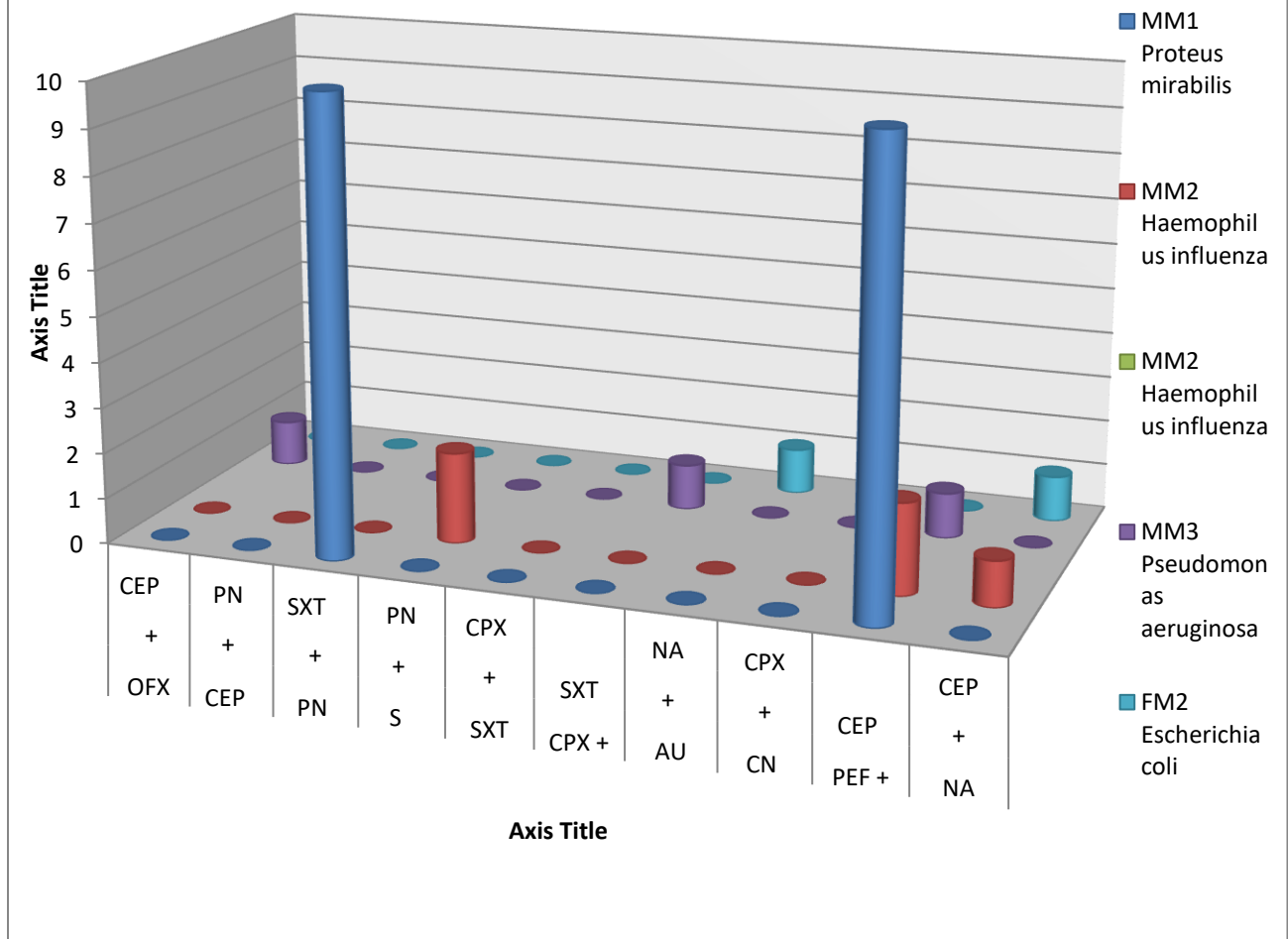
Fig 3b; Resistant synergy of Antibiotic Susceptibility (Modified Antibiotics) Pattern of Gram Negative Bacteria (GNB) Isolated From Used Nose/Face mask (Surgical Nose Mask).

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

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



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



Key: OFX= Tarivid [10mcg], CEP=Ceporex [10mcg], PN= Ampicilin [30mcg], S= Streptomycin [30mcg], SXT= Septrin [30mcg], CPX= Ciproflo[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 Surgical Nose/Face Mask

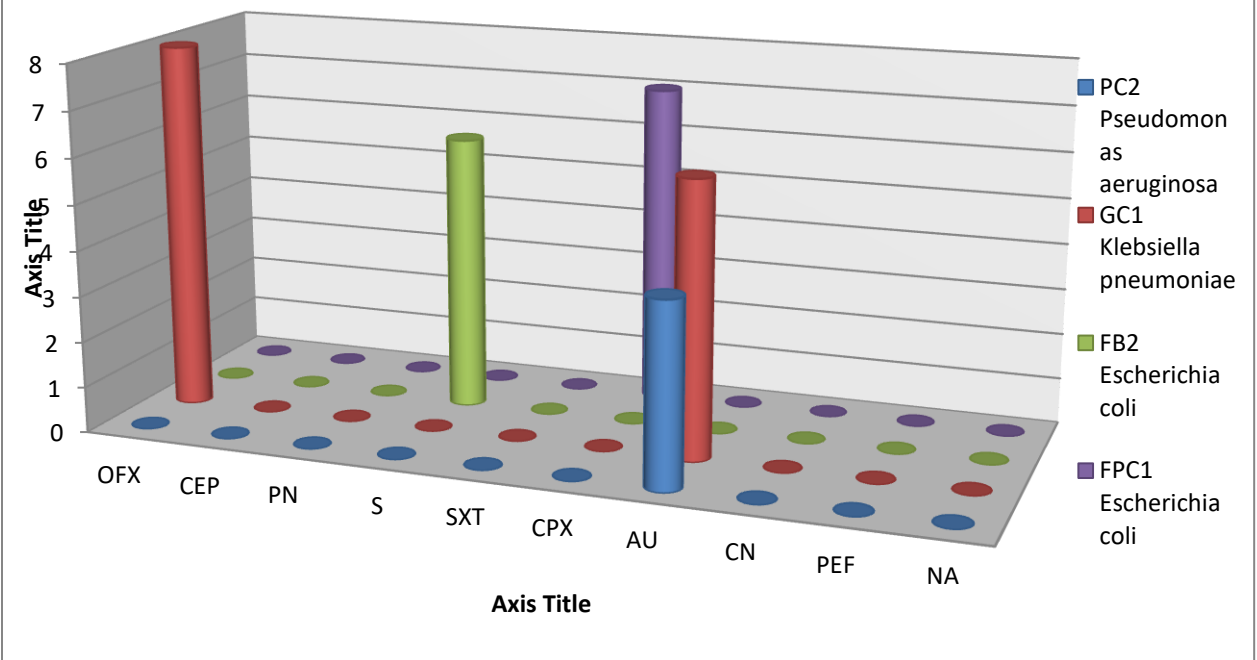
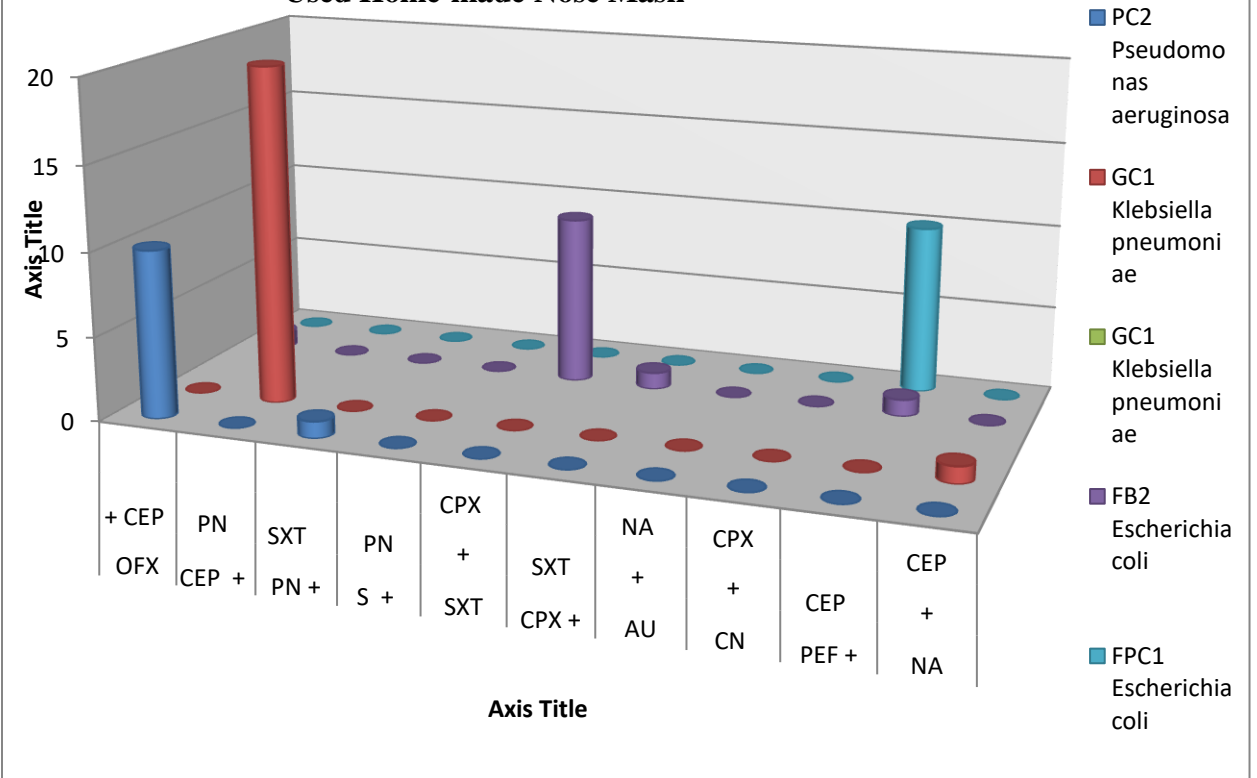


Fig 4b; Resistant synergy Antibiotic Susceptibility (Modified Antibiotics) Pattern of Gram Negative Bacteria (GNB) Isolated From Used Home-made Nose Mask



DISCUSSION

The purpose of this research work is to isolate, identify and characterized the Gram negative bacteria isolated from used home-made and surgical nose/face mask by local residents of Akungba Akoko, Ondo state, Nigeria. The World Health Organization (22) 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 (23) which will aid to prevent the scourge of some infectious disease and COVID 19 worldwide. But come to think of it, the nose mask has become another threat to life even more that the proposed prevention of Covid 19 pandemic.

Nose masks are physical barriers to respiratory droplets that may enter through the nose and mouth and to the expulsion of muco-salivary droplets from infected individuals (10), Local resident dispose the used nose mask indiscriminately and used and reused the nose mask without proper sterilization. The mask should be used once and discarded. Used and reused nose/face mask should not be encouraged. this create a false sense of protection against various diseases causative agent such as the Gran negative organism isolated from the used nose/ face mask during the course of this research work. Most of the Gram negative organisms isolated were pathogenic organism, this is another degree of infection This research work is an eye opener to the inherent danger of used and reused of nose mask.(25).

The use of adequate masks (example FFP2 and FFP3) and careful hygiene of hands and clothing together with correct information (e.g. do not touch the face if the hands are not sanitized or avoid shaking hands) are essential in the transmission of the responsible SARS-CoV2 virus of the current severe pandemic (24). People touch their eyes 15 to 20 times per hour on average, due to itchy, sweating or poorly fitted mask. This practice may contaminate the nose/face mask (22).to prevent cross contamination, the nose/face mask must be used once and disposed accordingly. Reused nose/face mask with sterilization, will converge a false sense of protection against SARS-CoV-2 (COVID-19) and other respiratory infection

Author 15) indicates that homemade nose masks (two layers, made of cotton) has poorer filtration capacities than surgical masks, due to higher moisture retention, the reuse of cloth mask i.e home made nose/face mask may increase the risk of infection. In this study, five (5) species of

Gram negative bacteria were isolated from 50(Fifty different used nose/face masks (twenty five surgical nose mask and twenty five Home-made) and were collected from both male and female user. The organisms that were isolated includes: *Proteus mirabilis*, *Escherichia coli*, *Haemophilus influenza*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*.

From this research work, Most commonly isolates were the Gram negative organisms with different level of resistant. *Escherichia coli* and was observed to be resistant to Ciproflox, Augmentin, Tarivid, Gentamycin and Reflaxine for used home- made nose mask and Reflaxine, Rentamicin, Augmentin and Tarivid for used Surgical nose masks. The Gram negative isolates from Surgical nose mask were resistant to Ampicilin. Augmentin, Seprin, Nalidixic Acid but Tarivid , Ceporex, Streptomycin, Ciproflox, Gentamycin and Reflaxine, while used home-made nose mask isolated organisms were resistant to Ceporex, Amplicin, Seprin and Nalidixic Acid. The resistant were clearly seen in the Gram negative organism, using this research work as case study, which is in accordance to literature that Gram-negative bacteria are more intrinsically resistant to antibiotics because they don't absorb the toxin into their insides cell wall (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(26) that is the reason a modified antibiotic becomes very imperative 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 (27). During this research work, the antibiotics were modified by synergizing the antibiotics, the result is not far from the finding of notable research(27), the isolated organisms were resistant to the modified by synergizing the antibiotics. The Gram negative may pose a serious threat to the public health is not adequately control.

Pseudomonas aeruginosa was also isolated from the nose /face mask, during the course of this research work, it was observed to be resistant to Tarivid, Ciproflox and Nalidixic acid and Augmentin and Gentamycin for used home-made and surgical 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 (28).

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 GNB may patients in the intensive care unit (ICU) at high risk and lead to high morbidity and mortality. it should be a major health concern if, Gram negative bacteria were randomly isolated from used home-made and surgical nose mask, this may be a greater public health threat to the Nigeria populace and the world at large. Common 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.

Used home-made and surgical nose/face mask become a false prevention to the said infection of **SARS-CoV-2 (COVID-19)** than a frequent case of self destruction of human health (26,27).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 bacteria cell wall, but with this new research of combining two or three antibiotics, the solution is imminent (30).

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 nose face /mask. Different types of mask such as surgical and home-made mask offer different level of protection to users (31,32,33)against **SARS-CoV-2 (COVID-19)** and other infectious disease, this research work advocate that home-made and surgical nose/face mask should be used once to prevent cross contamination of infection and the scourge of Gram negative bacteria. The reuse of home-made and surgical nose/face mask is a false sense of protection.

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 used home-made and surgical nose mask 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 wears and its disposal

must be followed especially by all those who work in the laboratory environment. Both home-made and surgical nose mask should be used adequately and worn once, stop the reuse of both home-made and surgical nose mask, it is better to use it once and not for long period, to converge the right sense of protection against **SARS-CoV-2 (COVID-19)** and other infectious disease, reuse will bath the populate with false sense of 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.

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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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