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MICROBIAL EXAMINATION OF DAILY USED FACE MASKS BY GODFREY OKOYE UNIVERSITY STUDENTS

ANIGBOGU, CHIEMERIE LUCIA

U17/NAS/MCB/208

PROGRAMME: MICROBIOLOGY

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FACULTY OF NATURAL SCIENCES AND ENVIROMENTAL STUDIES

GODFREY OKOYE UNIVERSITY, ENUGU NIGERIA

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A PROJECT RESEARCH SUBMITTED TO THE DEPARTMENT OF BIOLOGICAL SCIENCES IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF SCIENCE (B.Sc) DEGREE IN MICROBIOLOGY

SUPERVISOR: PROF. M.N. UNACHUKWU

JULY, 2021

CERTIFICATION PAGE

This is to certify that the research titled Microbial Contaminants Found On Daily Used Face Masks by Godfrey Okoye University students was carried out by ANIGBOGU, CHIEMERIE LUCIA with registration number U17/NAS/MCB/208 under the supervision of the department of biological sciences, Godfrey Okoye University Enugu Nigeria.

Prof. M.N. Unachukwu		15/10/2021
Supervisor	Signature	Date
Dr. C Onyia		19/10/2021
HOD, Department Of Biological Sciences	Signature	Date

DEDICATION

I dedicate this report first and foremost, to GOD almighty, my creator, my strong pillar, who has been there right from the onset till this very moment and has been my source of inspiration, knowledge, wisdom and understanding. Furthermore, I would like to dedicate this work to my ever supportive parents and siblings for their relentless support and compassion towards me because without them I would have been unable to accomplish this competitive phase of education.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF APPENDICES

ABSTRACT

The outbreak of different airborne diseases such as COVID19 has led to the wearing and extensive use of face masks to help control the spread of these diseases from one person to the other. Masks are designed in such a way that bacterial, viral and fungal cells cannot easily be transmitted from person to person. This study was aimed at determining the microorganisms that could be found on daily used face masks of Godfery Okoye students. A total of 20 face masks were used for this study. Ten(10) were distributed to male and female students and worn for 24hrs. Collection of sample was done using damp swab stick to swab the inner and front parts of the face masks. Isolation of bacteria and fungi was done by streaking the swabbed sticks on Nutrient agar and Potatoe Dextrose agar and incubated for 24hr and 48hrs respectively. Samples with growth were counted. Identification of the isolates was done by sub culturing to new media and incubated for 24hrs and using some biochemical tests such as gram staining, catalase test, oxidase testand Lactophenol cotton blue stain etc for the identification of the unknown isolates. From the result it was identified that the front part of the face mask had a higher number of microbes being fungi than on the inner part. Microorganisms such as *Streptococcus* spp., *Legionella* spp and *Staphylococcus* spp were the organisms identified from the inner part of the face mask. *Aspergillus* spp., *Fusarium* spp. and *Penicillium* spp. are the fungal species isolated from the outer part of the face mask. From the study it can be concluded that face masks can indeed prevent the transfer of organisms from person to person but it can also accumulate other microbes from the environment as a result of long usage.

CHAPTER ONE

INTRODUCTION

The best nonpharmaceutical interventions against disease spread via the respiratory route are broadly termed social or safe distancing measures, i.e., reducing close contact between individuals (Benzell *et al.*, 2020). Where safe distancing is not possible, personal protective equipment (PPE) is the accepted mode of self-protection. Masks and respirators are arguably the most important piece of PPE. They are a physical barrier to respiratory droplets that may enter through the nose and mouth and to the expulsion of mucosalivary droplets from infected individuals (Tang, 2009). Their role maybe particularly important in COVID-19, where infected individuals may be shedding virus while asymptomatic or presymptomatic carriers (He *et al.*, 2020).

There are many different types of face masks and respirators offering different levels of protection to users (Long *et al.*, 2020). Generally, masks do not fit tightly while respirators do. Masks and respirators may be reusable or disposable. Reusable ones include industrial-use half or full-face piece respirators with cartridge filter attached and homemade or commercial cloth masks; disposable ones include surgical masks, N95respirators, and KN95respirators. They all serve the general purpose of providing some form of protection against contaminants in the air, ranging from pollen to chemical fumes to pathogens. The filtering capacity, and hence the level of protection against pollutants and pathogens, depends on the materials used and the engineering design (Mueller *et al.*, 2020). Contaminants in the air differ vastly in size. SARS-CoV-2 has a size ranging from 60 to 140nm (Zhu *et al.*, 2020), smaller than bacteria, dust, and pollen. Therefore, masks and respirators made of materials with larger pore sizes, such as cotton

and synthetic fabric, will not be able to effectively filter these viruses or tiny virus-laden droplets, as compared with those made of materials with much smaller pore sizes. Likewise, masks and respirators made of or coated with water-resistant materials are more effective against large virus-laden respiratory droplets and fluid spills. In addition to filtering capacity, factors such as user comfort and breathability also vary across different models. For instance, although the tight-fitting N95 respirator has filtering capacity superior to surgical masks, they have lower breath-ability and may cause discomfort after hours of wearing (Zhu *et al*., 2020).

Mask-wearing can be effective in the containment of communicable diseases (Leung *et al*., 2020) and has thus become a new nor-mal in many societies in the COVID-19 pandemic. The surge in demand for surgical masks and respirators has led to a global shortage of supply and raw materials. As a result, many people have resorted to making their own masks, recycling used masks, or settling for masks offering less protection than actually needed. Researchers and industry players have therefore been working hard to address the issue of shortage, as well as to enhance the protection afforded by existing mask models. These efforts include (i) sourcing and engineering alternative materials with sufficient filtering capacity, (ii) engineering the design of masks and respirators for better protection, breathability, and user comfort, (iii) developing and engineering multifunctional masks and materials with hydro-phobic, antimicrobial, self-disinfecting, and even sensing properties, and (iv) exploring new technologies for efficient production and customization of masks, e.g., 3D printing (Swennen *et al*., 2020).

In schools, the main risk of infection for students and staff alike are the transmission from student to student and from student to staff and vice versa, contaminated instruments or equipments and the school environments (Luksamijarulkul *et al*., 2009).Students and staff who

have no symptoms of the disease or carriers of chronic disease are both potential sources and hosts of infectious agents. The sources of infectious agent are the normal endogenous microbial flora of the persons involved and the environmental sources such as air, water and the devices that have become contaminated (Luksamijarulkul *et al*., 2009).Indoor air quality in school and learning settings is an important issue. It affects the health of school staff based on evidence data about exposure to indoor air pollution, particularly in relation to allergies, asthma and respiratory diseases (Bennett and Parks, 2009). The aerosol particles of biological origin e.g., viruses, bacteria and fungal spores, have been associated with respiratory allergies, asthma, and several air-borne infections including influenza, tuberculosis, measles, mumps, chicken pox, and aspergillosis (Bonetta *et al.,* 2010) The adverse health effects of the biologic agents depend not only on the mass or number of the inhaled particles, but also on the infectivity of agents (Tunevall and Jörbeck, 2009)

Emerging and reemerging infections have emerged as a threat to human health in recent decade (Wong and Tan, 2019). Given how interconnected the world is today, a pathogen capable of human-to-human transmission can spark an outbreak far from where it originated. The virus causing the Middle East Respiratory Syndrome, for example, emerged in the Middle East but caused an outbreak in Korea. The world is in the midst of the COVID 19 pandemic, which is caused by the SARS-CoV-2virus. Lock downs and travel restrictions imposed to halt the spread of COVID-19 have led to devastating economic repercussions. The control of an infectious disease is based on knowledge of its mode of transmission. The recent COVID 19pandemiciscausedbythenovelcoro-navirus, SARS-CoV-2, which is transmitted largely by the respiratory route (videinfra)(Xie and Chen, 2020).

This study aimed to investigate the microbial contaminant in daily used face mask. We hypothesised that face masks, as a tool for reducing the bacterial shedding from the mouth, nose and face, may become a potential contamination sources when worn for an extended period of hours or days.

Face masks are materials made for the purpose of protecting the wearer from the invasion of external materials such as dusts from the environment, invasion of pathogenic microorganisms that can be transmitted from person to person through air droplets. It also prevents the exposure of some unwanted and most importantly human pathogens from sick persons from getting into the environment.

However the wearing of face mask could also be dangerous in that as it prevents microorganisms from being inhaled or getting into the human respiratory system it could serve as a home for hoarding microorganisms that can be consciously or unconsciously be carried and transmitted through by touching the infected masks with the hands. This study was to determine the extent of the microbes that can be trapped on these face masks.

1.1 Aim & Objectives of study:

To determine microorganisms on daily used face masks of Godfery okoye students

Objectives of study:

To isolate and identify the bacterial contaminants.

To quantify the microbial load

CHAPTER TWO

LITERETURE REVIEW

2.1 History and Cultural Background of Wearing Facemask

Wearing face masks in the community is like a religion, which has polarized people world-wide into true believers and sceptics. The zealots who want the wearing of masks made compulsory insist on their right to be protected against catching SARS-CoV-2.Opponents say the principle of autonomy gives them a right to decide whether or not they wear a mask and mandating the wearing of masks infringes their civil rights. It is not surprising that masks have generated such passionate appeals to conflicting rights. Masks have been with us for aeons and have spawned many contradictions. They have been worn for display or for disguise. They have been worn to woo or to wage war. They have been worn to hide or to identify. They have been used to protect or to punish.The earliest known masks are 9000 year old Neolithic stone masks from Judea, some of which were found in caves near the Dead Sea (Gannon, 2014). The purpose of these masks can only be speculated butsince Neanderthals buried their dead and engaged in symbolic and abstract thought over 50 000 years ago, it is possible that they were funerary masks. While we now use masks for protection against infection, the Scold's bridle was a particularly vicious iron muzzlein a metal frame known to have been used in 16th century Scot-land to silence women deemed to have been scolds or nags (Isaacs, 2020).

Venice is renowned for its decorative carnival masks, used to tantalize and be attractive. However, for physicians, the most memorable andmost sinister Venetian mask is that which features as part of the costume worn by mediaeval 'Plague Doctors'to protect them against the

plague or Black Death (Mussap, 2019). The costume comprised a beaked white mask, black hat and waxed gown. The beak was filled with herbs, thought to absorb noxious air, based on the Miasma Theory of disease propagation. The plague decimated Europe from the mid-14th century, each successive pandemic wave killing millions. Incidentally, Venice was a major seaport, and Venetians were among the first to establish the principles of quarantine; quarantenameant40 in Venetian and people on arriving ships were prohibited from disembarking for 40 days (Mussap, 2019)..

The controversy over wearing masks derives from a paucity of definitive studies. A systematic review and meta-analysis of public health methods to prevent person-to-person transmission concluded that face mask use could result in an 85% reduction in risk of infection (Chu *et al*., 2020). However, the evidence was much stronger for the use of face masks as part of personal protective equipment in health-care settings than in community settings, and stronger for N95/P2masks than for surgical masks and cotton masks. Furthermore, studies of the use of face masks in the community to protect against influenza have shown that compliance is an issue, even in Asian countries such as China (MacIntyre *et al*., 2016); in contrast to the health-care setting, there is a dearth of evidence for protective efficacy of face mask usein the community. While it is certainly possible that wearing masks would protect the public, it is also possible that they might actually be detrimental if people wear them for too long or under their chin, handle them when wet, or become blasé about hand hygiene through an illusion of protection (Isaacs *et al*., 2020).

Advocates for making it mandatory believe that everyone wear-ing masks will protect them from those unknown people infected with SARS-CoV-2 and will protect others if they themselves are

infected. Their advocacy may in part be motivated by anxiety: masks may make them feel safer. Currently some countries and some jurisdictions within countries where COVID-19 is widespread have decided that the likelihood that masks do more good than harm over-rides any concern about infringing autonomy and have mandated wearing masks in public. When mask-wearing is man-dated, the aim is also to protect others, not just the individual, anal-ogous to routine immunization providing community benefit. Wearing a mask is not a huge imposition, on the face of it hardly worthy of acts of civil disobedience. However, wearing a mask should not be a substitute for other proven public health measures: Physical distancing, practicing hand hygiene and, if symptomatic, getting tested for COVID-19 and not venturing out in public without receiving a negative test result (MacIntyre *et al*., 2016) Their desire for cheap, convenient, washable face coverings has spawned a culture of people making or buying designer cloth or silk masks, often as a distinctive fashion statement or to identify them symbolically as belonging to a particular group. We can find positives in this trend; involving children may give them a useful role and may help them cope with their anxieties about COVID-19. Until recently, Muslims were abused and attacked in many Western countries for covering their faces and hiding their identity. How ironic those Westerners have started to develop their own face coverings as a form of identity. History tells us that humans have been wearing masks for as long as we have been humans. Now a novel corona virus is writing a fascinating new chapter in the cultural history of human masks.

2.2 How do Masks Protect us Against Airborne Diseases?

2.2.1 The respiratory route of transmission.

A respiratory pathogen may be transmitted via three routes—contact, droplet, and airborne spread (Kutter *et al.*, 2018). Contact transmission may be direct (i.e., transfer of virus via contaminated hands) or indirect (i.e., via fomites). Fomites are objects or materials that may carry infection, and spread by fomites means spread by touch. Viruses do survive for some time on inanimate objects, although the viral load declines dramatically (Van *et al.*, 2020). If we touch a contaminated surface and then touch our eyes or nose, we may inoculate the virus into our mucosal surfaces. The role of touch in the spread of a respiratory virus is best exemplified by studies of the Respiratory Syncytial Virus (RSV). The spread of SARS-CoV-2via fomites has been elegantly demonstrated by real-world contact tracing, aided by closed-circuit cameras (Pung *et al.*, 2020).

Droplet spread and airborne spread are different modes of transmission of the virus through the air. Viruses released when an infected person coughs, sneezes, sings, talks, or merely exhales may be found in particles of varying sizes (Leung *et al.*, 2020). Generally, particles larger than 5μm were thought to fall to the ground within 1 metre. More recently, however, the "gas cloud" hypothesis has been proposed. Coughing, sneezing, or even exhaling produces mucosalivary drop-lets that exist as part of a cloud that "carries within it clusters of droplets with a continuum of droplet sizes"(Bourouiba, 2020. In combination with environmental factors, the "cloud" may be propelled up to 7–8 m. Wind speed, in particular, has been shown to play a role in determining the distance travelled by these particles (Bourouiba, 2020).Airborne spread occurs with pathogens found in exhaled droplets < 5μm in diameter. These particles remain afloat for

some time and are able to travel long distances. Respiratory viruses accepted as being capable of spread via the airborne route include measles and varicella zoster (chickenpox). These viruses have a largeR0, a feature thought to characterize spread by the airborne route. Interestingly, influenza, corona virus, and rhinovirus RNA, generally thought to be transmitted by the droplet route, can be found in exhaled particles smaller or larger than 5μm (Perencevich *et al.*, 2020). Further, viable influenza is present in particles smaller than 5μm. Hence, even viruses thought to be transmitted primarily by the respiratory droplet route may have the potential for airborne spread. Concern that SARS-CoV-2 may spread by the airborne route rose when it was shown to be viable for 3 hours in a drum that artificially kept particles afloat for several hours. It might be less well known that more basic processes like talking can also lead to the release of potentially infectious droplets and aerosols. Using laser light scattering, it was found that there were average emissions of about 1000 droplet particles per second during speech, with high emission rates of upto 10,000 droplet particles per second (Anfinrud*et al.*, 2020).

By fitting the time-dependent decrease in particle detected to exponential decay times, the droplet particle sizes and estimated viral load could be calculated. The authors estimate that 1min of loud speak-ing generates greater than 1000 droplets containing viruses (Stadnytskyi *et al.*, 2020). Alternatively, respiratory particles of between 0.5μmand 5μm could be imaged by aerodynamic particle sizing. When participants made the "Aah" sound, there were emissions of up to 330 particles per second. Taking into account that aerodynamic particle sizing measures particles under the detection limit of laser light scattering, these two methods can be seen to be complementary, and the total number of particles emitted could be even higher. In a separate study, droplet particle emission was shown to be directly proportional to loudness, with the number of particles emitted increasing from 6 particles per second when whispering to53

particles per second at the loudest talking. The number of particles generated varied greatly across individuals, raising the possibility of super spreaders who could be the primary spreaders of viruses by talking (Asadi *et al.*, 2019).

2.2.2 Mechanistic effect of wearing a mask.

Masks and other PPE items serve as a physical barrier to respiratory droplets. With imaging using laser light scattering, it was found that the number of flashes, which corresponds to the number of respiratory droplets, could be kept at background levels by covering the speaker's mouth with a slightly damp wash cloth (Anfinrud *et al.*, 2020). An *in-vitro* model with source and receiver mannequins was created to test the effect of the mask on filtering away radio labeled aerosol emitted from the source. Masking at the source mannequin was consistently more effective at lowering radio-labelled aerosols reaching the receiver mannequin, whereas the only experimental setup where the receiver mannequin could be equally well protected was if the receiver mannequin wore an N95 mask sealed with Vaseline (Patel *et al.*, 2016).Therefore, masks can act as a physical barrier and seem to be more effective when worn by the droplet emitting person. Masks have generally shown an effect in reducing microbial mission from infected patients. The surgical mask was tested for its ability to block the release of various viruses by studying the amount of virus present in the exhaled breath of patients. The investigators were able to collect particles separated by size (>or<5μm). A significant drop in corona-viruses in both larger and smaller particles was observed with the mask on. The mask reduced influenza viruses found in larger but not smaller particles. After wearing a mask, no corona virus was detected in all 11 patients, while influenza was detected in 1 patient's respiratory particles (out of 27).The mask did not lower rhinovirus counts in larger or smaller particles. This suggests that

surgical face masks can reduce the release of corona virus and influenza from an infected person. In an earlier study for influenza, participants were induced to cough, and with both surgical masks andN95 masks, there was no influenza that could be detected by reverse transcriptase-polymerase chain reaction (RT-PCR) for 9 infected patients (Johnson *et al.*, 2009).

When the exhaled influenza virus was separated into the fractions based on size, it was found that surgical masks were highly effective at removing influenza from the larger coarse fraction (≥5μm) but less effective from the fraction with smaller particles. Wearing masks has also been shown to protect individuals coming into contact with an infected person. In a survey of 5 hospitals in Hong Kong during SARS, hospital staff were asked about the protective measures they took and this information was correlated with whether they were infected by SARS. It was found that wearing masks was the single most important protective measure in reducing the chance of get-ting infected (p=0:0001), and the people who wore either surgical masks or N95 masks were not among the 11 infected staff. There were however 2 instances of people who worepaper masks being infected, suggesting that the type of masks was also important (Seto *et al.*, 2013. A study compared the effectiveness of N95 and surgical face masks against viral respiratory infections in healthcare workers. Healthcare workers had no significant difference in influenza infection outcomes when wearing N95 and surgical masks, suggesting that both types of medical masks could protect similarly (Radonovich *et al.*, 2019).

A meta-analysis was performed on clinical studies to explore the protective effect of masks. The risk ratio was calculated for the incidence of infection in the protected group vs. the unprotected group, where risk ratio < 1suggests a reduced risk. Wearing a mask protected individuals against influenza-like illness, showing a risk ratio of 0.34, with a 95% confidence interval between 0.14

and 0.82. Similar to the study above, surgical masks and N95 masks showed little difference in protection, with a risk ratio of 0.84 and a 95% confidence interval of 0.36-1.99 suggesting no significant difference in risk (Offeddu*et al.*, 2017).Recently, a modeling study performed by Eikenberry et al. based on COVID-19 infection data obtained in NewYork and Washington suggested that the broad adoption of face mask by the general public can significantly reduce com-munity transmission rate and death toll. Based on data obtained from 20thFebruary to 30thMarch, the cumulative death rate was projected to be reduced to a greater extent as more people wear masks over the next 2months. Therefore, the study concludes that community-wide adoption of face mask has great potential to help curtail community transmission and the burden of the COVID-19pandemic (Eikenberry *et al*, 2020).

2.3 Performance Criteria for Masks.

2.3.1 Commercial masks.

Face masks provide the user with protection against airborne particles, pathogens, secretions, and body fluids by physically filtering them from breathable air. According to the American Society of Testing and Materials (ASTM) F2100 standard, which specifies the performance requirements for materials used in medical face masks (ASTM, 2019), five performance characteristics have been identified. These are particulate filtration efficiency (PFE), bacterial filtration efficiency (BFE), fluid resistance, differential pressure, and flammability. As face masks are an integral part of the personal protective equipment (PPE) kit for medical use, these standardized characteristics ensure consistency in mask production and testing validation and help the end-user to make the most informed choice of mask for the intended application.

2.3.2 Particulate filtration efficiency (PFE).

This test measures the filtration efficiency of face masks towards mono-disperse particles under a constant air flow rate. For PFE testing, 0.1μm polystyrene latex particles are used according to FDA guidance at airflow velocities of 0.5-25 cm/s as recommended by the ASTM F2299 standard, for quantifying the filtration efficiency of materials used in facial masks (STM, 2017). Light scattering is used to quantify the particle count in the upstream feed (Mu) prior to filtration, as well as that in the downstream filtrate (Md). The filtration efficiency (E), often expressed as a percentage, can be calculated with Equation (1):

2.3.2 Equation

It thus follows that the higher the value of E, with a corresponding smaller P, indicates a better ability of the mask material to filter submicron particles. While theF2299 standard allows consistent comparison of the PFE value of different materials used for face masks, it does not access the effectiveness of the overall design of the facemask, nor the quality of the mask's seal to the wearer's face.

2.3.3 Bacteria filtration efficiency (BFE).

This test quantifies the performance of the mask material in filtering out bacteria when challenged with an aerosol of *Staphylococcus aureus*, as recommended by the ASTM F2101 standard. *S. aureus* was chosen for its clinical relevance as one of the leading causes of nosocomial infections acquired in a hospital or healthcare facility (Valaperta *et al.*, 2010). To perform the test, an aerosolized liquid suspension of *S. aureus* (mean particle size of3:0±0:3μm) is delivered to the target filter sample at a constant flow rate of 1 ft3/min (or 28.3 L/min). The

aerosol is then drawn through a six-stage Andersen sampler. Each tier contains an agar plate which acts as a medium for the growth of any bacteria which passes through the filter material to form visible colonies on the plates. A control is also performed under identical conditions in the absence of the filter specimen (Valaperta *et al.*, 2010)..

For surgical masks, a minimum BFE of 95% BFE is required. It should be noted that other than the AS6TM specifications, some mask manufacturers quantify BFE ratings with the modified Greene and Vesley method (Greene and Vesley, 2012) which measures the effectiveness of the mask in preventing bacteria from passing through when worn on a human test subject's face. This method is not comparable with ASTM F2101 and is not recommended by ASTM for comparison. The ASTM F2101 method possesses numerous advantages, including a highly reproducible testing procedure, the ability to tightly control the mean bacteria aerosol particle size, and has not been modified for many years, which provides a consistent set of standards for comparing across many different filter materials assessed at different times (Labs, 2020). However, like the ASTM F2299 standard for PFE, the ASTM F2101 standard for BFE does not evaluate the fit, design, and facial-sealing properties of the mask.

2.3.4 Viral filtration efficiency (VFE).

The viral filtration efficiency (VFE) is another parameter used by mask manufacturers for marketing and in FDA 510(k) applications for certain N95filtering face piece respirators, although itis not currently recognized as a standard test method by ASTM and hence is not a requirement for mask evaluation. The VFE test utilizes the same procedure and setup as recommended by ASTM F2101 for BFE (Labs, 2020). The bacteriophage ΦX174, which infects only E. coli bacteria, is used as the challenge virus that is aerosolized to form3:0±0:3μm virus-

containing water droplets (not individual viruses). Unlike the BFE test, the agar plates in the Andersen sampler are first inoculated with E. coli, and areas in contact with the viral droplets become clear as the bacteria cells are lysed to form plaques. The VFE value is calculated by comparison with a control without the filter material as described above for BFE.

2.3.5 Fluid resistance.

Fluid resistance evaluates the mask's ability to act as a barrier to the transfer of fluids from its outer to its inner layers due to spraying or splashing. According to the ASTM F1862 standard, 2 mL of synthetic blood, containing a red dye for visual detection and a thickening agent for stimulating blood flow properties, is dispensed against a complete medical mask specimen at different velocities (ASTM, 2017). These velocities correspond to different blood pressures of 80 mmHg (Level 1, venous blood pressure), 120 mmHg(Level 2, arterial pressure), and 160 mmHg (Level 3, high pressures occurring during trauma or under surgical conditions with high-pressure irrigation), assuming the facemask is within 300 mm of the blood vessel puncture. The pass/fail determinations are based on visually detecting penetration of the synthetic blood to the inner layer. To simulate actual usage conditions, i.e., breathing, which creates high humidity (thus affecting fluid resistance), and mask material, the test specimens are also preconditioned at high relative humidity of (85 ± 5) % at (21 ± 5)°C.

2.3.6 Differential pressure (DP).

This parameter, otherwise known as "delta P,"measures the ability of the mask material to restrict airflow through it, giving an objective indication of the mask's breathability. Typically, it is determined by measuring the difference in air pressure on both sides of the mask material

using a manometer at a constant airflow rate, and the difference in pressure is divided by the surface area of the sample, according to the MIL-M-36954 standard (ASTM, 2017).As such, DP is usually expressed in units of mm H2O/cm2,where a lower value (i.e., smaller difference in pressure on both sides) indicates greater breathability, feels cooler to the wearer, and hence gives an overall better comfort level. ASTM requires that moderate and high barrier masks have a DP value of<5.0, while low barrier masks have DP < 4:0.It is noteworthy that a trade-offexists between DP and fluid resistance for the same design and fit of the wearer: generally, an increase in resistance to synthetic blood penetration also results in a greater pressure drop across the mask layers and hence reduces breathability (ASTM, 2017).

2.3.7 Flammability.

Hospitals contain numerous sources of ignition, such as heat, oxygen, and fuel sources. As the natural and synthetic fibers making up the mask materials are flammable, these can pose potential risks to the wearer due to the speed and intensity of flame spreading. Mask flammability is assessed in accordance with the 16 CFR Part 1610standards, typically performing the tests on 5-10 test samples. In a nutshell, the mask specimen is first cut into the defined dimension of50 × 150mm, and then mounted and secured onto a specimen holder. Thereafter, the mounted specimen is conditioned in a desiccating oven at (105 ± 3)°C for 30 minutes, before it is then transferred to the test chamber. A stable butane flame of fixed length (16 mm) is then impinged upon the sample for exactly 1.0 s. The burn time, i.e., the time taken for the flame to travel up the specimen till a stop device is triggered, is then registered. According to the ASTM F2100 Standard for Performance of Materials Used in Medical Face Masks (ASTM, 2019), the masks need to meet the requirements of Class 1flammability, with an average

burn time of≥3.5s.In addition to these aforementioned standardized tests, the medical face masks should be tested according to ISO10993-5 and 10, which specifies cytotoxicity and skin sensitivity test methods, respectively, to ensure the materials are not harmful to the wearer (ISO, 2010).

2.4 Microorganisms of Importance to Face Mask Wearing.

Certain microorganisms that exist in our environment and within the human system are of prime target. Bacteria and fungi as well as viruses are constantly being recycled in the air therefore aerosols, they can be inhaled and have the potential to cause disease hence the need to wear mask. Masks serves as protective membrane against these microbes thereby harboring these microbes at the surface. Also microbes from the human system can be exhaled from the nose and mouth and are trapped by the masks from getting out to the environment. Some of these microbes are discussed below:

2.4.1 Fungi

Fungal cells usually die when they travel through the atmosphere due to the desiccating effects of higher altitudes. However, some particularly resilient fungal bioaerosols have been shown to survive in atmospheric transport despite exposure to severe UV light conditions (Tang and Julian, 2009). Although bioaerosol levels of fungal spores increase in higher humidity conditions, they can also be active in low humidity conditions and in most temperature ranges. Certain fungal bioaerosols even increase at relatively low levels of humidity. Eg *Aspergillus* spp., *Penicillum* spp.

2.4.2 Bacteria

Unlike other bioaerosols, bacteria are able to complete full reproductive cycles within the days or weeks that they survive in the atmosphere, making them a major component of the air biota ecosystem. These reproductive cycles support a currently unproven theory that bacteria bioaerosols form communities in an atmospheric ecosystem (Smets *et al.*, 2016). The survival of bacteria depends on water droplets from fog and clouds that provide bacteria with nutrients and protection from UV light The four known bacterial groupings that are abundant in aeromicrobial environments around the world include Bacillaceae, Actinobacteria, Proteobacteria, and Bacteroidetes.

2.4.2.1 Bacillus species

Bacillus (Latin "stick") is a genus of Gram-positive, rod-shaped bacteria, a member of the phylum *Firmicutes*, with 266 named species. The term is also used to describe the shape (rod) of certain bacteria; and the plural Bacilli is the name of the class of bacteria to which this genus belongs. *Bacillus* species can be either obligate aerobes: oxygen dependent; or facultative anaerobes: having the ability to continue living in the absence of oxygen. Cultured *Bacillus* species test positive for the enzymecatalase if oxygen has been used or is present (Turnbull, 2010).

Bacillus can reduce themselves to oval endospores and can remain in this dormant state for years. Endospore can be easily carried in the air to different surfaces such as wooden surfaces walls of the working or learning environments as well as on equipments used in schools and hospitals. The spores of this organism can also find its way on face masks due to the fact that it

could easily be transported by air. The endospore of one species from Morocco is reported to have survived being heated to 420 °C. Endospore formation is usually triggered by a lack of nutrients: the bacterium divides within its cell wall, and one side then engulfs the other. They are not true spores (i.e., not an offspring). Endospore formation originally defined the genus, but not all such species are closely related, and many species have been moved to other genera of the *Firmicutes* (Madigan and Martinko, 2015). Only one endospore is formed per cell. The spores are resistant to heat, cold, radiation, desiccation, and disinfectants. *Bacillus anthracis* needs oxygen to sporulate; this constraint has important consequences for epidemiology and control. In vivo, *B. anthracis* produces a polypeptide (polyglutamic acid) capsule that kills it from phagocytosis. The genera *Bacillus* and *Clostridium* constitute the family *Bacillaceae*. Species are identified by using morphologic and biochemical criteria. Because the spores of many *Bacillus* species are resistant to heat, radiation, disinfectants, and desiccation, they are difficult to eliminate from medical and pharmaceutical materials and are a frequent cause of contamination. Not only they are they resistant to heat, radiation, etc., but they are also resistant to chemicals such as antibiotics (Graham *et al.,* 2020). This resistance allows them to survive for many years and especially in a controlled environment. Ubiquitous in nature, *Bacillus* includes both free-living (nonparasitic) species, and two parasiticpathogenic species. These two *Bacillus* species are medically significant: *B. anthracis* causes anthrax; and *B. cereus* causes food poisoning. Many species of *Bacillus* can produce copious amounts of enzymes, which are used in various industries, such as in the production of alpha amylase used in starch hydrolysis and the proteasesubtilisin used in detergents. *B. subtilis* is a valuable model for bacterial research. Some *Bacillus* species can synthesize and secrete lipopeptides, in particular surfactins and mycosubtilins.[8][9]

2.4.2.2 Actinobacteria

The Actinobacteria are a phylum of Gram-positive bacteria. They can be terrestrial or aquatic (Servinet *al*., 2008). They are of great economic importance to humans because agriculture and forests depend on their contributions to soil systems. In soil they help to decompose the organic matter of dead organisms so the molecules can be taken up anew by plants. While this role is also played by fungi, Actinobacteria are much smaller and likely do not occupy the same ecological niche. In this role the colonies often grow extensive mycelia, like a fungus would, and the name of an important order of the phylum, Actinomycetales (the actinomycetes), reflects that they were long believed to be fungi. Some soil actinobacteria (such as *Frankia*) live symbiotically with the plants whose roots pervade the soil, fixing nitrogen for the plants in exchange for access to some of the plant's saccharides. Other species, such as many members of the genus *Mycobacterium*, are important pathogens. Beyond the great interest in Actinobacteria for their soil role, much is yet to be learned about them. Although currently understood primarily as soil bacteria, they might be more abundant in fresh waters. Actinobacteria is one of the dominant bacterial phyla and contains one of the largest of bacterial genera, *Streptomyces* (Michael, 2010)*Streptomyces* and other actinobacteria are major contributors to biological buffering of soils. They are also the source of many antibiotics.

Actinobacteria are normally present in the gums and are the most common cause of infection in dental procedures and oral abscesses. Many *Actinomyces* species are opportunistic pathogens of humans and other mammals, particularly in the oral cavity (Madigan and Martinko, 2015). In rare cases, these bacteria can cause actinomycosis, a disease characterized by the formation of abscesses in the mouth, lungs, or the gastrointestinal tract.Actinomycosis is most frequently

caused by *A. israelii*, which may also cause endocarditis, though the resulting symptoms may be similar to those resulting from infections by other bacterial species (Adalja and Vergis. 2010).*Aggregatibacteractinomycetemcomitans* has been identified as being of note in periodontal disease.

The genus is typically the cause of oral-cervicofacial disease. It is characterized by a painless "lumpy jaw". Lymphadenopathy is uncommon in this form of the disease. Another form of actinomycosis is thoracic disease, which is often misdiagnosed as a neoplasm, as it forms a mass that extends to the chest wall. It arises from aspiration of organisms from the oropharynx. Symptoms include chest pain, fever, and weight loss. Abdominal disease is another manifestation of actinomycosis. This can lead to a sinus tract that drains to the abdominal wall or the perianal area. Symptoms include fever, abdominal pain, and weight loss (Sahli*et al.*, 2009)*Actinomyces* species have also been shown to infect the central nervous system in a dog "without history or evidence of previous trauma or other organ involvement (Couto*et al.*, 2010).

2.4.2.3 *Staphylococcus* spp

Staphylococcus is a genus of Gram-positivebacteria in the family Staphylococcaceae from the order Bacillales. Under the microscope, they appear spherical (cocci), and form in grape-like clusters. *Staphylococcus* species are facultative anaerobic organisms (capable of growth both aerobically and anaerobically).

The name was coined in 1880 by Scottish surgeon and bacteriologist Alexander Ogston (1844–1929), following the pattern established five years earlier with the naming of *Streptococcus*. It combines the prefix "staphylo-" (from Ancient Greek: σταφυλή, romanized: *staphylē*, lit. 'bunch

of grapes'), and suffixed by the Modern Latin: *coccus*, lit. 'spherical bacterium' (from Ancient Greek: κόκκος, romanized: *kókkos*, lit. 'grain, seed, berry') (Liddell*et al.*, 2011).

Staphylococcus includes at least 40 species. Of these, nine have two subspecies, one has three subspecies, and one has four subspecies. Many species cannot cause disease and reside normally on the skin and mucous membranes of humans and other animals. *Staphylococcus* has been found to be a nectar-inhabiting microbe. They are also a small component of the soil microbiome (Madigan and Martinko, 2015).

2.4.2.4 *Escherichia coli*

Escherichia is a genus of Gram-negative, non-spore-forming, facultatively anaerobic, rod-shaped bacteria from the family Enterobacteriaceae (Madigan*et al.*, 2015). In those species which are inhabitants of the gastrointestinal tracts of warm-blooded animals, *Escherichia* species provide a portion of the microbially derived vitamin K for their host. A number of the species of *Escherichia* are pathogenic.[4] The genus is named after Theodor Escherich, the discoverer of *Escherichia coli*. *Escherichia* are facultative aerobes, with both aerobic and anaerobic growth, and an optimum temperature of 37 °C. *Escherichia* are usually motile by flagella, produce gas from fermentable carbohydrates, and do not decarboxylatelysine or hydrolyze arginine.Species include *E. albertii*, *E. fergusonii*, *E. hermannii*, *E. senegalensis*, *E. marmotae* and most notably, the model organism and clinically relevant *E. coli*. *Shimwelliablattae* was formerly classified in this genus (Priest and Barker, 2009).

E. coli normally grow in soil and in the large intestines of many mammals, including humans. Most strains of *E. coli* do not cause disease, but instead help animals get vitamins and

digestfood. Some strains of *E. coli* cause sickness in people. *E. coli* are not usually in food or water. When food has not been prepared with clean equipment, *E. coli* can grow in the food. When *E. coli* are found in water, this may mean that the water has touched sewage.

2.4.3 Viruses

The air transports viruses and other pathogens. Since viruses are smaller than other bioaerosols, they have the potential to travel further distances. In one simulation, a virus and a fungal spore were simultaneously released from the top of a building; the spore traveled only 150 meters while the virus traveled almost 200,000 horizontal kilometers (Núñez*et al.*, 2016).

In one study, aerosols (<5 μm) containing SARS-CoV-1 and SARS-CoV-2 were generated by an atomizer and fed into a Goldberg drum to create an aerosolized environment. The inoculum yielded cycle thresholds between 20 and 22, similar to those observed in human upper and lower respiratory tract samples. SARS-CoV-2 remained viable in aerosols for 3 hours, with a decrease in infection titre similar to SARS-CoV-1. The half-life of both viruses in aerosols was 1.1 to 1.2 hours on average. The results suggest that the transmission of both viruses by aerosols is plausible, as they can remain viable and infectious in suspended aerosols for hours and on surfaces for up to days.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Site

The study was carried out at Godfrey Okoye University Microbiology laboratory, at Thinkers corner, Enugu, Nigeria.

3.2 Collection of Samples.

A total of 20 face masks were distributed to 20 students (10 boys and 10 girls) to wear for a period of 24hrs. The face masks were aseptically taken using a sterile bag (zip-lock bag) and taken to the lab for analysis. Swab sticks damped in normal saline were used to swab Outer and inner parts of the face masks for culturing.

3.4 Media Preparation

The media was prepared as follows 20 plates of Nutrient agar, Makoncey agar and Patotoe Dextrose agar, for bacteria and fungi isolation respectively were prepared using the manufacturers' description and was dissolved with distilled water in a conical flask and autoclaved at 121°C for 15min. After autoclaving, the medium was allowed to cool and poured into 20 Petri dishes for each medium and allowed to gel, then dried using a hot air oven.

3.5 Isolation of Bacteria from used Face Masks

The swab sticks were inoculated into these prepared media plates. Inoculation was done using streak plate method. The plates were incubated aerobically at 37°C for 24hours. Plates with growth were selected and those without growth were further incubated for 48 hrs.

3.5 Isolation of Pure Culture

Distinct colonies from the inoculated plates were selected using inoculating loops and subsequently sub cultured on Nutrient agar plates and incubated for 37^{0}C for 24 hours to confirm that they were pure cultures of the isolates. Nutrient agar slants were prepared according to manufacturer's guide. The pure isolates were streaked on the agar slants aseptically. The agar slants were stored in the refrigerated at 2-8^{o}C.

3.6 Identification of Bacterial Isolates

Pure cultures were then identified as described by Cheesbough, M. (2006) using morphological methods such as colonial appearances, Gram staining biochemical tests such as, catalase tests, spore staining, sugar fermentation tests, etc.

3.6.1 Gram staining:

Smear of the isolates were prepared and heat-fixed on a clean grease free slide. The smears were stained by flooding it for one minute with crystal violet. This was washed out with a gentle running tap water. The slides were flooded with Lugos iodine and left for one minute. This was washed off with water and the slides were decolorized with acetone, washed off immediately with water and counter stained with Safranin solution for about one minute. Finally, the slides were washed with tap water, allowed to dry, and observed under oil immersion objective at 100x objective.

3.6.2 Lactophenol cotton blue test:

A drop of lacto phenol blue was placed on a grease free clean microscopic slide and using a well flamed inoculating needle, the test organism was picked and thinly spread onto the slide

containing lactophenol blue. Then a cover slip was carefully and slowly placed at the edge of the lactophenol in order to prevent air bubbles from being trapped under the cover slip. The slide was then viewed under the microscope using 10× and 40× objective lens.

3.6.3 Catalase test

A microscopic slide was placed in a petri-dish. Using a sterile inoculating loop, a colony of the pure bacteria isolates were placed on the slide. A drop of 3% hydrogen peroxide (H_2O_2) was placed onto the slide where the isolates were smeared. The slides were checked for immediate bubbling.

3.6.4 Coagulase test

A drop of distilled water was placed on each end of a slide and used to emulsify a colony of the test organism in each of the drops on the slides. Not more than a loop-full of plasma was added to each of the suspensions, and gently mixed. Afterwards, it was observed for clumping of the organisms within 10 seconds. No plasma was added to the second suspension on the other end of the slide. This was used to differentiate any granular appearance of the organism from true coagulase clumping.

3.6.5 Indole production test

According to Cheesebrough (2006), the Tryptone water method of detecting indole was used. The test organism in a bijou bottle containing 3 ml of sterile Tryptone water was inoculated and incubated at 35–37°C for up to 48 h. Indole was tested for by adding 0.5 ml of Kovac's reagent and gently shook, after which it was examined within 10 minutes for a red colour in the surface layer.

3.6.6 Oxidase test

A small piece of filter paper was soaked in Gaby and Hadley oxidase test reagent and dried Using an inoculating loop, a well-isolated colony from pure 24-hour culture was picked and rubbed onto filter paper and observed for color change (Shields and Cathcart, 2010).

3.6.7 Methyl red test

The MR-VP broth of 7 ml in each test tube was prepared by according to manufacturer's guide. Using sterile inoculating loop, a pure isolate was inoculated into the tubes and incubated at 37°C. After 48-hours incubation, 5 drops of methyl red indicator was added directly into the culture tubes to observe the immediate development of a red Color.

3.6.8 Voges Proskauer test

The colony of interest to be analyzed from the pure culture was suspended in the VP/MR medium and incubated at 30-37°C for 24-48 hours. After incubation, 0.2 ml of 40% KOH was added and then 0.6 ml of alpha-naphtol solution and then observed for colour change.

CHAPTER 4

4.0 RESULTS

Table 4.1 Total Bacterial Count from Girls' Mask Samples

Samples	Bacteria count Cfu/ml		No of isolates	
	Outer	Inner	Outer	Inner
GA	-	-	-	-
GB	$1.0x10^2$	$1.0x10^1$	2	1
GC	$2.2x10^1$	$1.1x10^1$	1	1
GD	-	-	-	-
GE	$6.1x10^1$	$2.1x10^2$	2	1
GF	$3.1x10^2$	-	2	-
GG	$1.2x10^2$	$1.1x10^1$	1	1
GH	-	-	-	-
GI	$2.4x10^2$	-	3	-
GJ	$3.2x10^1$	$2.3x10^1$	2	1
Total			**13**	**5**

KEYWORD:- G- girls, A-J – alphanumerical representation of the 10 girls (i.e GA- first girl, GB- second girl, GJ-tenth girl), inner- represents the inner part of the masks, Outer- represents the outer part of the face masks.

Result Interpretation:- Table 4.1 shows the total Bacterial Count from samples gotten from the outer and inner parts of face masks distributed to the ten(10) girls. This table shows the that samples; GA,GD,GH- showed no bacterial contamination on both Outer and inner.

GF, GI- showed no bacterial contamination on the inner.

GB, GC, GE, GG, GJ- showed bacterial contamination on both Outer and inner.

Table 4.1.1 Total Fungal Count from Girls' Mask Samples

Samples	No of isolates	
	Outer	Inner
GA	-	-
GB	1	-
GC	1	-
GD	-	-
GE	-	-
GF	1	-
GG	1	1
GH	-	-
GI	2	-
GJ	1	1
Total	**7**	**2**

KEYWORD:- G- girls, A-J – alphanumerical representation of the 10 girls (i.e GA- first girl, GB- second girl, GJ-tenth girl), inner- represents the inner part of the face masks, Outer- represents the outer part of the face masks.

Result Interpretation:- Table 4.1.1 shows the total Fungal Count from samples gotten from the outer and inner parts of face masks distributed to the ten(10) girls. This table shows the that samples;:- GA,GD,GE,GH:-showed no fungal contamination on both Outer and inner.

GB, GC, GF, GI:- showed fungal contamination on the Outer.

GG, GJ:- showed fungal contamination on both Outer and inner.

Table 4.2 **Total Bacterial Count from Boys' Mask Samples**

Samples	Bacteria count Cfu/ml		No of isolates	
	Outer	Inner	Outer	Inner
BA	-	-	-	-
BB	$1.0x10^2$	$1.0x10^3$	1	2
BC	$2.2x10^2$	$1.1x10^1$	1	1
BD	$2.3x10^1$	$2.0x10^1$	2	1
BE	$4.2x10^1$	-	2	-
BF	$1.2x10^2$	$1.0x10^2$	1	1
BG	-	-	-	-
BH	$3.3x10^2$	-	2	-
BI	-	-	-	-
BJ	$4.2x10^2$	$2.1x10^2$	2	1
Total			**11**	**6**

KEYWORD:- B- boys, A-J – alphanumerical representation of the 10 boys (i.e BA- first boy, BB- second boy, BJ-tenth boy), inner- represents the inner part of the face masks, Outer- represents the outer part of the face masks.

Result Interpretation:- Table 4.2 shows the total Bacterial Count from samples gotten from the outer and inner parts of face masks distributed to the ten(10) boys. This table shows the that samples;:- BA,BG,BI- showed no bacterial contamination on both Outer and inner.

BB,BC,BD,BF,BJ:- showed bacterial contamination on both Outer and inner.

BE,BH:- showed growth on the Outer part but none on the inner.

Table 4.2.1 Total Fungal Count from Boys' Mask Samples

Samples	No of isolates	
	Outer	Inner
BA	-	-
BB	1	-
BC	1	-
BD	1	-
BE	-	-
BF	1	1
BG	-	-
BH	2	-
BI	-	-
BJ	-	-
Total	**6**	**1**

KEYWORD:- B- boys, A-J – alphanumerical representation of the 10 boys (i.e BA- first boy, BB- second boy, BJ-tenth boy), inner- represents the inner part of the face masks, Outer- represents the outer part of the face masks.

Result Interpretation:- Table 4.1 shows the total Fungal Count from samples gotten from the outer and inner parts of face masks distributed to the ten(10) boys. This table shows the that samples;**:-**BA,BE,BG,BI,BJ:-showed no fungal contamination on both Outer and inner,

BB,BC,BD,BH;- showed fungal contamination on Outer but not on the inner.

BF:- showed fungal contamination on both Outer and inner.

4.3 Biochemical and morphological identification of bacterial isolates from girls

Samples	Parts of the mask	Colonial morphology	Microscopy		Biochemical tests						Probable organisms
			Arrangement	Gram stain	Motility test	Indole test	Catalase test	Coagulase test	Oxidase test	Methyl red test	Voges proskauer	
GB	Inner	Yellow, shiny	Cocci	+ve	-	+	+	-	-	+	-	*Streptococcus spp*
		Pink, creamy	Rod	-ve	+	+	+	-	-	+	-	*Legionella spp*
	Outer	Cream, smooth	Rod	-ve	-	+	+	-	-	+	-	*E. coli*
GC	Inner	Yellow, shiny	Cocci	+ve	-	+	+	-	-	+	-	*Streptococcus spp.*
	Outer	Yellow, moist	Cocci	+ve	+	-	+	+	-	+	+	*Staphylococcus spp.*
GE	Inner	Yellow, shiny	Cocci	+ve	-	+	+	-	-	+	-	*Streptococcus spp.*
	Outer	Cream, smooth	Rod	-ve	-	+	+	-	-	+	-	*E. coli*
		Yellow, moist	Cocci	+ve	+	-	+	+	-	+	+	*Staphylococcus spp.*
GF	Outer	Pink, moist	Rod	-ve	-	-	+	-	-	-	+	*Klebsiella spp*
		Yellow, moist	Cocci	+ve	+	-	+	+	-	+	+	*Staphylococcus spp.*
GI	Outer	Pink, creamy	Rod	-ve	+	+	+	-	-	+	-	*Legionella spp*
	Outer	Yellow, moist	Cocci	+ve	+	-	+	+	-	+	+	*Staphylococcus spp.*
		Pink, creamy	Rod	-ve	+	+	+	-	-	+	-	*Legionella spp*
GJ	Inner	Yellow, shiny	Cocci	+ve	-	+	+	-	-	+	-	*Streptococcus spp.*
	Outer	Pink, moist	Rod	-ve	-	-	+	-	-	-	+	*Klebsiella spp*
		Cream, smooth	Rod	-ve	-	+	+	-	-	+	-	*E. coli*
		Yellow, moist	Cocci	+ve	+	-	+	+	-	+	+	*Staphylococcus spp.*

Result interpretation: Table 4.3 shows the identification of bacterial isolates gotten from the inner and outer parts of the face masks distributed to the Ten(10) girls. Identification was done based on their Colonial morphology, Microscopy and Biochemical examination. Out of the Ten(10) samples, 6 had bacterial growth and a total of 5 bacteria were isolated, these include; *Staphylococcus* spp., *Legionella* spp., *Streptococcus* spp., *Klebsiella* spp. and *Escherichia coli.*

Keys: **:- G-** girls, A-J – alphanumerical representation of the 10 girls (i.e GA- first girl, GB- second girl, GJ-tenth girl), inner- represents the inner part of the face masks, Outer- represents the outer part of the face masks, +: positive – negative

Table 4.4: Biochemical and Morphological identification of the isolates for boys

Samples	Parts of the mask	Colonial morphology	Microscopy		Biochemical tests						Probable organisms
			Arrangement	Gram stain	Motility test	Indole test	Catalase test	Coagulase test	Oxidase test	Methyl red test	Voges proskauer	
BB	Inner	Yellow, shiny	Cocci	+ve	-	+	+	-	-	+	-	*Streptococcus spp*
		Cream, smooth	Rod	-ve	-	+	+	-	-	+	-	*E. coli*
	Outer	Yellow, moist	Cocci	+ve	+	-	+	+	-	+	+	*Staphylococcus spp.*
BC	Inner	Yellow, shiny	Cocci	+ve	-	+	+	-	-	+	-	*Streptococcus spp.*
	Outer	Yellow, smooth	Cocci	+ve	+	-	+	+	-	+	+	*Staphylococcus spp.*
BD	Inner	Yellow, moist	Cocci	+ve	+	-	+	+	-	+	+	*Staphylococcus spp.*
	Outer	Pink, moist	Rod	-ve	-	-	+	-	-	-	+	*Klebsiella spp*
		Cream, smooth	Rod	-ve	-	+	+	-	-	+	-	*E. coli*
BE	Outer	Cream, smooth	Rod	-ve	-	+	+	-	-	+	-	*E. coli*
		Yellow, moist	Cocci	+ve	+	-	+	+	-	+	+	*Staphylococcus spp.*
BF	Inner	Yellow, shiny	Cocci	+ve	-	+	+	-	-	+	-	*Streptococcus spp.*
	Outer	Pink, moist	Rod	-ve	-	-	+	-	-	-	+	*Klebsiella spp*
BH	Outer	Pink, cream	Rod	-ve	+	+	+	-	-	+	-	*Legionella spp*
		Cream, smooth	Rod	-ve	-	+	+	-	-	+	+	*Escherichia coli*
		Yellow, moist	Cocci	+ve	+	-	+	+	-	+	+	*Staphylococcus spp.*
BJ	Inner	Yellow, shiny	Cocci	+ve	-	+	+	-	-	+	-	*Streptococcus spp.*
	Outer	Pink, moist	Rod	-ve	-	-	+	-	-	-	+	*Klebsiella spp*
		Cream, smooth	Rod	-ve	-	+	+	-	-	+	-	*E. coli*

Result interpretation: Table 4.4 shows the identification of bacterial isolates gotten from the inner and outer parts of the face masks distributed to the Ten(10) boys. Identification was done based on their Colonial morphology, Microscopy and Biochemical examination. Out of the Ten(10) samples, 6 had bacterial growth and a total of 5 bacteria were isolated, these include; *Staphylococcus* spp., *Legionella* spp., *Streptococcus* spp., *Klebsiella* spp. and *Escherichia coli*

Keys: NA B- boys, A-J – alphanumerical representation of the 10 boys (i.e BA- first boy, BB- second boy, BJ-tenth boy), inner- represents the inner part of the face masks, Outer- represents the outer part of the face masks, +: positive – negative

4.5: Morphological And Biochemical Identification Of Fungal Isolates from girl's .

Samples	Parts of the mask	Colonial morphology	Microscopy	Biochemical tests	Probable organisms
			Arrangement	Lactophenol cotton blue test	
GB	Outer	Dark brown, thicker	Vessicle	+	*Aspergillus spp.*
GC	Inner	Dark brown, thicker	Vessicle	+	*Aspergillus spp.*
	Outer	Dark brown, thicker	Vessicle	+	*Fusarium spp.*
GF	Outer	Dark, thick	Flat	+	*Penicillium spp.*
GG	Inner	Dark brown, thicker	Vessicle	+	*Aspergillus spp.*
	Outer	Dark brown, thicker	Vessicle	+	*Aspergillus spp.*
GI	Outer	Dark, thick	Flat	+	*Penicillium spp.*
		Dark brown, thicker	Vessicle	+	*Fusarium spp.*
GJ	Inner	Dark brown, thicker	Vessicle	+	*Aspergillus spp.*

Result interpretation: Table 4.6 shows the identification of fungal isolates gotten from the inner and outer parts of the face masks distributed to girls. Identification was done based on their Colonial morphology, Microscopy and Biochemical examination. A total of 3 fungi were isolated, these include; *Aspergillus* spp., *Fusarium* spp., *Penicillium* spp.

4.6: Morphological And Biochemical Identification Of Fungal Isolates from boy's .

Samples	Parts of the mask	Colonial morphology	Microscopy	Biochemical tests	Probable organisms
			Arrangement	**Lactophenol cotton blue test**	
BB	Outer	Dark brown, thicker	Vessicle	+	*Aspergillus spp.*
BC	Outer	Dark brown, thicker	Vessicle	+	*Aspergillus spp.*
BD	Outer	Dark, thick	Flat	+	*Penicillium spp.*
BF	Inner	Dark, thick	Flat	+	*Penicillium spp.*
	Outer	Dark brown, thicker	Vessicle	+	*Aspergillus spp.*
BH	Outer	Dark brown, thicker	Vessicle	+	*Fusarium spp.*
BJ	inner	Dark brown, thicker	Vessicle	+	*Aspergillus spp.*

Result interpretation: Table 4.6 shows the identification of fungal isolates gotten from the inner and outer parts of the face masks distributed to boys. Identification was done based on their Colonial morphology, Microscopy and Biochemical examination. A total of 3 fungi were isolated, these include; *Aspergillus* spp., *Fusarium* spp., *Penicillium* spp.

Table 4.7: Frequency of occurrence of bacterial isolates

Isolates	No of isolates	Frequency of occurrence(%)
Streptococcus spp	8	22.86
Escherichia coli	8	22.86
Staphylococcus spp	10	28.57
Klebsiella spp	5	14.28
Legionella spp	4	11.43
	35	100

Result interpretation: : According to the table above, it can be shown that *Staphylococcus* spp. Is the highest occurring bacteria isolate with a frequency of 28.57% followed by, *Streptococcus* spp. and *Escherichia coli* with a frequency of 22.86%, then *Klebsiella* spp. with a frequency of 14.28% and lastly, *Legionella* spp with a frequency of 11.43%.

Table 4.8: **Frequency of occurrence of fungal isolates**

Isolates	No of isolates	Frequency of occurrence (%)
Aspergillus spp.	9	56.25
Fusarium spp.	3	18.75
Penicillium spp.	4	25
	16	100

Result interpretation: According to the table above, it can be shown that *Aspergillus spp.* Is the highest occurring fungi isolates with a frequency of 56.25% followed by, *Penicillium spp.* with a frequency of 25% and then *Fusarium spp.* with a frequency of 18.75.

CHAPTER FIVE

5.1 DISCUSSION

From the result in table 4.1 and table 4.2 which shows total bacterial count and number of isolates from the samples in boys and girls respectively, it was observed that the microbial load obtained from the culture of the Outer part of the face mask was higher than the numbers obtained from the inner part of the face mask, This could be attributed to the fact that the Outer part are easily exposed to the environment hence are easily contaminated by organisms in the air and also organisms that can be transported by the activities of the hand. Micro-organisms such as *Streptococcus spp., Legionella spp* and *Staphylococcus spp* were mainly the organisms isolated from the inner part of the face masks this could be attributed to the fact that they are mainly normal flora of the mouth.

Table 4.7 shows the frequency of occurrence of bacterial isolates with *Staphylococcus* spp. being the highest occurring bacteria isolate with a frequency of 28.57% followed by, *Streptococcus* spp. and *Escherichia coli* with a frequency of 22.86%, then *Klebsiella* spp. with a frequency of 14.28% and lastly, *Legionella* spp with a frequency of 11.43% while Table 4.8 shows the frequency of occurrence of fungal isolates with that *Aspergillus spp.* being the highest occurring fungi isolates with a frequency of 56.25% followed by, *Penicillium spp.* with a frequency of 25% and then *Fusarium spp.* with a frequency of 18.75.

There was higher number of bacteria isolated than fungi this comprises organisms from the saliva and aerosols. A higher number of bacterial and fungal isolates that were obtained from the

group of samples of girls was higher than that of the boys and there is no reason for this other than it's a condition of chance.

A systematic review and meta-analysis of public health methods to prevent person-to-person transmission concluded that face mask use could result in an 85% reduction in risk of infection (Chu *et al.*, 2020). However, the evidence was much stronger for the use of face masks as part of personal protective equipment in health-care settings than in community settings, and stronger for N95/P2masks than for surgical masks and cotton masks as observed in this study.

While it is certainly possible that wearing masks would protect the public, it is also possible that they might actually be detrimental if people wear them for too long or under their chin, handle them when wet, or become blasé about hand hygiene through an illusion of protection (Isaacs *et al.*, 2020). This was the observation in this study with the prevalence of fungi being more than bacteria in some groups.

Therefore, masks can act as a physical barrier and seem to be more effective when worn by the droplet emitting person. Masks have generally shown an effect in reducing microbial mission from infected patients.

5.2 Conclusion

This present study revealed that the high bacterial contamination on the outside area of the used face masks had significantly positive correlation with bacteria and fungi that could be found in air. From the findings it was observed that wearing of facemasks can help prevent the spread of pathogenic microbes but longer usage is advised because as more moisture are being built the more the face mask is at risk of being contaminated hence increases the chances of re-occurring infection in human.

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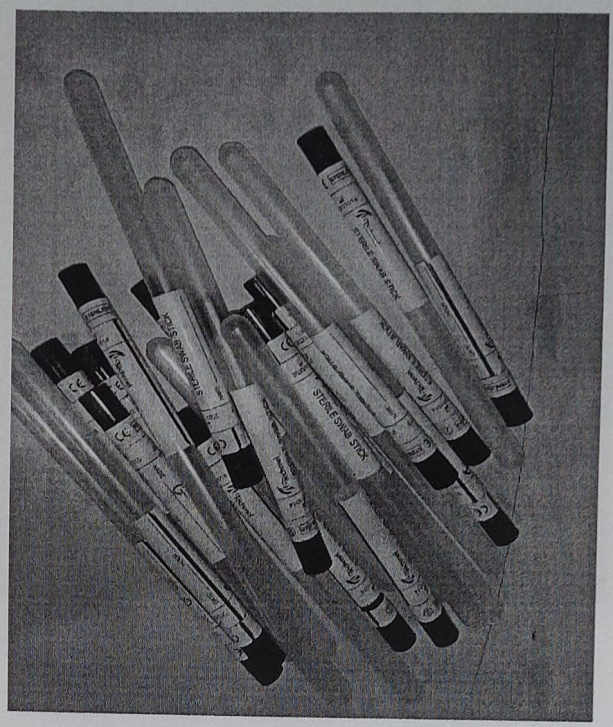
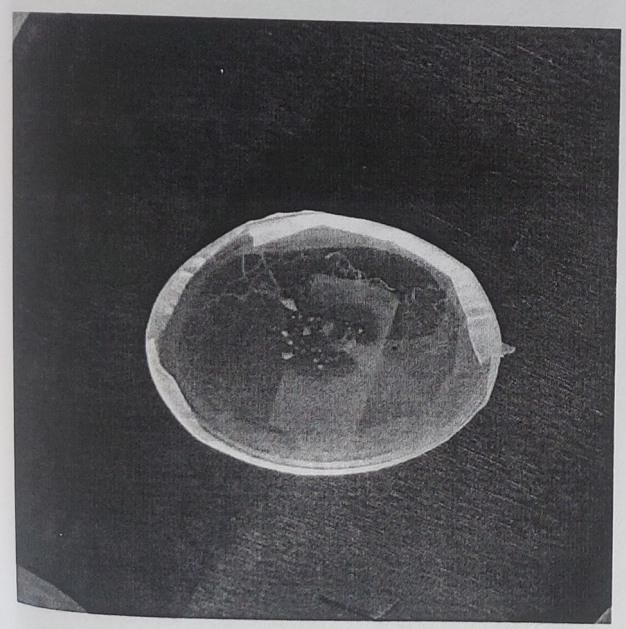
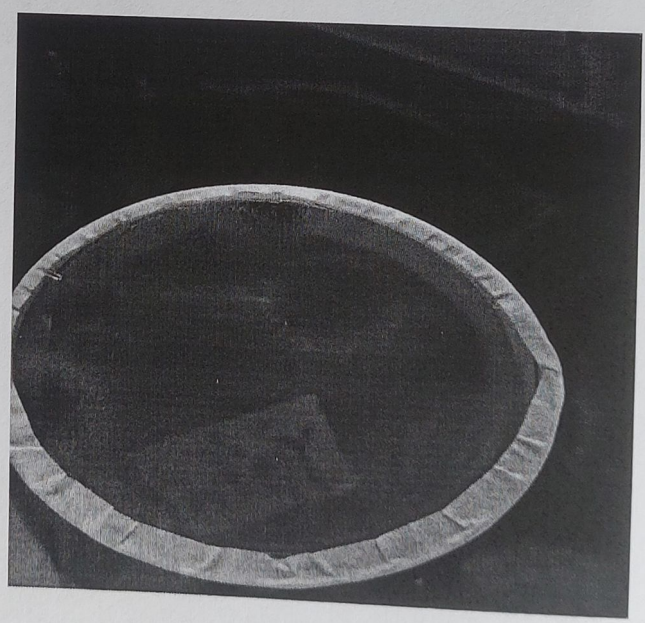
APPENDIX

Appendix I: image of face masks used by participants in this study collection of

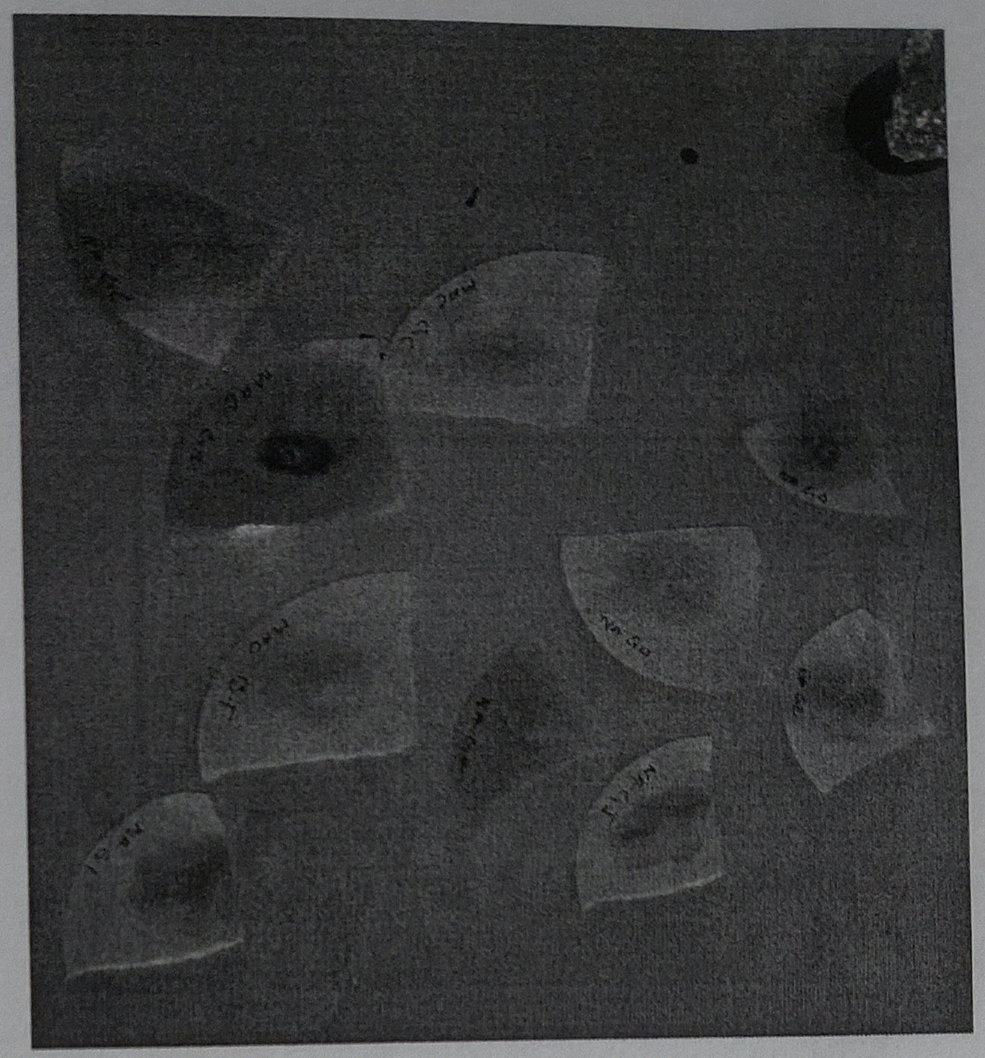


Appendix II: image of swab sticks used for sample

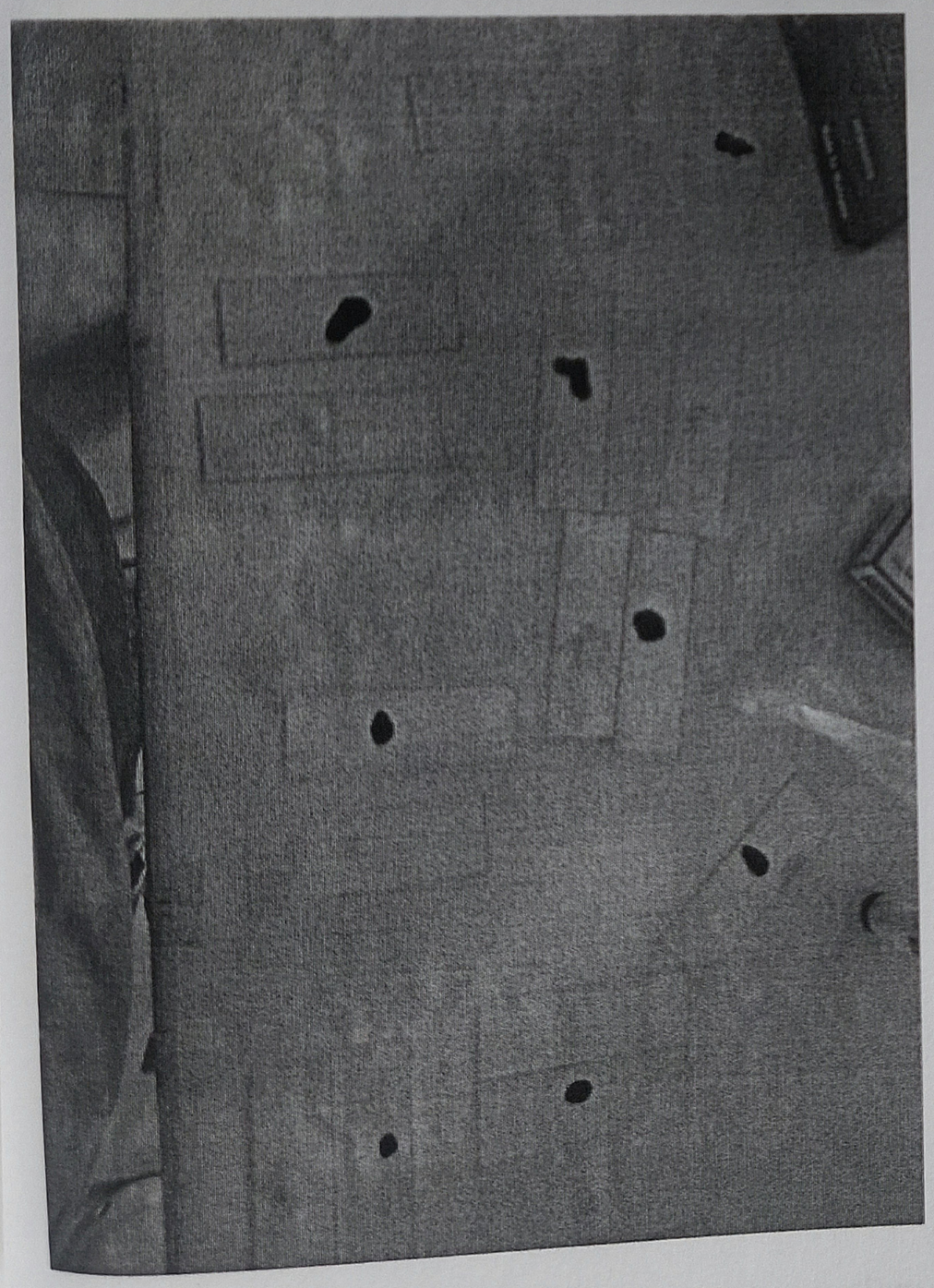




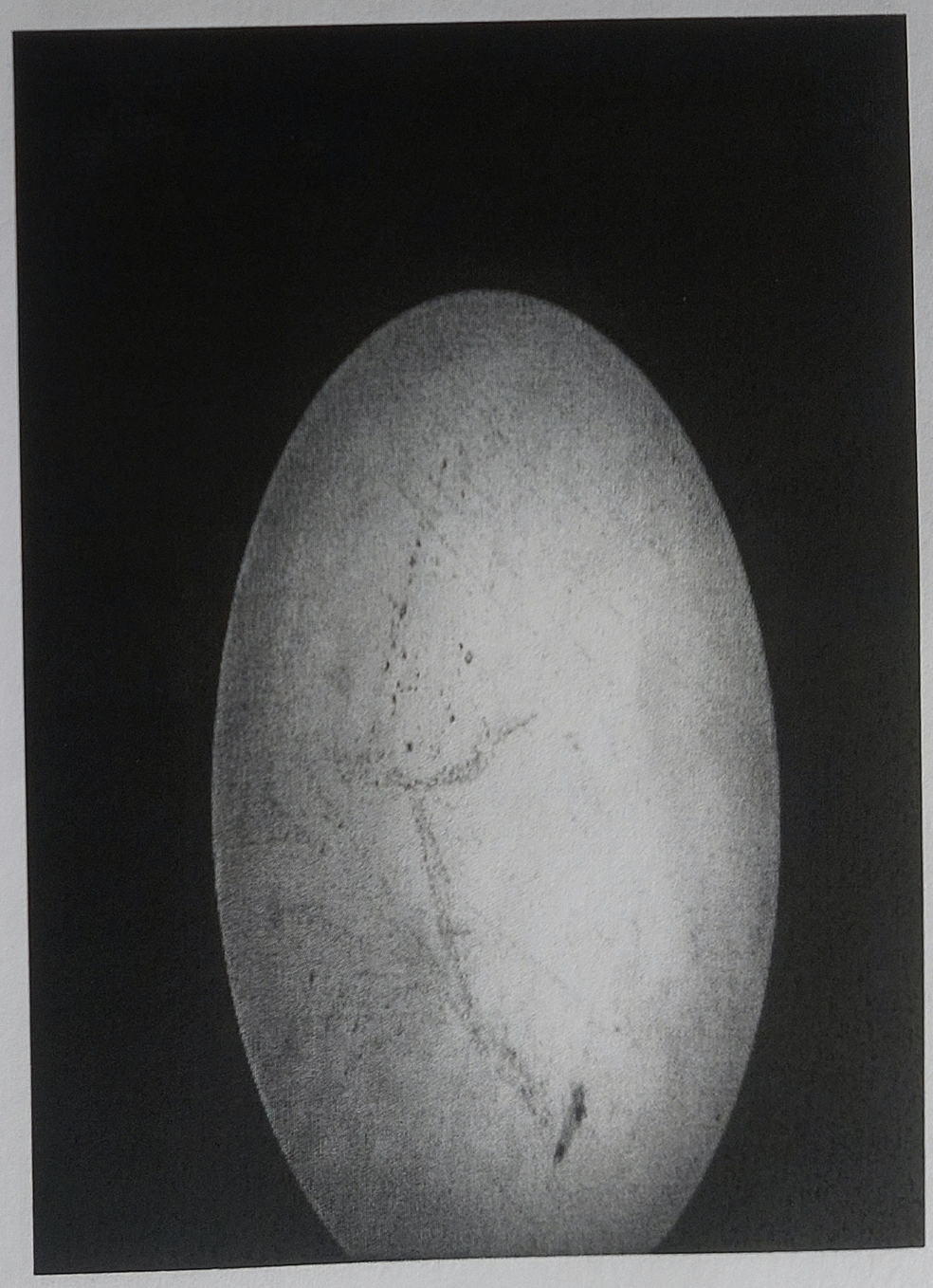
Appendix III: Image of aspergillus spp.



Appendix IV: image of penicillium spp

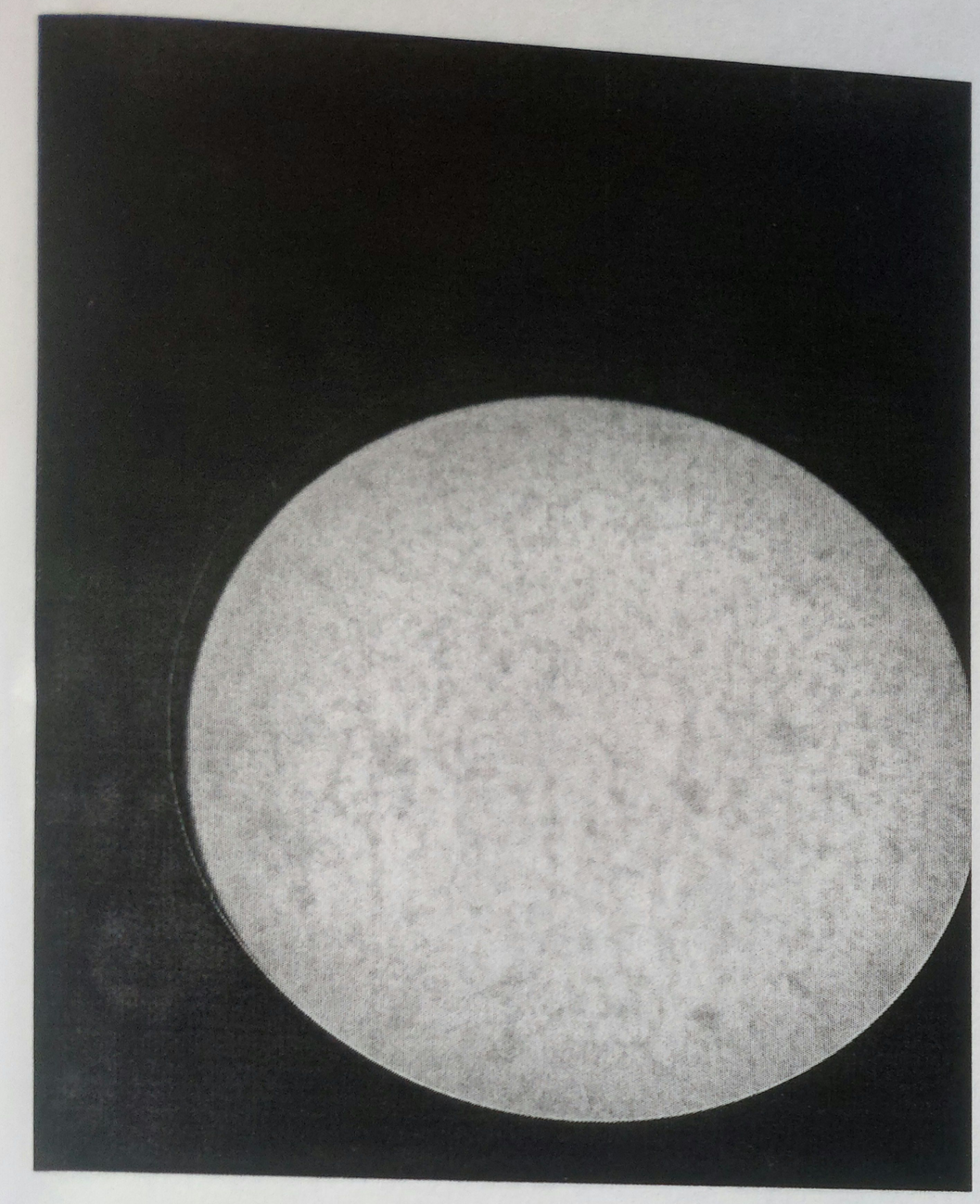


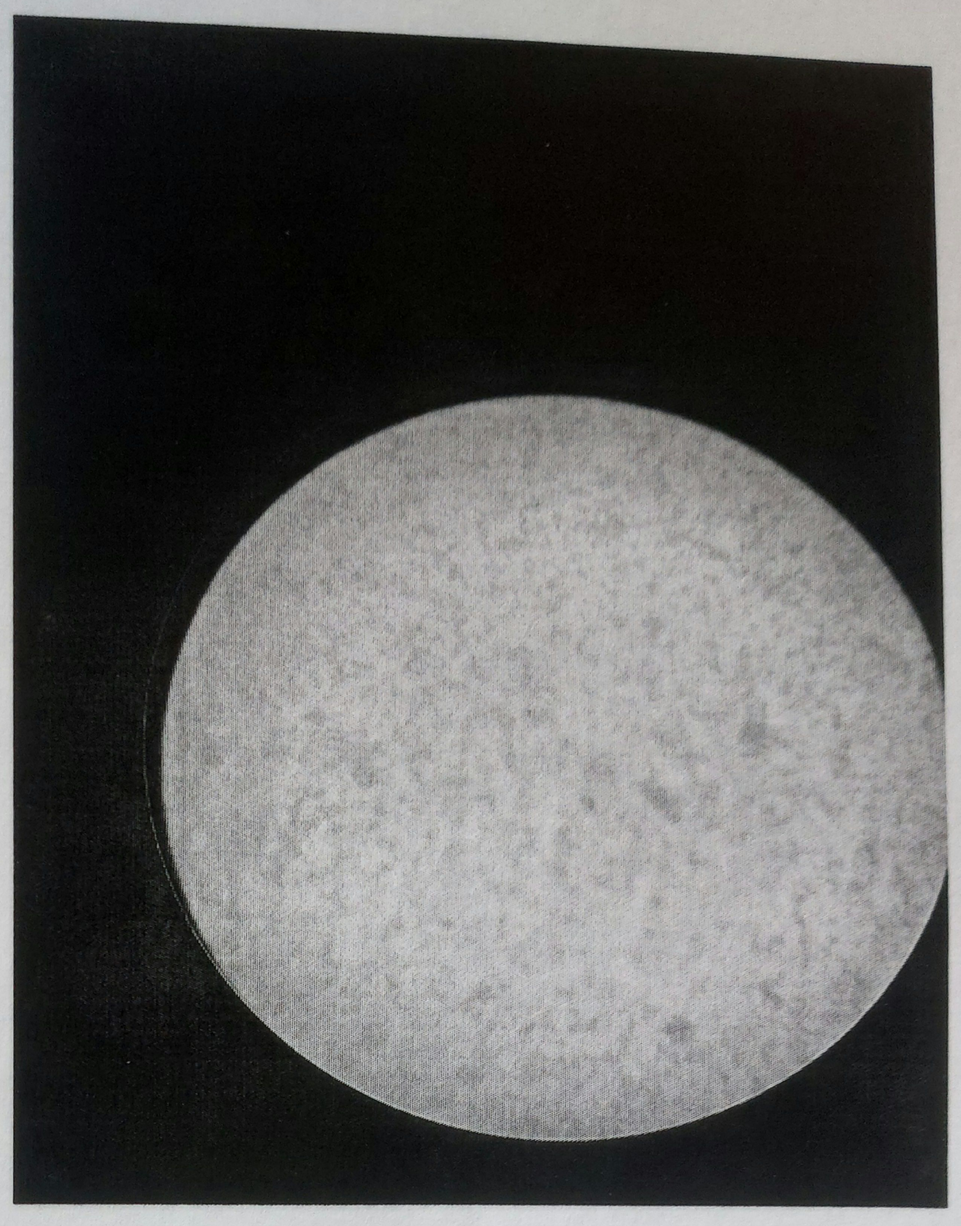
Appendix V: image of oxidase test done



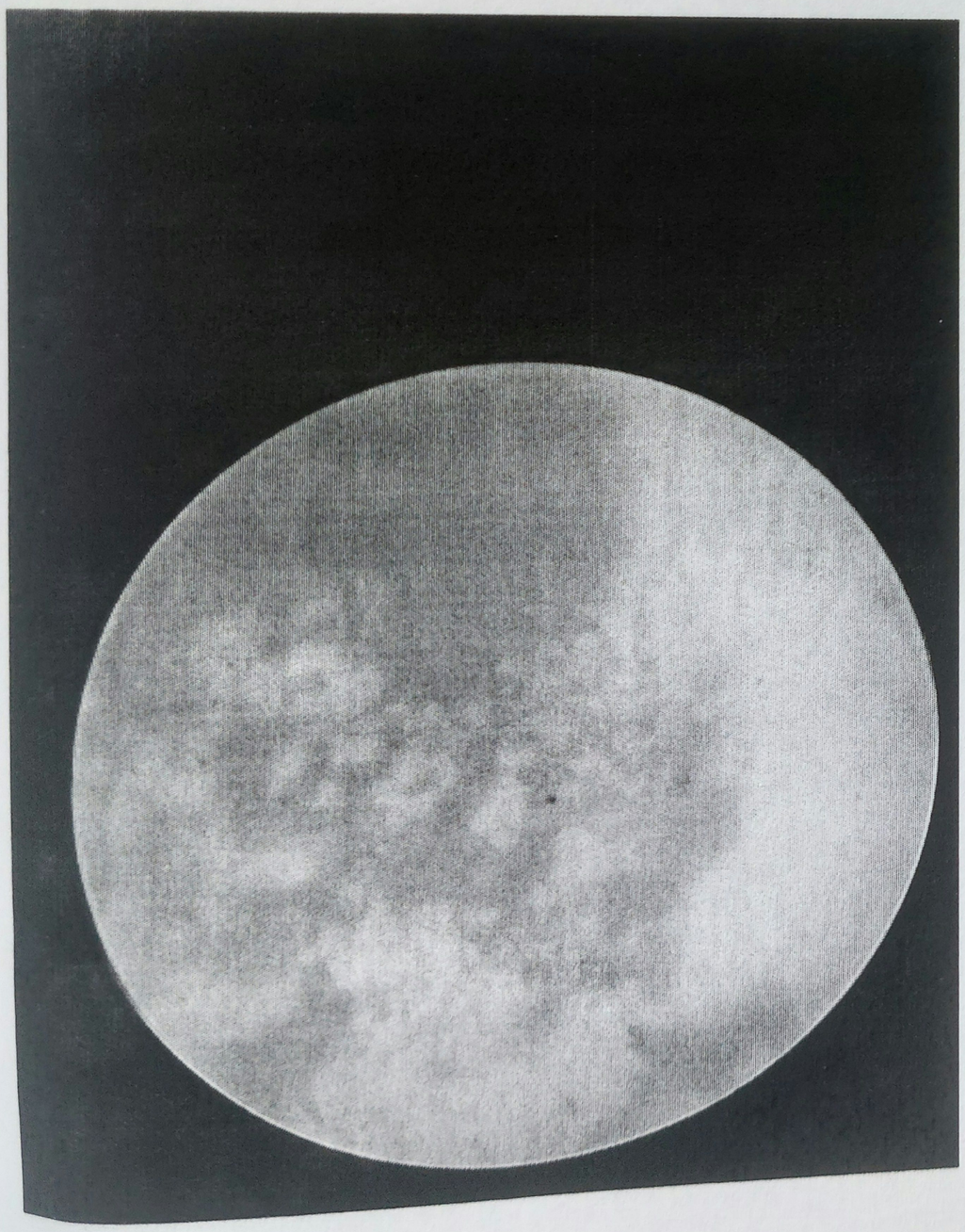
Appendix VI: image of coagulase testdone

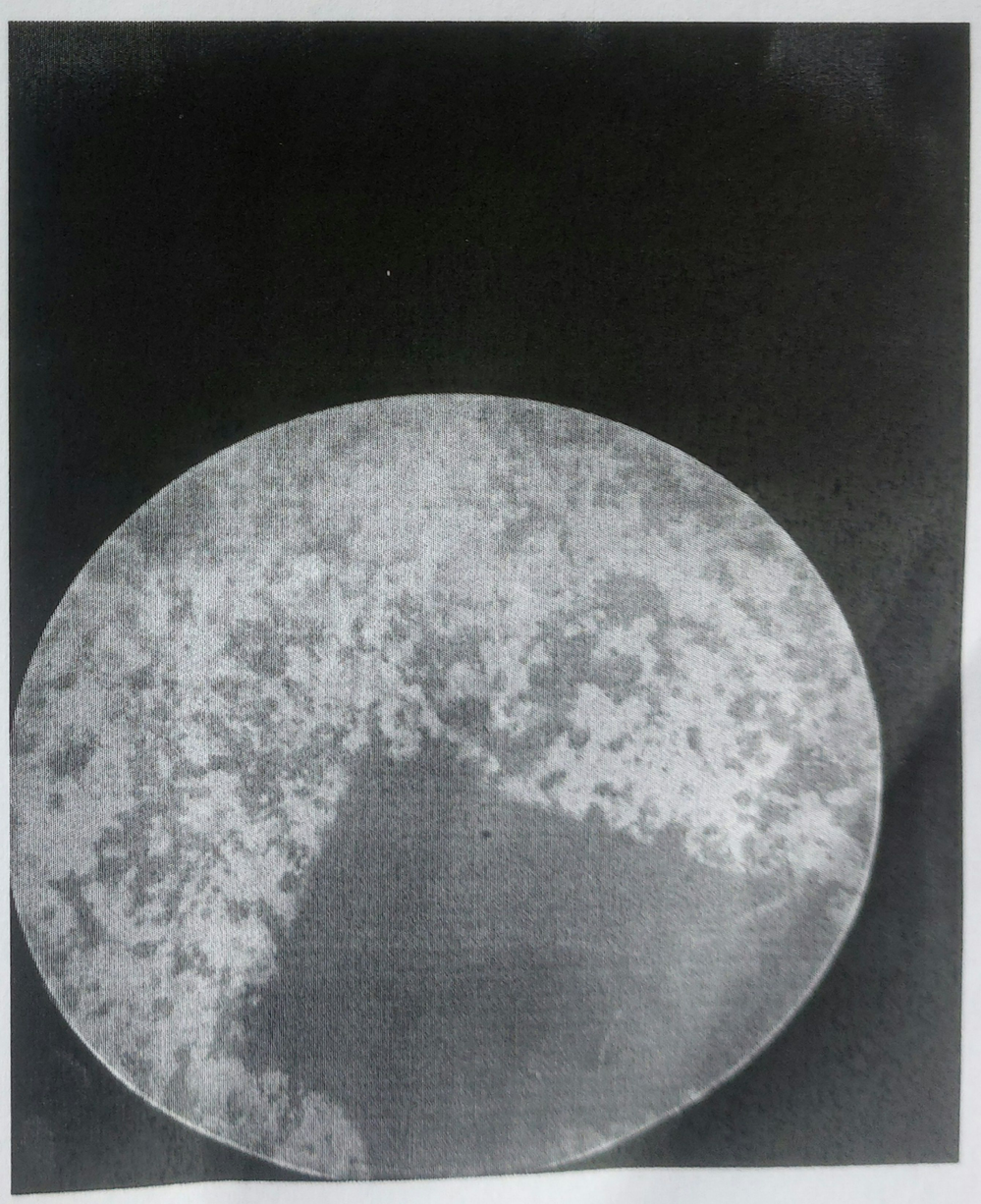
Appendix VII: Microscopic view of Penicillum spp





Appendix VIII: microscopic view Rod organism





Appendix IX; microscopic view cocci organisms