CHARACTERIZATION OF AIRBORNE BACTERIAL ISOLATE FROM NIGER DELTA DEVELOPMENT COMMISSION (NDDC), MALE HOSTEL OF UNIVERSITY OF BENIN, BENIN CITY, EDO STATE.

BY

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BENIN CITY

DECEMBER, 2019

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A PROJECT SUBMITTED TO THE DEPARTMENT OF MICROBIOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF SCIENCE DEGREE (B.Sc. HONS) IN MICROBIOLOGY.

DECEMBER, 2019

CERTIFICATION

This is to certify that the project work was carried out by **EPHRAIM OGHENEKEVWE EVUMOVWE** with matriculation number **LSC1507884** in the Department of Microbiology, Faculty of Life Sciences, University of Benin, Benin City under the supervisor of

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DR. (MRS.) I. B. IDEMUDIA **DATE**
(PROJECT SUPERVISOR)

APPROVAL

This project was carried out by under the supervision of **Dr. (Mrs.) R. O. Okojie** in partial fulfilment for the award of a Bachelor of Science (B.Sc.) degree in Microbiology

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PROF S. E. OMONIGHO **DATE**
(HEAD OF DEPARTMENT)

DEDICATION

This project is dedicated to God Almighty my creator, my strong pillar, my source of inspiration, wisdom, knowledge and understanding. He has been the source of my strength throughout this program and on His wings only have I soared. I also dedicate this work to my loving parents; Mr. and Mrs. Evumovwe who has encouraged me all the way and whose encouragement has made sure that I give it all it takes to finish that which I have started. God bless you.

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My profound gratitude goes to God almighty for his strength, wisdom, and guidance all through my period of study and for making this work a success.

I am also grateful to the Head of Department of Life Sciences **Prof. S. E. Omonigho**, I wish to acknowledge and appreciate my supervisor **Dr. (Mrs.) I. B. Idemudia** for her immense contribution, dedication and commitment to the success of this work. It is a pleasure being under you ma.

Nobody has been more important to me in the pursuit of this project than the members of my family. I would like to thank my parents, whose love and guidance are with me in whatever I pursue. They are the ultimate role models.

God Bless you all.

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ABSTRACT

Introduction: The study was conducted to enumerate, identify the total airborne bacterial isolates from rooms in Niger Delta Development Commission (NDDC) male hostel, University of Benin, Benin City, Edo State and determine the frequency of occurrence as well as antibiotic sensitivity profiles of these bacterial isolates.

Materials and Methods: Samples were collected in duplicates and a total of 36 airborne samples were collected from six rooms. Using sedimentation technique, open Petri-dishes containing nutrient agar fortified with nystatin to inhibit fungal growth and enhance bacterial proliferation was applied to enumerate and isolate airborne bacterial isolates. Standard cultural, morphological and biochemical procedures were used to characterize the isolates. Antibiotics susceptibility profiles were determined using standard disk diffusion methods.

Results: The total plate count for bacterial enumeration obtained from the sampling sites ranged from 8.1×10^{-3} to 1.0×10^{-3} and expressed in colony forming units (cfu). Result of cultural, biochemical and morphological test showed *Staphylococcus aureus* and *Bacillus subtilis* according to Bergey's manual of determinative bacteriology. The antibiotics resistant pattern of *Staphylococcus aureus* expressed resistance to Amoxicillin Clavulanate (AUG), Cefotaxime (CTX), Imipenem/Cilastatin (IMP), Ofloxacin (OFX), Nalidixic Acid (NA), Nitrofurantoin (NF), Nitrofurantoin (NF), Cefuroxime (CXM), Ceftriaxone Sulbactarm (CRO), Gentamycin (GN), Cefuroxime (CXM), Imipenem/Cilastatin (IMP), Levofloxacin (LBC), Cefexime (ZEM), Ampiclox (ACX) which was a higher number of antibiotics and sensitive on Ofloxacin (OFX), Azithromycin (AZN), Gentamycin (GN) and Erythromycin (ERY). *Bacillus subtilis* showed resistance to Amoxicillin Clavulanate (AUG), Cefotaxime (CTX), Imipenem/Cilastatin (IMP), Ofloxacin (OFX), Gentamycin (GN), Nalidixic Acid (NA), Nitrofurantoin (NF), Nitrofurantoin (NF), Cefuroxime (CXM), Ceftriaxone Sulbactarm

(CRO), Gentamycin (GN), Azithromycin (AZN), Ofloxacin (OFX), Cefuroxime (CXM), Imipenem/Cilastatin(IMP), Levofloxacin (LBC), Cefexime (ZEM), Ampiclox (ACX) and Erythromycin (ERY). Significantly, the various air sampling sites of the rooms indicated the presence of bacteria with majority showing multiple antibiotics resistance.

Conclusion: The assessment of indoor air quality is essential in determining bacterial air pollution. Bacterial isolates obtained can be used to estimate the health hazard posed and create standards for air quality for both the indoor environment. These bacterial isolates obtained from the sampling sites during this research calls for attention as they are of public health concern and suggesting risk of exposure of students to airborne bacterial contamination.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

Indoor environments are fundamental environmental factors capable of impacting health. Air quality of indoor environments is one of the main factors affecting health, wellbeing and productivity of people who inhale 14m^3 of the air every day (Brochu *et al*., 2006), and spend between 80-95% of their lives indoors (Dacarro *et al*., 2003).

The air inhaled by people in indoor environment is abundantly populated with microorganisms such as bacteria, fungi and moulds which form so called bioaerosols (Yassin *et al*., 2010). Bioaerosols are always present in our environment and pose no problem in most cases when the air quantities are kept within reasonable limits. A review made by WHO on the number of epidemiological studies showed that, there is sufficient evidence for an association between indoor dampness-related factors and a wide range of effects on respiratory health, including asthma development, asthma exacerbation, respiratory infections, cough, wheeze and dyspnea (WHO, 2010). Many common indoor air quality problems are associated with improperly operated and maintained heating, ventilating and air conditioning (HVAC) systems, overcrowding, moisture incursion and dampness, presence of outside air pollutants, and the presence of internally generated contaminants such as use of cleaning and disinfecting supplies and aerosol products, off-gassing from materials in the building, and use of mechanical equipment. People, organic dust, various materials stored in the buildings remains the major sources of biological contamination of indoor air (Amengialue *et al*., 2017)). Dust serve as a vehicle in transporting microorganisms from outside environment to the rooms by sweeping, walking and wind. Many different species may dominate the indoor environment depending on amount of viscosity, temperature, lighting, and food present in that environment (Dumala and Dudzinska, 2013).

Air, as a non-renewable resource, supplies us with the energy production requirement, oxygen which is essential for our bodies to live. Pollution of the air is the introduction of chemicals, particulate matter or biological materials into the atmosphere, capable of causing discomfort, disease or death to humans, damage to other living organisms including food crops. Since air is an important medium for the spread of infectious and allergic triggers which can result to undesirable effects on human beings, the control of the microbial charge became an important key to define the environmental quality of ambient media surrounding wide human populations which are largely exposed to indoor air during their daily activities (Soto *et al*., 2009). Indoor air quality (IAQ), as the name implies, is a term used to assess the quality of the air in indoor environment like offices and other building environments. In a normal indoor environment, the quantity of microorganisms should be significantly lower than outdoor levels. Possible sources of biological contamination of indoor air include: people, organic dust, various materials stored in the buildings, and the air inflowing from the ventilation and air conditioning systems (Kalwasińska*et al*., 2012). Depending on the amount of viscosity, temperature, lighting, and food available, different species may become dominant (Dumala and Dudzinska, 2013). The presence of bacteria and fungi in indoor air pose a serious problem from the point of view of health protection and environmental engineering. Dust, a good vehicle of airborne contamination may arise from human activities such as sweeping, movements, waving of handkerchief and bed making. Sneezing has been described as the most vigorous mechanism of generating millions of droplet into the environment with the larger falling to the ground and smaller ones evaporate and remain suspended as nuclei (Awosika*et al*., 2012). Although pathogenic species are rather scarce in the air, some relevant microorganisms travel by aerial transmission and are involved in serious processes causing pneumonia and other diseases. Aerial fungi are much more important than bacteria as agents for allergic diseases. Air sampling of microorganisms is a

popular method of conducting microbial examinations, as it allows a direct toxicological evaluation. The problem of environmental pollution is enormous and various attempts have been made to establish facilities for its control and regulation in various parts of the world. Results have shown from various studies that microbial characterization of different indoor air environments differ greatly in time, season, location and ventilation (Udochukwu*et al*., 2015).

Bioaerosols are airborne particles usually defined as particulate matter of plant, soil, animal or human origin, containing microorganisms or organic compounds (Thorne *et al*., 2003). The particles become airborne when suspended in air as a result of wind, turbulence, coughing, traffic etc. When in air, the particles may adhere to tiny droplets of water or dust material, creating bioaerosols that can reside in the air for longer periods of time, depending on the weather conditions and the sizes of the particles (Francoise, 2002). Particles below 5µm remain suspended in the air stream for long periods of time, and they are of primary concern because they penetrate deep into our lungs when respired, potentially causing infections (Webber *et al*., 2008).

Sneezing has been found to be one of the strongest way of producing millions of droplet into the environment with the maximum falling to the ground and smaller amount evaporate and remain suspended as nuclei (Awosika *et al*., 2012). Improper temperature and relative humidity conditions can also present problems, especially concerning comfort (Dumala and Dudzinska, 2013). Thus microbological air quality is a salient issue that must be considered when indoor workplaces are designed to provide a safe environment.

1.2 AIM AND OBJECTIVES

This study was aimed at assessingthe airborne bacterial isolates from Niger Delta Development Commission (NDDC) male hostel, University of Benin, Benin City, Edo State.

OBJECTIVES;

1. enumeratethe total airborne bacteria isolated from rooms in Niger Delta Development Commission (NDDC) male hostel, University of Benin, Benin City, Edo State.
2. isolate and identify the bacterial isolates.
3. determine the frequency of occurrence of bacterial isolates
4. determine the antibiotics sensitivity profile of the bacterial isolates.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Bioaerosols

The study of airborne microorganisms and their effect on human health and the environment is known as aerobiology. In recent years, research in this field has increased because of growing awareness of the variety of health problems potentially caused by airborne microorganisms. An aerosol is a suspension of microscopic solid and/or liquid particles in air or gas. Biological aerosols (bioaerosols) are single microorganisms or clumps of microorganisms attached to solid or liquid particles suspended in the air (Downes *et al*., 2001). Organisms present in bioaerosols can be bacteria, yeasts, molds, spores of bacteria and molds, microbial fragments, toxins, metabolites, viruses, parasites and pollen.

Bioaerosols generally range in size from 0.5 μm to 50μm in diameter (Kang *et al*., 1989). Microorganisms in bioaerosols may attach to dust particles or may survive as free floating particles surrounded by a coating of dried organic or inorganic material. They cannot multiply in bioaerosols due to a lack of nutrients, but these aerosols can travel in the air for great distances. Location and environmental conditions such as humidity, density and temperature have a great effect on the type of population and amount of microorganisms in the air. Some of the major sources of bioaerosols are humans (by sneezing, coughing and talking), animals, vegetation and dust particles (Al-Dagal and Fung, 1990).

Microorganisms can become aerosolized from environmental sources such as worker activity, water spraying, sink and floor drains, air conditioning systems, and different food processing systems (Downes *et al*., 2001).

Aerosols display intricate aerodynamic behavior resulting from various combinations of physical factors such as Brownian motion, electric gradient, gravitational field, inertia force,

electromagnetic radiation, particle density, temperature gradient and humidity. The behavior of bioaerosols is dominated by physical and biological factors.

Physical factors affect where and how many bioaerosol particles will reach a specific surface. The most important biological factor is the ability of the bioaerosol particle to withstand lethal or sublethal stress or damage as it is dispersed in the air. Some of these stresses are created during aerosol generation, dispersion and landing or collection. These stresses are usually sublethal, but when joined with other environmental stressors like temperature, dehydration, irradiation, oxidation and pollution the effect is often lethal (Kang *et al*., 1989).

Bioaerosol particle size is one of the main factors affecting aerodynamic behavior. Vegetative bacterial cells usually will not survive long in air unless they have a protective medium surrounding them or unless relative humidity and temperature are favorable. Vegetative bacteria are usually present in the air in lower numbers than bacterial and mold spores. Bioaerosols generated from the environment are usually bacterial spores, yeasts and molds, but during food processing the main sources of contamination are vegetative bacteria like *Staphylococcus spp*. and *Micrococcus* spp. (Downes *et al*., 2001). *Escherichia coli* exhibits rapid death at low relative humidity (<50%) and temperatures between 15^0C and 30^0C (Al-Dagal and Fung, 1990) Aerosolization is stressful enough for vegetative bacteria so it is important to reduce additional stress caused by collection procedures and growth media used during air sampling. When bioaerosols have been subjected to mechanical or physical damage their recovery on selective media is reduced. Bioaerosol research includes generation, collection, storage and analysis of aerosols. In addition to cell injury some other factors that will influence bioaerosol collection are strain of the microorganism, growth conditions, aerosol generation, aerosol particle size and collection method (Kang *et al*., 1989).

Bioaerosols can be found in all outdoor and indoor environments, and they often contain bacteria, virus or fungi, which may be pathogenic or non-pathogenic, viable or dead (Thorne

et al. 2003). The viability of the airborne microorganisms is dependent on measureable factors like relative humidity, solar irradiance and temperature, in addition to special properties of the bacteria themselves, like endospore-forming capability and pigment content (Gilbert and Duchaine, 2009).

The interest in bioaerosol exposure has increased over the last few decades, both due to the emerging understanding of its association with a wide range of adverse health effects, and due to the fear of bioterrorism. In hospitals, it could be important to quality test the air because it is a serious and widespread problem that patients acquire infections through the airborne route during hospital stay (Greatorex *et al*., 2010). For safety reasons, monitoring the air at public places could help minimizing the proportions of potential bioaerosol attacks. An example of a bioterrorism event propagated by bioaerosols was the anthrax attacks in the United States in 2001 (Centers for Disease Control and Prevention, 2001). However, spread of anthrax is self-limiting, as it is not likely to infect other people than those directly exposed. A scenario even more dangerous than a new anthrax attack would be the spread of a contagious agent at a crowded public place, like a subway station, where every infected person will transmit the disease to others after leaving the station (Henderson *et al*., 1999). Biological agents that are easy to spread and capable of infecting human, causing incapacitation or death, can be considered biological threat agents (Centers for Disease Control and Prevention, 2007).

In order to minimize the consequences of a bioterrorism attack, early detection of the dispersed threat agent is necessary. Continual monitoring of the airborne environment for detection of specific agents is possible, but false positive results are likely to occur due to low background levels of naturally-occurring threat agents (Wyatt, 2009). Airborne fungi are of health concerns, as they are associated with allergy and respiratory diseases (Hope and Simon, 2007). However, biological threat agents dispersed in a bioterrorism attack are more likely to

be bacteria, toxins or virus, according to the list of critical biological agents obtained from Centers for Disease Control and Prevention (Khan *et al*., 2002).

Relatively many bioaerosol characterization studies have been conducted in hospitals, schools, farm buildings and other industry buildings, but few studies have been conducted in subway stations or train stations (Abdel Hameed and Awad, 2002). A study performed in Beijing in 2010 investigated bacteria concentrations in different airborne environments, and found that the cultivable bacteria concentrations were significantly higher in train and metro stations than in hospitals, offices and in outdoor city centre (Dong and Yao, 2010). However, among different studies performed in stations, the obtained results are not directly comparable due to use of different air sampling devices and analysis methods (Sudharsanam *et al*., 2008).

2.2 Bioaerosol sampling

Sampling techniques

Three basic sampling methods exist for collection of airborne microorganisms: filtration, impingement and impaction. Most air sampling devices in use rely on techniques that force surrounding air into the device, where airborne particles are departed from the air stream. These are active air samplers, and the airborne particles can be deposited onto a solid medium (impaction), into a liquid (impingement) or onto a filter (filtration) (Sullivan and Krieger, 2001). Methods for collection can also be passive. An example is use of settling plates, where particles deposit due to gravity. However, gravitational collection is not appropriate for quantitative analyses of airborne microorganisms, as sampling efficiency is highly dependent on motion in the surrounding environment (Pasquarella, 2000).

2.2.1 Impaction

When considering active air sampling, impaction is a sampling method that separates airborne particles from the airflow by leading the airflow into the device, where the particles deviate from the air flow and impacts on a medium. Petri dishes with culture medium are often used as they can be incubated directly for microbiological growth studies after sampling (Horrocks *et al.,* 2009). This makes impaction appropriate for culture-dependent studies, but insufficient for culturing independent studies (Gilbert and Duchaine, 2009). However, there is a high risk of overloading the plates with growing cultures, introducing error when estimating the microorganism concentrations. Therefore, impaction sampling is more suitable for less contaminated bioaerosol environments, or requires shorter sampling time (Horrocks *et al.,* 2009).

2.2.2 Impingement

The principle of impingement is similar to that of impaction, but here the particles impact a liquid when the air flow abruptly changes its direction, and not a solid medium. The impingement method may induce less physical stress to the collected microorganisms than impaction, making impingement suitable for microbiological studies of viable microorganisms. Impingers also have the advantage of being able to sample for long time intervals, and the collection in liquid makes multiple analyses possible per sample, including both culturing dependent studies and culture-independent studies by use of molecular biological techniques (Horrocks *et al*., 2009).

Impingers have been used for collection of airborne bacteria, fungi and virus (Sullivan and Krieger, 2001). However, similar to the impaction method, impingement sampling is most efficient at capturing large particles (>10 μm). Smaller particles (< 10 μm) might also be captured in the liquid, but are likely to re-aerosolize as a consequence of liquid medium evaporation (Moineau *et al*., 2008).

2.2.3 Filtration

Filtration relies on collecting airborne particles by passing air through a porous medium. Depending on the sizes and charges of the particles, in addition to the streamline of the airflow though the filter, the particles will divide from the airflow and impact on the filter surface. This is a relatively simple and effective method for collection of airborne microorganisms (Moineau *et al.,* 2008; Horrocks *et al.,* 2009). An advantage of filtration over impaction and impingement is that filters can be designed for efficient collection of particles with aerodynamic sizes less than 0.5 µm (Moineau *et al.,* 2008).

Collection of airborne particles on a filter usually requires extraction of the particles into liquid prior to analysis, although gelatin filters can be placed onto agar plates for direct growth studies. An advantage of the samples extracted in liquid is that they can be tested by multiple assays, and both microbiological culturing analyses and molecular biological analyses are possible (Gilbert and Duchaine, 2009). Filter samplers have potential for collection of excessive amounts of airborne microorganisms by long sampling periods or use of high flow rate. However, a drawback of the method is desiccation of microorganisms, resulting in loss of viability. The loss of viability is affected by the sampling time and humidity, appreciating that meteorological conditions should be measured while collecting filter air samples (Horrocks *et al*., 2009).

Different types of filter samplers differ mainly in composition and pore size of their filters. The filter material affects collection efficiency, but the yields obtained are also affected by the extraction efficiency of the filter in use (Hameed and Awad, 2002).

2.3 The choice of sampling equipment

Airborne particle harvesting for analysis purposes have been performed with a wide variety of sampling devices, and new instruments are continually being developed. Choice of sampling device is highly dependent on the aim of the study. For microbiological analyses of

viable airborne microorganisms, sampling devices that maintain the viability of the microorganisms throughout the sampling process are required. On the other hand, high-flow sampling devices can be more efficient for molecular biological studies, where viability is not a requirement (Gilbert and Duchaine, 2009).

Other criteria that should be considered when choosing sampling device are ease of operation and transportation, cost, sampler reliability and optimum particle size range of the device (Horrocks *et al.,* 2009). For analysis of bioaerosols potentially causing respiratory diseases, it is important to choose a sampling device approved for collection of particles below 10µm (Webber *et al.,* 2008). In general, the same kinds of sampling devices have been approved for analysis of airborne fungi and bacteria, whereas modifications have been necessary in order to detect viruses in the air samples (Gilbert and Duchaine, 2009). However, there has been a lack of standard protocols for treatment of air samples, making result comparison between different studies difficult (Sudharsanam *et al.,* 2008).

2.4 Microbiological techniques

Traditional microbiological techniques rely on culturing as a means to enumerate and characterize microorganisms from samples (Gilbert and Duchaine,2009). Some great advantages of microbiological culture studies, compared to culture independent studies, are the possibility for testing the viable bacteria found for pathogenic potential, metabolic requirements and endospore-forming capability.

2.5 Culture-dependent quantification

Culture-dependent methods are relatively easy and cheap to perform, and they are applicable to quantification of bacteria, fungi and viruses. For culturing of bacteria and fungi, use of semi-solid growth media is most common. The microorganisms may be collected onto the growth medium directly from air when using impaction-based sampling methods. When sampling is performed by impingement systems, the liquid sample must be spread onto the

growth medium prior to culturing. For filter samples, the filter must be extracted in liquid buffer and spread onto the growth medium prior to culturing (Schafer and Jensen, 1998).

Growth media appropriate for growth of the microorganisms of interest need to be selected. TSA is often used for enumeration of bacteria, while malt extract agar is commonly used for culturing of fungi (Schafer and Jensen, 1998). However, no single growth medium is suitable for all sorts of bacteria or all sorts of fungi, meaning that only the microorganisms able to grow and multiply on the chosen growth media are found and quantified. These cultivable microorganisms are estimated to represent about 1 percent of the total amount of viable and nonviable microorganisms in the sample (Ludwig *et al*., 1995).

Culturing of viruses is performed on cellular growth cultures, consisting of bacteria culture, animal tissue or human tissue. Viruses need to infect living cells in order to multiply, which can be observed as clear spots in the culture. These clear spots are called plaques and each plaque represents one initial viral particle. Counting the plaques gives a measure of the number of initial viruses in the sample poured onto the growth culture, given as plaque forming units. Bacteria and fungi form colonies when incubated on a growth medium, and each colony represents one single initial microorganism poured onto the medium. Counting the colonies gives a measure of the initial number of microorganisms in the sample, given as colony forming units. It is common to select for only bacteria or fungi, because the growth of fungi is likely to mask many bacterial colonies present. Growth inhibitors for fungi, like cyclohexamide, can be added to the growth media when bacteria counts are in focus (Horrocks *et al*., 2009).

Problems related to viable counts are for example quantification error that occurs if large numbers of colonies (above 300) are grown on one culture plate. The colonies are then likely to merge, and one colony might represent several initial microorganisms. If the microorganisms were sampled directly on the culture plate by impaction methods, the

sampling time must be reduced in order to reduce the number of colonies. Serial dilutions are needed if impingement or filter sampling resulted in too many colonies. Dilutions do however induce some error and should be avoided if possible (Horrocks *et al.,* 2009).

2.6 Traditional taxonomical classification

Characterization of microorganisms by culture-dependent methods is the traditional approach for classification. The science of classification is called taxonomy, where the objective is to classify living organisms based on similarities and differences between the organisms (Schafer and Jensen,1998). The classification can permit species-level identification when using appropriate numbers of characteristics tests per microorganism.

Culture-dependent methods for characterization include incubation of microorganisms for studying their growth appearance. Formation of endospores is a characteristic property of some bacteria and fungi, and can be tested for by heat shocking the microorganisms prior to culturing. Bacteria can be classified more closely based on the results from biochemical, physiological and nutritional tests, which evaluate characteristics like temperature optimum, pH tolerance, modes of metabolism (Schafer and Jensen, 1998). Culture-dependent methods for characterization can also include use of differential media and selective media. Differential media contain indicators that permit the recognition of microorganisms with particular metabolic activities. Growth on blood agar is an example, where growth of bacteria that degrade hemoglobin are detected (Coffin *et al*., 1994). Selective media contain compounds that inhibit the growth of particular microorganisms. Growth media with antibiotics are examples of selective media, were only bacteria with antibiotic resistance are able to grow (Martinko, 2006). Hemolytic activity and antibiotic resistance are of health concern, and can be tested for in order to investigate for pathogenic potential in an environment.

For viruses, the need for specific animal or human tissue for reproduction has made large-spectrum characterization of viruses from air samples difficult. For bacteria and fungi, the species not able to grow on the medium provided or under the particular incubation conditions in use, are excluded from the study (Noakes *et al.,*2011). The microbiological techniques give information about types and quantities of cultivable microorganisms in samples, but no information is to be obtained about the fraction of non-cultivable microorganisms.

Airborne contaminants are also known as bioaerosols and include bacteria, fungi, viruses and pollen. These may be present in the air as solid (dust) or as liquid (condensation and water). An aerosol is a two-phase system of gaseous phase (air) and particulate matter (dust, pathogens), thus making an important bacterial vehicle. Pathogenic bacteria attach to dust particles and condensation, and travel around the processing facility. This contaminated air comes in contact with food products, containers, equipment and other food contact surfaces during processing. According to the Food and Drug Administration (FDA), the food industry must reduce product contamination by reducing airborne microorganisms (Heldman, 1974).

Airborne contaminants are the causative agents of human illness due to ingestion of contaminated foods and also reduce product shelf life resulting in an economic loss. A typical analysis was on swabbing of equipment is to determine the sanitation level of food processing plants. This method does not always provide an effective enumeration of airborne contaminants. Air sampling is more effective because it collects aerosols settled on equipment and food contact surfaces. Through air sampling, food processing facilities can identify airborne contamination due to air contact with food products (Heldman, 1974). Preferably, an air sampler would be able to collect all of the viable microorganisms per unit volume of air, but this is not possible because not all airborne cells can be physically separated from the air without killing them during sampling (Kang *et al*., 1989). Methods for

the detection of viable airborne microorganisms include sedimentation, impaction on solid surfaces, impingement in liquids, filtration, centrifugation, electrostatic precipitation and thermal precipitation. Impaction methods are usually used because they obtain higher recovery rates than other air sampling methods and can be used in situations where bioaerosol levels might be low (Downes *et al*., 2001).

It is incredible to keep airborne bacteria, yeast and mold in food processing areas at a zero level. Some of the major sources of contamination in food processing facilities are wastewater, rinse water and spilled product that become aerosolized. Airborne bacteria, yeast and mold are generated in processing facilities by heating, ventilation and air conditioning systems (HVAC). These systems contribute airborne microorganisms under normal operation because they provide fertile areas for growth due to moisture. Worker activity, equipment operation, sink and floor drains, and high pressure spraying are also major sources of bioaerosols (Heldman, 1974).

Worker activity, talking, sneezing and coughing create dust particles and air disturbances creating airborne microorganisms. The workers' contribution of airborne bacteria depends on their health, condition of clothing, hygiene and location in processing facility. Equipment operation contributes to variations in microorganism levels. Airborne bacteria increase with the use of conveyor systems which cause bacterial aerosols that adhere to conveyor surfaces (Ren *et al*., 1992).

The direction of airflow is important in the control of bioaerosol contamination and it should always be counter current to that of the product flow. Barriers like walls and doors are used to separate clean and unclean areas (Rahkio *et al*., 1997). Sink and floor drains can harbor microorganisms because they are humid and contain nutrients from wastewater which provide a fertile growth environment. Flooding of drains causes microorganisms on the surface to become aerosolized and air disperses them, causing increased levels of aerosolized

bacteria in the food processing facility (Ren *et al*., 1992). High pressure spraying also causes an increased level of aerosolized bacteria after spraying. The extent of this increase depends on the condition of floor, water pressure and the amount of water used.

High temperature and humidity in the processing room increase microbial growth, but if the environment is controlled, bacterial growth can be minimized (Ren *et al*., 1992).

Intense husbandry practices and long term residence of cattle in feedlots and pens provide great opportunity for microorganisms to affix to hoofs and hides. Research regarding airborne contamination levels in meat processing facilities indicates airborne microbes are a potential source of microbiological contamination in various meat products. Higher concentrations of airborne bacteria exist in the back-splitting area than in the weighing section in pork slaughterhouses (Rahkio *et al*., 1997). The skin or hide of slaughtered animals can be a source of airborne bacteria in slaughterhouses. Many processes during cattle slaughtering are associated with the creation of bioaerosols. The attachment of specific pathogenic and nonpathogenic bacteria to carcass surfaces after hide removal is usually immediate (Jericho *et al*., 2000).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Sampling location

This study was carried in the University of Benin, from July 2019-September 2019. The air quality assessment was done at the Niger Delta DevelopmentCommission Male Hostel, University of Benin. The sample location was situated at Latitude of 6^{o} 20' 1.32 N and Longitude of 5^{o} 36' 0.53" in Ugbowo, Benin City, Edo State, Nigeria (Saria *et al*., 2013).

3.2 Samples collection: This study was done at Niger Delta Development Commission Male Hostel and the sampling technique for assessingthe air borne microbial contamination was a sedimentation technique using open Petri-dishes containingNutrient agar media fortified with an antifungal agent (Nystatin) to inhibit the growth or proliferation of fungi. This media was exposed at room air for 1-2 h then closing the Petri-dishes. Immediately after collection of samples, the Petri-dishes were taken to the laboratory of Microbiology, Faculty of Life Sciences, University of Benin, for further bacteriological analysis.

3.3 Identification of bacteria: The identification of bacteria was based on morphological characteristics and biochemical tests carried out on the isolates. Morphological characteristics observed for each bacteria colony after 24 h of growth included colony appearance; shape, elevation, edge, optical characteristics, consistency, colony surface and pigmentation. Biochemical characterizations were done according to the method of Fawole and Oso (2004). Some of the key tests for identification include the following:

3.3.1 Triple Sugar Iron Agar (TSI):

Triple Sugar Iron Agar has three sugar (Lactose, Sucrose, and Glucose) and also iron; and it contains Agar as solidifying agent (TSI is a semi-solid media having slant and butt).

Beef extract	3.0 g
Yeast extract	3.0 g
Peptone	20.0 g
Glucose	1.0 g
Lactose	10.0 g
Sucrose	10.0 g
Ferrous sulfate or ferrous ammonium sulfate	0.2 g
NaCl	5.0 g
Sodium thiosulfate	0.3 g
Phenol red	0.024 g
Agar	13.0 g
Distilled water	1,000 mL

Composition of TSI agar Medium

- **0.1% glucose**: If only glucose is fermented, only enough acid is produced to turn the butt yellow. The slant will remain red
- **1.0 % lactose/1.0% sucrose:** If lactose or sucrose or both sugar are fermented, a large amount of acid will produce which turns both butt and slant yellow. So the appearance

of yellow color in both slant and butt indicates that the isolate has the ability to ferment lactose or sucrose or both.

- **Iron** (ferrous sulfate): Indicator of H_2S formation
- **Phenol red:** Indicator of acidification (It is yellow in acidic condition and red under alkaline conditions).
- It also contains peptone which acts as a source of nitrogen. (Remember that whenever peptone is utilized under aerobic condition ammonia is produced).

Preparation of TSI agar medium

- Combine the ingredients, and adjust the pH to 7.3
- Boil to dissolve the agar and dispense into tubes.
- Sterilize by autoclaving at 121°C for 15 minutes
- Cool in a slanted position to give a 2.5 cm butt and a 3.8 cm slant.

Procedure for Triple Sugar Iron Agar (TSI) Test

1. With a sterilized straight inoculation needle, well-isolated colony touch was used obtained.
2. Inoculatedinto the TSI agar by first stabbing through the center of the medium to the bottom of the tube and then was streaked on the surface of the agar slant.
3. Cap was loosely closed and incubate the tube at 35°C in ambient air for 18 to 24 hours.

Interpretation of Triple Sugar Iron Agar Test

1. Lactose (or sucrose) fermentationindicated a large amount of acid production, which turned the phenol red indicator yellow both in butt and in the slant. Some organisms generated gases, which produced bubbles/cracks on the medium.
2. Lactose not fermented but the small amount of glucose was the oxygen-deficient buttturned yellow, but on the slant the acid produced was then oxidized to

carbon dioxide and water by the organism and the slant was red (alkaline or neutral pH).

3. Neither lactose/sucrose nor glucose was fermented, both the butt and the slant turned red. The slant became a deeper red-purple (more alkaline) as a result of production of ammonia from the oxidative deamination of amino acids.
4. H_2S was produced, the black color of ferrous sulfide was seen.
5. Alkaline slant/no change in butt (K/NC) i.e Red/Red = glucose, lactose and sucrose non-fermenter.
6. Alkaline slant/Alkaline butt (K/K) i.e Red/Red = glucose, lactose and sucrose non-fermenter
7. Alkaline slant/acidic butt (K/A); Red/Yellow = glucose fermentation only, gas (+ or -), H_2S (+ or -).
8. Acidic slant/acidic butt (A/A); Yellow/Yellow = glucose, lactose and/or sucrose fermenter gas (+ or -), H_2S (+ or -).

3.3.2 Motility Test

A sterile needle was used to pick a loop of a 24 h old culture and was stabbed onto nutrient agar in glass vials. The vials were incubated at 37°C for 24-48 h. Non-motile bacteria had growth confined to the stab line with definite margins without spreading to surroundings area while motile bacteria gave diffused growth extending from the surface (Olutiola *et al*., 2000).

3.3.3 Gram Staining

Gram's staining was done to find the reactions of the bacterial isolates to Gram reagents. A smear was prepared and heat fixed. The crystal violet (primary stain) stain was flooded over the fixed culture for 60 seconds, the stain was washed with water. The iodine solution was added onto the smear for 60 seconds, pour off and

rinsed with water. A few drops of decolorizer (ethyl alcohol) was added and washed with water immediately after 5 seconds and finally safaranin (Secondary stain) was added for 60 seconds and washed, the smear was allowed to air dry. After drying the slide was mounted under microscope and observed. The stain differentiates bacterial species into two groups; Grampositive bacteria, which take up crystal violet dye (primary stain) and are stained violet and Gram-negative, which pick up safranin (Secondary stain) are thus stained red after decolourization with alcohol.

3.3.4 Catalase Test

A small quantity of 24 h old culture was transferred into a drop of 3% Hydrogen peroxide solution on a clean slide with the aid of sterile inoculating loop. Gas seen as white froth indicates the presence of catalase enzyme (Cheesbrough, 2006).

3.3.5 Coagulase Test

Coagulase is an enzyme capable of coagulating certain blood plasma, notably human and rabbit plasma. This test differentiates pathogenic from non-pathogenic *Staphylococcus* sp., the test was carried out using 18-24 h old culture. A loopful of isolated bacterium was emulsified with normal saline solution on a microscope slide. A drop of undiluted plasma was added to the suspension and stirred for five seconds. A coagulase-positive result was indicated by clumping of colonies together (Olutiola *et al*., 2000).

3.3.6 Indole Test

Tryptone broth (5 mL) was placed into different test tubes after which a loopful of the bacterial isolates was inoculated into the test tubes, leaving one of the test tubes uninoculated to serve as control. The test tubes were then incubated at 37°C for 48 h. After incubation, 0.5 mL of Kovac's reagent was added and shaken gently; it was allowed to stand for 20 min to permit the reagent to rise. A red or red-violet colour at

the top surface of the tube indicates a positive result while yellow colouration indicates a negative result (Cheesbrough, 2006).

3.3.7 Citrate Test

This test detects the ability of an organism to use citrate as a sole source of carbon and energy. About 2.4 g of citrate agar was dissolve in 100 mL of distilled water. About ten milliliter (10 mL) of citrate medium was dispensed into each tubes and covered, then sterilized and allowed to cool in a slanted position. The tubes were inoculated by streaking the organisms once across the surface. A change from green to blue indicates utilization of the citrate.

3.3.8 Oxidase Test

A piece of filter paper was soaked with few drops of oxidase reagent. Sterile inoculating loop was used to pick a colony of the test organism and smeared on the filter paper. If the organism is oxidase producing, the phenylenediamine in the reagent will be oxidized to a deep purple colour (Cheesbrough, 2006).

3.4 Antibiotics Susceptibility Testing

Laboratory standards were followed to carry out the Antimicrobial susceptibility testing using disk diffusion method. Commonly used antibiotics were selected, based on their general known effectiveness against bacterial infections. The discs used for screening Gram positive and negative bacteria contained the following antibiotics with the respective concentrations: ampicillin (10 µg), cefotaxime (30µg), chloramphenicol (30µg), ciprofloxacin (5 µg), gentamicin (10 µg), nalidixic acid (30 µg), nitrofuratoin (200µg), tetracycline (30µg), penicillin (15 µg), flucloxacillin (5 µg), cloxacillin (10 µg), erythromycyin (5 µg), ceftriaxone (30 µg) and cotrimoxazole (25 µg) (Mast Diagnostics, Mast Group Ltd., Merseyside, U.K.).

The bacterial suspension was compared to the 0.5McFarland standard. This comparison was made more easily with tubes viewed against a sheet of white paper on which sharp black lines are drawn. The bacterial suspension appeared to be the same density as the 0.5 McFarland.

The McFarland standard was prepared by adding 0.5mL of 0.048 M BaCl2 to 99.5mL of 0.18 M H_2SO_4 with constant stirring. The test was performed by applying the bacterial culture on Mueller-Hinton agar plate using a sterile swab and plates incubated for 16-24 h at 35^{o}C prior to determine results. The zones of growth inhibition around each of the antibiotics disks are measured in the nearest millimeter. The diameters of the zones are related to the susceptibility of the isolates.

CHAPTER FOUR

RESULT

Enumeration of Airborne Bacterial Isolates

The present study was conducted to enumerate, isolate and identify airborne bacterial isolates as well as determine the frequency of occurrence of bacterial isolates and determine the antibiotics sensitivity profile of the bacterial isolates in some selected hostel rooms in the Niger Delta Development Commission (NDDC) male hostel, University of Benin. It was observed that all the sections sampled showed diverse bacterial loads. In accordance with this study, frequent movements of students from immediate-outdoor to the indoor environments decisively influenced the diversity and abundance of the isolated bacteria. In this context, samples collected from different sections were significantly matched to the bacteria isolated from each room.

This study of indoor airborne bacteria synchronized with previous research on the temporal variation which are valuable for understanding what drives the assembly of biological communities and for predicting how these communities may change in the future (Shade *et al*., 2013). In the atmosphere, bacterial communities vary over multiple time scales including seasonal, inter-day, and diurnal (Després*et al*., 2012). Time series analyses have provided key information about the influence of terrestrial and aquatic sources in structuring atmospheric bacterial communities. However, it is not known over which time scales they are most variable, and how this variation may be related to changes in inputs from source environments or to environmental conditions. Most studies of bacterial dynamics in the atmosphere have focused on changes in the concentration of cells rather than their taxonomic composition. This is largely because most studies have used culture-based methods, which miss the majority microbial diversity (Rappé and Giovannoni, 2003). Culture-independent methods have been applied to bacterial communities in the atmosphere only in recent years.

Variation in the concentration of cells in the atmosphere has been measured over seasonal and diurnal time scales (Despres*et al*., 2012). Potential mechanisms that contribute to high densities of cells in the atmosphere include the presence of a strong source from anthropogenic activity such as tilling and crop harvesting (Lighthart 1984).

Result of this research showed significant differences in sampling sites as shown in Table 1. Room 104 had the highest airborne bacterial load(7.2×10^{-3}) in July and a decline in August(1.0×10^{-3}) with slight increased in September (4.1×10^{-3}) due to the inflow of students in and out of the hostel as well as humidity. Room 303 recorded bacterial load of (5.7×10^{-3}) in July, declined in August (1.0×10^{-3}) and was stable in September (1.0×10^{-3}).This changes in bacterial load was due to high waste generated and indiscriminate disposal of waste. Room 107 in July (1.4×10^{-3}) followed an increased sequence in August and September (1.4×10^{-3}) and (3.4×10^{-3}) respectively. Room 237 in July revealed higher incidence of (8.1×10^{-3}) and an increase in August (3.4×10^{-3}) and (1.9×10^{-3}) in September. In July Room 307 had (6.5×10^{-3}) and increased in August (1.0×10^{-3}) with drastic decline in September(9.0×10^{-3}). Eventually, Room 233 had (1.8×10^{-3}) and increased in August to (5.3×10^{-3}) with decline in September (1.7×10^{-3}). Graphical representation of airborne microbial load for individual rooms are shown below in figure 1, 2, 3 and 4. The inflow of air through the immediate-outdoors and other openings, like the doors which are been engaged daily and almost every minute by the students alike contributed to the high frequency of bacteria obtained in this study. This data portrayed the understanding that airborne microbial dynamics in built environments can influence the indoor airborne bacterial communities due to outdoor air source and ventilation.

When considering the sources of bacterial isolates in air, they can originate from human activities (coughing, sneezing, talking, food preparation, house or floor cleaning), Air sampling can be influenced by temperature, humidity, wind velocity as well as time of day. If

the air sampling was carried out early in the morning when there's more human activity, there is a possibility of obtaining higher numbers of bacteria. Even though all movement was avoided during sampling periods, it takes a while for bacteria to settle out of the air after all activities have ceased.

In fact, this result implies that the air of polluted hostel environment was the major cause of this airborne bacterial isolates. Moreover, *Staphylococcus aureus* and Bacillus subtilis are considered as environmentally hardy microorganisms (Davis *et al*., 2012) and they could remain viable in dry environments for at least a week to three months or longer (Beard-Pegler et al., 1988; Boyce, 2007; Dietze*et al*., 2001).

The result of this research is in agreement with previous studies that human exposure to microorganisms suspended in the air is associated with a wide range of major, adverse health effects. However, there is no international standard available for maximum levels of bioaerosols in the air, due to variations in human responses to exposure and difficulties in recovering potentially hazardous microorganisms in routine sampling (Gizaw*et al*., 2016). Research conducted by a WHO expert group on assessment of the health risks of biological agents in indoor environments has suggested that the total microbial load should not exceed 1000 CFU m−3 (WHO, 2016), whereas Polish proposals for the mesophilic bacteria suggest 5000 CFU m−3 for public service buildings.

Isolation and Identification of Airborne Bacterial Isolates

Nutrient agar plate fortified with nystatin (antifungal agent) was used to isolate these bacterial isolates through sedimentation technique using open Petri-dish. Cultural, morphological characterization of isolates as presented in Table 2 in this study showed that *Bacillus subtilis and Staphylococcus aureus* were predominant bacterial isolates identified.*Bacillussubtilis* indicated no gas production during sugar fermentation test, was motile on motility test medium, positive (+) during gram staining test with white moist

colonies on agar plates.*Staphylococcusaureus* showed positive on gram stain, non-motile and no fermentation on sugar test.

Frequency of Bacterial Airborne Occurrence

During July 2019 to September 2019 air samples were collected from 6 hostel rooms. The frequency of occurrence was observed to be higher in September. Total bacterial colony counts were performed for each sample. Taking all the statistical analysis results into account the frequency of occurrence accessed through total bacterial colony count results in hostel rooms were statistically different. This trend was observed in all hostel rooms. Therefore, in all situations, the average of hostel room results was considered as indoor. Since both of these rooms are located inside the same hostel building, it is evident that factors that can affect the number of bacteria present in the air are more or less similar. Analyzing indoor total bacterial colony count data, it was statistically different in some rooms. In fact, the average colony count in some rooms was higher than in others. The hostel air environment is a favorable place for bacterial survival and proliferation mainly due to rapid air flow. In addition, indoor bacterial populations mainly depend on the vicinity due to flow of outdoor air from the environment, human activities are the major factors that influence bacterial populations. Therefore, the data presented in this study provides evidence for an indoor source for culturable bacteria in hostels.

Antibiotics Susceptibility Profile of the Bacterial Isolates

The following antibiotics were used to access the antibiotic profile of *Staphylococcus aureus* and *Bacillus subtilis*; Amoxicillin Clavulanate (AUG),Cefotaxime (CTX), Imipenem/Cilastatin (IMP), Ofloxacin (OFX), Gentamycin (GN), Nalidixic Acid (NA), Nitrofurantoin (NF), Nitrofurantoin (NF), Cefuroxime (CXM), Ceftriaxone Sulbactarm (CRO), Gentamycin (GN), Azithromycin (AZN), Ofloxacin (OFX), Cefuroxime (CXM),

Imipenem/Cilastatin (IMP), Levofloxacin (LBC), Cefexime (ZEM), Ampiclox (ACX) and Erythromycin (ERY).

Antibiotic Resistance Patterns of *Staphylococcus aureus* expressed resistance to Amoxicillin Clavulanate (AUG), Cefotaxime (CTX), Imipenem/Cilastatin (IMP), Ofloxacin (OFX), Nalidixic Acid (NA), Nitrofurantoin (NF), Nitrofurantoin (NF), Cefuroxime (CXM), Ceftriaxone Sulbactarm (CRO), Gentamycin (GN), Cefuroxime (CXM), Imipenem/Cilastatin (IMP), Levofloxacin (LBC), Cefexime (ZEM), Ampiclox (ACX) which was a higher number of antibiotics and sensitive on Ofloxacin (OFX), Azithromycin (AZN), Gentamycin (GN) and Erythromycin (ERY). *Bacillus subtilis*showedshowed resistance to Amoxicillin Clavulanate (AUG), Cefotaxime (CTX), Imipenem/Cilastatin (IMP), Ofloxacin (OFX), Gentamycin (GN), Nalidixic Acid (NA), Nitrofurantoin (NF), Nitrofurantoin (NF), Cefuroxime (CXM), Ceftriaxone Sulbactarm (CRO), Gentamycin (GN), Azithromycin (AZN), Ofloxacin (OFX), Cefuroxime (CXM), Imipenem/Cilastatin(IMP), Levofloxacin (LBC), Cefexime (ZEM), Ampiclox (ACX) and Erythromycin (ERY) as shown in (Table 3).

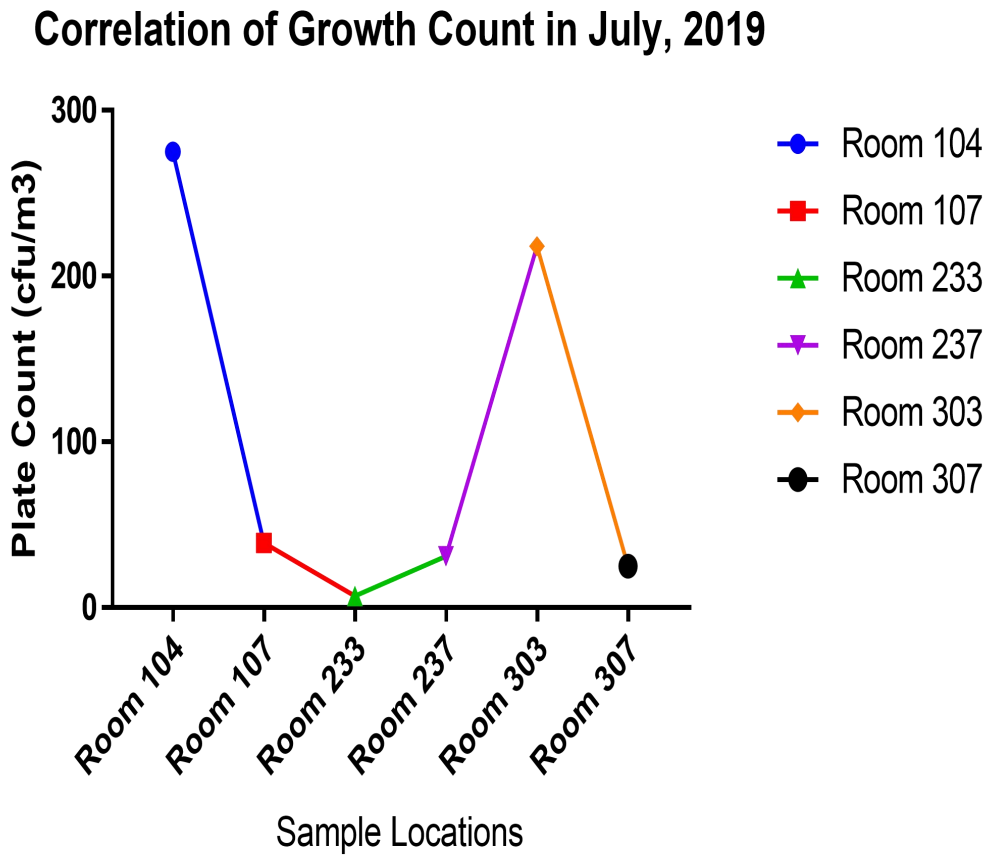

Figure 4.1: Correlation of Airborne Incidence in July, 2019

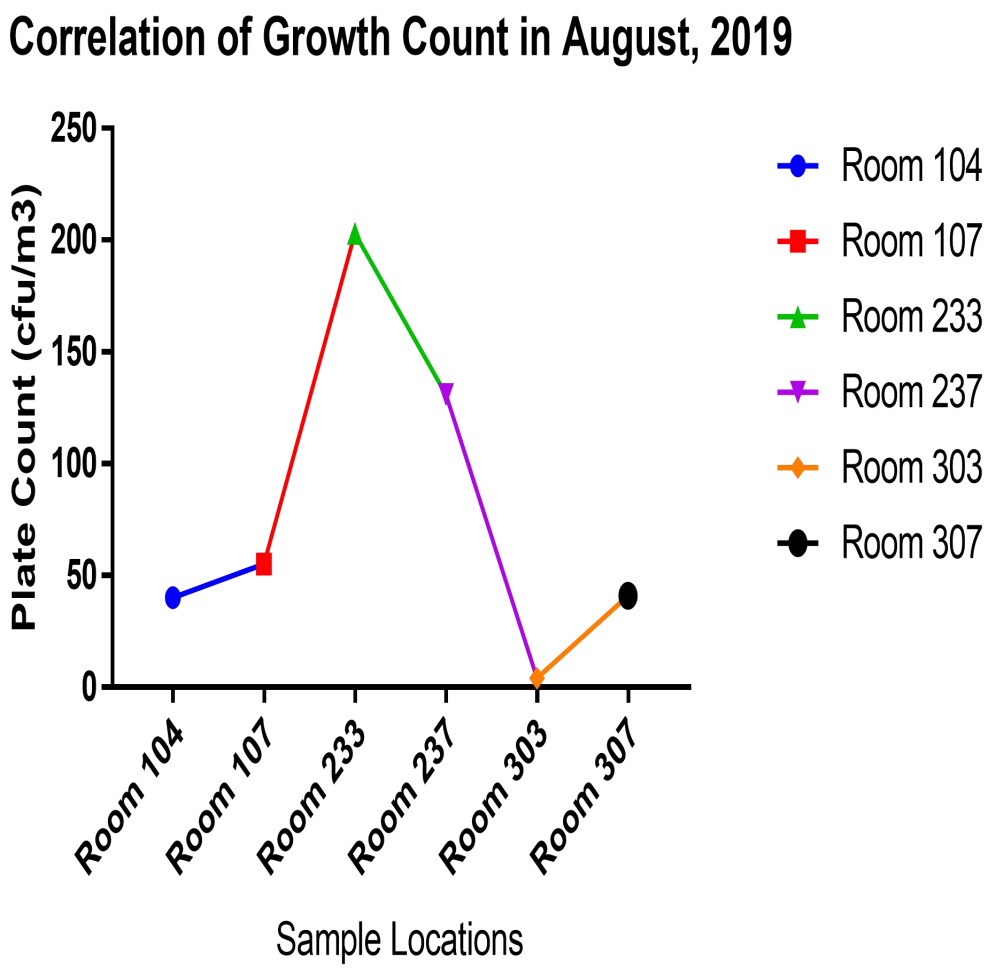

Figure 4.2: Correlation of Airborne Incidence in August, 2019

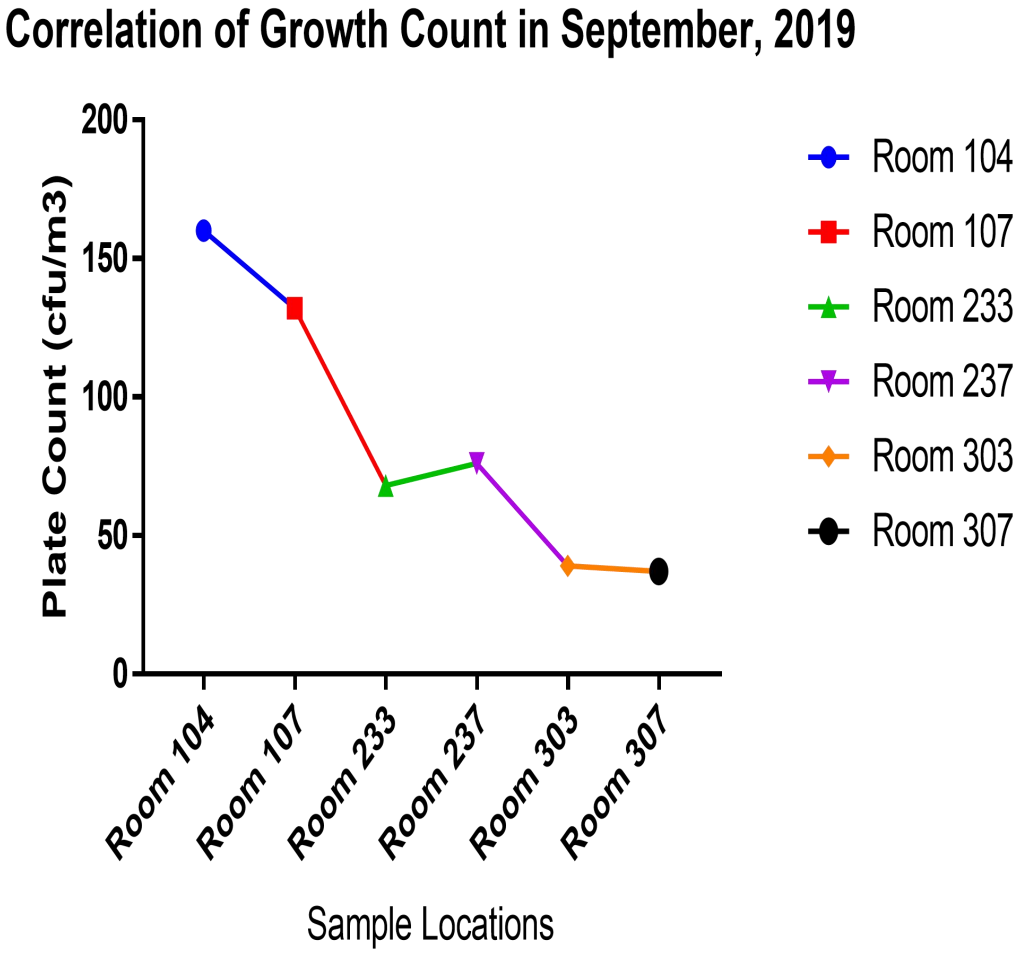

Figure 4.3: Correlation of Airborne Incidence in September, 2019

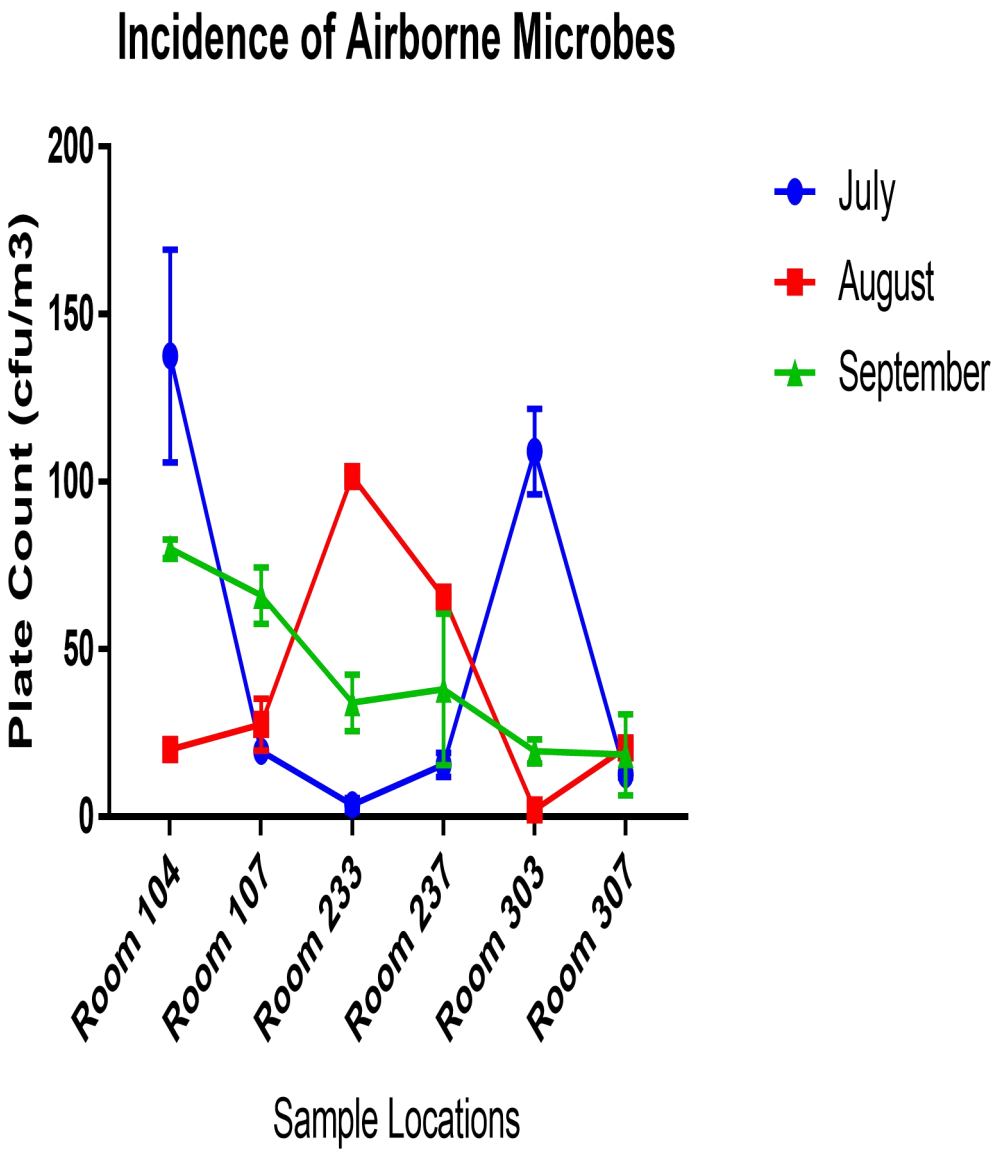

Figure 4.4: Correlation of Airborne Incidence in July, August and September, 2019

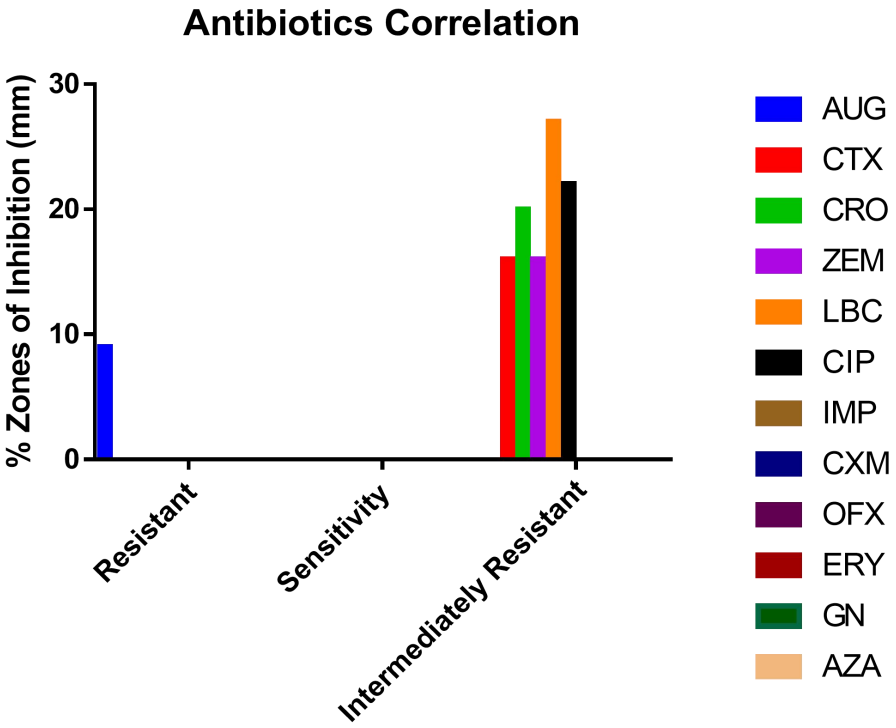

Figure 4.5: Antibiotics Correlation on *Bacillus subtilis*

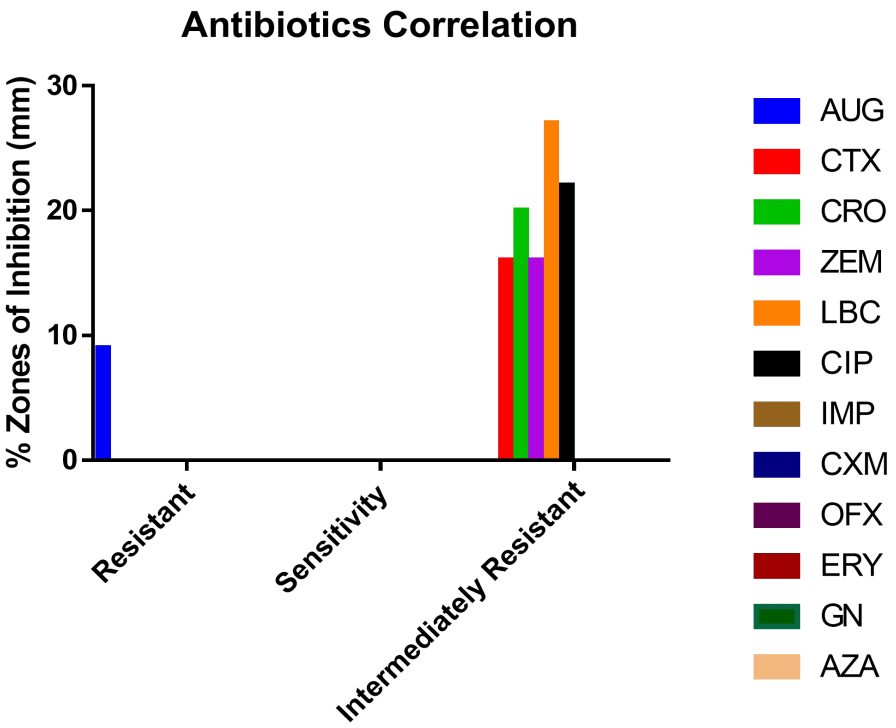

Figure 4.6: Antibiotics Correlation on *Staphylococcus aureus*

Table 1

	Sampling Location	Sum (∑X)	Standard Deviation (σ)	Mean (μ)	Variance (σ^2)	Cfu/m^3	SEM (σx)	Confidence Level	Margin of Error	% Error
July	Room 104	275	22.5	137.5	506.25	$7.2x10^{-3}$	15.91	99.99% $3.891\sigma_{\bar{x}}$	137.5 ± 61.905	± 45.02%
	Room 107	39	1.5	19.5	2.25	$1.4\ x10^{-3}$	1.06	99.9999% $4.892\sigma_{\bar{x}}$	19.5 ± 5.189	± 26.61%
	Room 233	7	1.5	3.5	2.25	$1.8\ x10^{-2}$	1.06	99.9999% $4.892\sigma_{\bar{x}}$	3.5 ± 5.189	± 148.25%
	Room 237	31	2.5	15.5	6.25	$8.1\ x10^{-2}$	1.77	99.9999% $4.892\sigma_{\bar{x}}$	15.5 ± 8.648	± 55.79%
	Room 303	218	9	109	81	$5.7\ x10^{-3}$	6.36	99.9999% $4.892\sigma_{\bar{x}}$	109 ± 31.132	± 28.56%
	Room 307	25	0.5	12.5	0.25	$6.5\ x10^{-2}$	0.35	99.9999% $4.892\sigma_{\bar{x}}$	12.5 ± 1.73	± 13.84%
August	Sampling Location	Sum (∑X)	Standard Deviation (σ)	Mean (μ)	Variance (σ^2)	Cfu/M^3	SEM (σx)	Confidence Level	Margin of Error	% Error
	Room 104	40	2	20	4	$1.0X10^{-3}$	1.41	99.9999% $4.892\sigma_{\bar{x}}$	20 ± 6.918	± 34.59%
	Room 107	55	5.5	27.5	30.25	$1.4\ X10^{-3}$	3.89	99.9999% $4.892\sigma_{\bar{x}}$	27.5 ± 19.025	± 69.18%
	Room 233	203	1.5	101.5	2.25	$5.3\ X10^{-3}$	1.06	99.9999% $4.892\sigma_{\bar{x}}$	101.5 ± 5.189	± 5.11%
	Room 237	131	0.5	65.5	0.25	$3.4\ X10^{-3}$	0.35	99.9999% $4.892\sigma_{\bar{x}}$	65.5 ± 1.73	± 2.64%
	Room 303	4	1	2	1	$1.0\ X10^{-2}$	0.71	99.9999% $4.892\sigma_{\bar{x}}$	2 ± 3.459	± 172.96%
	Room 307	41	0.5	20.5	0.25	$1.0\ X10^{-3}$	0.35	99.9999% $4.892\sigma_{\bar{x}}$	20.5 ± 1.73	± 8.44%
	Sampling Location	Sum (∑x)	Standard Deviation	Mean (μ)	Variance (σ^2)	Cfu/m^3	SUM (σx)	Confidence Level	Margin of Error	% Error

			(σ)						
September	Room 104	160	2	80	4	$4.1X10^{-3}$	1.41	99.9999% $4.892\sigma_{\bar{x}}$	80 $\pm$ 6.918	$\pm$ 8.65%
	Room 107	132	6	66	36	$3.4\ X10^{-3}$	4.2	99.9999% $3.891\sigma_{\bar{x}}$	66 $\pm$ 16.508	$\pm$ 25.01%
	Room 233	68	6	34	36	$1.7\ X10^{-3}$	4.2	99.9999% $4.892\sigma_{\bar{x}}$	34 $\pm$ 20.755	$\pm$ 61.04%
	Room 237	76	16	38	256	$1.9\ X10^{-3}$	11.31	99.9999% $4.892\sigma_{\bar{x}}$	38 $\pm$ 55.347	$\pm$ 145.65%
	Room 303	39	2.5	19.5	6.25	$1.\ X10^{-3}$	1.77	99.9999% $4.892\sigma_{\bar{x}}$	19.5 $\pm$ 8.648	$\pm$ 44.35%
	Room 307	37	8.5	18.5	72.25	$9.6\ X10^{-2}$	6.01	99.9999% $4.892\sigma_{\bar{x}}$	18.5 $\pm$ 29.403	$\pm$ 158.93%

TABLE 2: Cultural, Morphological and Biochemical Characterization of Bacterial Isolates

Colony on NA	H2O Gas	Motility	Gram Stain	Catalase	Citrate	Indole	Coagulase	Oxidase	Urease	Isolate identity
White, Moist	-	+	+	+	-	-	-	-	-	*Bacillus Subtilis*
White, Smooth, Creamy and Round	-	-	+	+	-	-	-	-	-	*Staphylococcus aureus*

Table 3: Antibiotics Susceptibility pattern of airborne bacterial isolates in NDDC hostel

Isolates	AUG	CTQ	CRO	ZEM	LBC	CIP	IMP	CXM	OFX	AZN	GN	FCRY
Bacilussubtilis	R	R	R	R	R	R	R	R	R	R	R	R
Staphylococcus aureus	R	R	R	R	R	R	R	S	S	S	S	S

Statistical Analysis

Bacterial growth data obtained from plate counts were subjected to statistical analyses and were performed using SPSS package version 17.0 and GraphPad prism version 6 software.

Discussion

The assessment of indoor air quality is essential in determining microbial air pollution. Information on number and type of air borne microorganisms can be used to estimate the health hazard posed and create standards for air quality for both the indoor and outdoor environment. This study investigated the bacterial qualities indoor air of hostel in University of Benin. The results of this study have revealed fairly high bacterial loads in the hostel atmosphere. The research findings have shown that bacterial isolates were detected in all the rooms.

Staphylococcus aureus and *Bacillus subtilis*were obtained from this study in normal microbial flora of air. Previous studies have identified these organisms in aerosols of different environments such as living rooms, hospitals, class rooms, offices, childcare centres and in residences. In this study, biochemical method was applied to identify bacterial isolates derived from hostel air samples as well as antibiotics susceptibility profile was assessed.

There is no invariable standard in world to show tolerable maximum airborne fungi loads. Governmental and private organizations have different values for acceptable fungal bioaerosol concentrations (Rao *et al*., 2011). The number of colony forming unit (CFU/m^3) varies widely; from < 25 to more than 300. The quantitative interpretation of the results describing the air quality in this study was evaluated.

According to this research, all the hostel rooms that were included in the study were not in hygienic conditions and the outer environment was contaminated and was the majorly the cause of the indoor air quality. These might be because of students, visitors, and the high density of

students. Beside these, the environmental factors, mainly, inadequate disinfection, inappropriate and insufficient ventilation system might also contribute to the high bacterial loads of the hostel rooms.In isolation of airborne microbes in student hostels, the extent of airborne microbial contamination was high.

These are living biological contaminants can be transmitted by infected people, indoor air, and they can also travel through the air and get inside buildings (Cheng *et al*., 2014). Bacteria species like *Staphylococcus* sp are found on human skin scales (Del *et al.*, 2014). *S. aureus* are emitted from the nasopharynx of normally healthy individuals when the person talks, and are commonly found in air, water, the skin (Ewing and Green, 2011). *Pseudomonas*sp has been reportedly associated with wet surfaces of air- conditioning systems, cooling coils, drain pans and sump pumps (Tokuyasu, *et al*., 2012). Poppert, *et al*. (2005) reported the isolation and characterization of thirty-one microorganisms from different laboratory locations including walls, tables and floor. Results showed that the microbial population is higher in some rooms than others (Table 4.6 and 4.7). This might be attributed to the fact that more people enter or live in those rooms. Mitsuko *et al*. (2005) discovered that presence of bacteria in a room indicates the presence of people and their levels may get high when the room is heavily populated.

The antibiotics susceptibility test in this research also conforms with previous research that Penicillium is associated with infections including pneumonia especially in immunocompromised individuals was isolated in this study. Penicillium was also isolated from tables and walls by Tim *et al.* (2004).

The resistance of the bacterial isolates to most of the antibiotics tested in this study were of medical and public health importance. Both Gram positive and Gram negative bacteria considerable rates of resistance to different classes of antibiotics. Most of the antibiotic classes

were used as treatment options by clinicians in case of an infection. Interestingly, gentamicin and tetracycline showed a level of effectiveness especially against some of the Gram positive bacteria, and these antibiotics might be considered as parts of the treatment regimen in the study area.

CONCLUSION

The various air sampling sites showed the presence of bacteria, though with low levels of contamination within the range of 1.0×10^{-3} to9.6×10^{-3}CFU/m^3. Thus, students living in this hostels are at risk of exposure to airborne bacteria. Although the majority seems opportunistic, they may have pathogenic potentials with significant consequences. The strength of this study is that it unravels the level of bacterial contamination and subsequent antibiotic susceptibility profiles of hostel environments.

From the sampling results a lot of relationships between different factors that can influence on the amount of bacteria can be seen. Firstly, it was observed that concentration of bacteria in the air depends on number of people in the room and the out-door environmental status which greatly affected the indoor air quality due to indiscriminate disposal of waste. Also in that room the wardrobes are situated where the conditions for bacterial proliferation were encouraged, so it is also a source of air pollution, because people carry bacterial spores on them from out-wears.

Nowadays, quality of the air which we inhale indoors has great and adverse influence to the lungs health. Lungs are very feeble and can be easily damaged by polluters which are contains in the air. These polluters increase the risk of appearance of sickness like asthma, allergy, chronic bronchitis, lungs cancer etc.

Biological contaminants like bacteria can be a cause of bad indoor quality. They could be found worldwide. There are a lot of causes of its appearance; one of them is high moisture indoors, because all microorganisms are in need of water. So that is why moisture control is a key to the clean indoor air.

In present study, there are a lot of methods that can help in prevention of appearance of microorganisms, such as regular sanitary cleaning of indoor and outdoor environment whichis

rather cheap and everyone can afford it. Thus, it is recommended to control bacterial spores which are highly resistant, interventions through installation of good ventilation systems, regular disinfected floors, systematic checking of the indoor air and ventilation systems.

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APPENDICIES
APPENDIX I

JULY, 2019
Room 104
Standard Deviation, σ: **22.5**
Count, N: 2
Sum, Σx: 275
Mean, μ: 137.5
Variance, σ^2: 506.25

Steps

$$\sigma = \sqrt{\frac{1}{N}\sum_{i=1}^{N}(x_i - \mu)^2}.$$

$$\sigma^2 = \frac{\Sigma(x_i - \mu)^2}{N}$$

$$= \frac{(160 - 137.5)^2 + \ldots + (115 - 137.5)^2}{2}$$

$$= \frac{1012.5}{2}$$

$$= 506.25$$

$$\sigma = \sqrt{506.25}$$

$$= 22.5$$

Margin of Error (Confidence Interval)
The sampling mean most likely follows a normal distribution. In this case, the standard error of the mean (SEM) can be calculated using the following equation:

$$\sigma_{\bar{x}} = \frac{\sigma}{\sqrt{N}} = 15.909902576697$$

Based on the SEM, the following are the margins of error (or confidence intervals) at different confidence levels. Depending on the field of study, a confidence level of 95% (or statistical significance of 5%) is typically used for data representation.

Confidence Level	Margin of Error	Error Bar
68.3%, $\sigma_{\bar{x}}$	137.5 ±15.91 (±11.57%)	
90%, $1.645\sigma_{\bar{x}}$	137.5 ±26.172 (±19.03%)	
95%, $1.960\sigma_{\bar{x}}$	137.5 ±31.183 (±22.68%)	
99%, $2.576\sigma_{\bar{x}}$	137.5 ±40.984 (±29.81%)	
99.9%, $3.291\sigma_{\bar{x}}$	137.5 ±52.359 (±38.08%)	
99.99%, $3.891\sigma_{\bar{x}}$	137.5 ±61.905 (±45.02%)	

99.999%, 4.417$\sigma_{\bar{x}}$	137.5 ±70.274 (±51.11%)	
99.9999%, 4.892$\sigma_{\bar{x}}$	137.5 ±77.831 (±56.60%)	

Room 107
Standard Deviation, σ: **1.5**
Count, N: 2
Sum, Σx: 39
Mean, μ: 19.5
Variance, σ^2: 2.25

Steps

$$\sigma = \sqrt{\frac{1}{N}\sum_{i=1}^{N}(x_i - \mu)^2}.$$

$$\sigma^2 = \frac{\Sigma(x_i - \mu)^2}{N}$$

$$= \frac{(18 - 19.5)^2 + \ldots + (21 - 19.5)^2}{2}$$

$$= \frac{4.5}{2}$$

$$= 2.25$$

$$\sigma = \sqrt{2.25}$$

$$= 1.5$$

Margin of Error (Confidence Interval)
The sampling mean most likely follows a normal distribution. In this case, the standard error of the mean (SEM) can be calculated using the following equation:

$$\sigma_{\bar{x}} = \frac{\sigma}{\sqrt{N}} = 1.0606601717798$$

Based on the SEM, the following are the margins of error (or confidence intervals) at different confidence levels. Depending on the field of study, a confidence level of 95% (or statistical significance of 5%) is typically used for data representation.

Confidence Level	Margin of Error	Error Bar
68.3%, $\sigma_{\bar{x}}$	19.5 ±1.061 (±5.44%)	
90%, 1.645$\sigma_{\bar{x}}$	19.5 ±1.745 (±8.95%)	
95%, 1.960$\sigma_{\bar{x}}$	19.5 ±2.079 (±10.66%)	
99%, 2.576$\sigma_{\bar{x}}$	19.5 ±2.732 (±14.01%)	
99.9%, 3.291$\sigma_{\bar{x}}$	19.5 ±3.491 (±17.90%)	

99.99%, 3.891$\sigma_{\bar{x}}$	19.5 ±4.127 (±21.16%)	
99.999%, 4.417$\sigma_{\bar{x}}$	19.5 ±4.685 (±24.03%)	
99.9999%, 4.892$\sigma_{\bar{x}}$	19.5 ±5.189 (±26.61%)	

Room 233
Standard Deviation, σ: **1.5**
Count, N: 2
Sum, Σx: 7
Mean, μ: 3.5
Variance, σ^2: 2.25

Steps

$$\sigma = \sqrt{\frac{1}{N}\sum_{i=1}^{N}(x_i - \mu)^2}.$$

$$\sigma^2 = \frac{\Sigma(x_i - \mu)^2}{N}$$

$$= \frac{(5 - 3.5)^2 + \ldots + (2 - 3.5)^2}{2}$$

$$= \frac{4.5}{2}$$

$$= 2.25$$

$$\sigma = \sqrt{2.25}$$

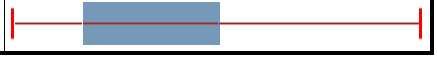
$$= 1.5$$

Margin of Error (Confidence Interval)
The sampling mean most likely follows a normal distribution. In this case, the standard error of the mean (SEM) can be calculated using the following equation:

$$\sigma_{\bar{x}} = \frac{\sigma}{\sqrt{N}} = 1.0606601717798$$

Based on the SEM, the following are the margins of error (or confidence intervals) at different confidence levels. Depending on the field of study, a confidence level of 95% (or statistical significance of 5%) is typically used for data representation.

Confidence Level	Margin of Error	Error Bar
68.3%, $\sigma_{\bar{x}}$	3.5 ±1.061 (±30.30%)	
90%, 1.645$\sigma_{\bar{x}}$	3.5 ±1.745 (±49.85%)	
95%, 1.960$\sigma_{\bar{x}}$	3.5 ±2.079 (±59.40%)	

99%, 2.576$\sigma_{\bar{x}}$	3.5 ±2.732 (±78.06%)	
99.9%, 3.291$\sigma_{\bar{x}}$	3.5 ±3.491 (±99.73%)	
99.99%, 3.891$\sigma_{\bar{x}}$	3.5 ±4.127 (±117.92%)	
99.999%, 4.417$\sigma_{\bar{x}}$	3.5 ±4.685 (±133.86%)	
99.9999%, 4.892$\sigma_{\bar{x}}$	3.5 ±5.189 (±148.25%)	

Room 237

Standard Deviation, σ: **2.5**
Count, N: 2
Sum, Σx: 31
Mean, μ: 15.5
Variance, σ^2: 6.25

Steps

$$\sigma = \sqrt{\frac{1}{N}\sum_{i=1}^{N}(x_i - \mu)^2}.$$

$$\sigma^2 = \frac{\Sigma(x_i - \mu)^2}{N}$$

$$= \frac{(13 - 15.5)^2 + \ldots + (18 - 15.5)^2}{2}$$

$$= \frac{12.5}{2}$$

$$= 6.25$$

$$\sigma = \sqrt{6.25}$$

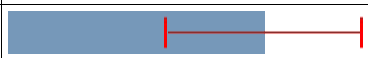
$$= 2.5$$

Margin of Error (Confidence Interval)
The sampling mean most likely follows a normal distribution. In this case, the standard error of the mean (SEM) can be calculated using the following equation:

$$\sigma_{\bar{x}} = \frac{\sigma}{\sqrt{N}} = 1.7677669529664$$

Based on the SEM, the following are the margins of error (or confidence intervals) at different confidence levels. Depending on the field of study, a confidence level of 95% (or statistical significance of 5%) is typically used for data representation.

Confidence Level	Margin of Error	Error Bar
68.3%, $\sigma_{\bar{x}}$	15.5 ±1.768 (±11.40%)	

90%, 1.645$\sigma_{\bar{x}}$	15.5 ±2.908 (±18.76%)	
95%, 1.960$\sigma_{\bar{x}}$	15.5 ±3.465 (±22.35%)	
99%, 2.576$\sigma_{\bar{x}}$	15.5 ±4.554 (±29.38%)	
99.9%, 3.291$\sigma_{\bar{x}}$	15.5 ±5.818 (±37.53%)	
99.99%, 3.891$\sigma_{\bar{x}}$	15.5 ±6.878 (±44.38%)	
99.999%, 4.417$\sigma_{\bar{x}}$	15.5 ±7.808 (±50.38%)	
99.9999%, 4.892$\sigma_{\bar{x}}$	15.5 ±8.648 (±55.79%)	

Room 303
Standard Deviation, σ: **9**
Count, N: 2
Sum, Σx: 218
Mean, μ: 109
Variance, σ^2: 81

Steps

$$\sigma = \sqrt{\frac{1}{N}\sum_{i=1}^{N}(x_i - \mu)^2}.$$

$$\sigma^2 = \frac{\Sigma(x_i - \mu)^2}{N}$$

$$= \frac{(100 - 109)^2 + \ldots + (118 - 109)^2}{2}$$

$$= \frac{162}{2}$$

$$= 81$$

$$\sigma = \sqrt{81}$$

$$= 9$$

Margin of Error (Confidence Interval)
The sampling mean most likely follows a normal distribution. In this case, the standard error of the mean (SEM) can be calculated using the following equation:

$$\sigma_{\bar{x}} = \frac{\sigma}{\sqrt{N}} = 6.3639610306789$$

Based on the SEM, the following are the margins of error (or confidence intervals) at different confidence levels. Depending on the field of study, a confidence level of 95% (or statistical significance of 5%) is typically used for data representation.

Confidence Level	Margin of Error	Error Bar

68.3%, $\sigma_{\bar{x}}$	109 ±6.364 (±5.84%)	
90%, 1.645$\sigma_{\bar{x}}$	109 ±10.469 (±9.60%)	
95%, 1.960$\sigma_{\bar{x}}$	109 ±12.473 (±11.44%)	
99%, 2.576$\sigma_{\bar{x}}$	109 ±16.394 (±15.04%)	
99.9%, 3.291$\sigma_{\bar{x}}$	109 ±20.944 (±19.21%)	
99.99%, 3.891$\sigma_{\bar{x}}$	109 ±24.762 (±22.72%)	
99.999%, 4.417$\sigma_{\bar{x}}$	109 ±28.11 (±25.79%)	
99.9999%, 4.892$\sigma_{\bar{x}}$	109 ±31.132 (±28.56%)	

Room 307
Standard Deviation, σ: **0.5**
Count, N: 2
Sum, Σx: 25
Mean, μ: 12.5
Variance, σ^2: 0.25

Steps

$$\sigma = \sqrt{\frac{1}{N}\sum_{i=1}^{N}(x_i - \mu)^2}.$$

$$\sigma^2 = \frac{\Sigma(x_i - \mu)^2}{N}$$

$$= \frac{(13 - 12.5)^2 + \ldots + (12 - 12.5)^2}{2}$$

$$= \frac{0.5}{2}$$

$$= 0.25$$

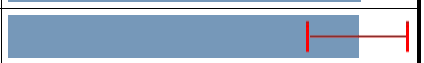
$$\sigma = \sqrt{0.25}$$

$$= 0.5$$

Margin of Error (Confidence Interval)
The sampling mean most likely follows a normal distribution. In this case, the standard error of the mean (SEM) can be calculated using the following equation:

$$\sigma_{\bar{x}} = \frac{\sigma}{\sqrt{N}} = 0.35355339059327$$

Based on the SEM, the following are the margins of error (or confidence intervals) at different confidence levels. Depending on the field of study, a confidence level of 95% (or statistical significance of 5%) is typically used for data representation.

Confidence Level	Margin of Error	Error Bar
68.3%, $\sigma_{\bar{x}}$	12.5 ±0.354 (±2.83%)	
90%, $1.645\sigma_{\bar{x}}$	12.5 ±0.582 (±4.65%)	
95%, $1.960\sigma_{\bar{x}}$	12.5 ±0.693 (±5.54%)	
99%, $2.576\sigma_{\bar{x}}$	12.5 ±0.911 (±7.29%)	
99.9%, $3.291\sigma_{\bar{x}}$	12.5 ±1.164 (±9.31%)	
99.99%, $3.891\sigma_{\bar{x}}$	12.5 ±1.376 (±11.01%)	
99.999%, $4.417\sigma_{\bar{x}}$	12.5 ±1.562 (±12.49%)	
99.9999%, $4.892\sigma_{\bar{x}}$	12.5 ±1.73 (±13.84%)	

August, 2019
Room 104
Standard Deviation, σ: **2**
Count, N: 2
Sum, Σx: 40
Mean, μ: 20
Variance, σ^2: 4

Steps

$$\sigma = \sqrt{\frac{1}{N}\sum_{i=1}^{N}(x_i - \mu)^2}.$$

$$\sigma^2 = \frac{\Sigma(x_i - \mu)^2}{N}$$

$$= \frac{(22 - 20)^2 + \ldots + (18 - 20)^2}{2}$$

$$= \frac{8}{2}$$

$$= 4$$

$$\sigma = \sqrt{4}$$

$$= 2$$

Margin of Error (Confidence Interval)
The sampling mean most likely follows a normal distribution. In this case, the standard error of the mean (SEM) can be calculated using the following equation:

$\sigma_{\bar{x}} = \sigma = 1.4142135623731$

[redacted]
√N

Based on the SEM, the following are the margins of error (or confidence intervals) at different confidence levels. Depending on the field of study, a confidence level of 95% (or statistical significance of 5%) is typically used for data representation.

Confidence Level	Margin of Error	Error Bar
68.3%, $\sigma_{\bar{x}}$	20 ±1.414 (±7.07%)	
90%, $1.645\sigma_{\bar{x}}$	20 ±2.326 (±11.63%)	
95%, $1.960\sigma_{\bar{x}}$	20 ±2.772 (±13.86%)	
99%, $2.576\sigma_{\bar{x}}$	20 ±3.643 (±18.22%)	
99.9%, $3.291\sigma_{\bar{x}}$	20 ±4.654 (±23.27%)	
99.99%, $3.891\sigma_{\bar{x}}$	20 ±5.503 (±27.51%)	
99.999%, $4.417\sigma_{\bar{x}}$	20 ±6.247 (±31.23%)	
99.9999%, $4.892\sigma_{\bar{x}}$	20 ±6.918 (±34.59%)	

Room 107
Standard Deviation, σ: **5.5**
Count, N: 2
Sum, Σx: 55
Mean, μ: 27.5
Variance, σ^2: 30.25

Steps

$$\sigma = \sqrt{\frac{1}{N}\sum_{i=1}^{N}(x_i - \mu)^2}.$$

$\sigma^2 = \frac{\Sigma(x_i - \mu)^2}{N}$

$= \frac{(22 - 27.5)^2 + \ldots + (33 - 27.5)^2}{2}$

$= \frac{60.5}{2}$

$= 30.25$

$\sigma = \sqrt{30.25}$

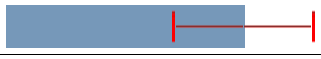
$= 5.5$

Margin of Error (Confidence Interval)

The sampling mean most likely follows a normal distribution. In this case, the standard error of the mean (SEM) can be calculated using the following equation:

$$\sigma_{\bar{x}} = \frac{\sigma}{\sqrt{N}} = 3.889087296526$$

Based on the SEM, the following are the margins of error (or confidence intervals) at different confidence levels. Depending on the field of study, a confidence level of 95% (or statistical significance of 5%) is typically used for data representation.

Confidence Level	Margin of Error	Error Bar
68.3%, $\sigma_{\bar{x}}$	27.5 ±3.889 (±14.14%)	
90%, $1.645\sigma_{\bar{x}}$	27.5 ±6.398 (±23.26%)	
95%, $1.960\sigma_{\bar{x}}$	27.5 ±7.623 (±27.72%)	
99%, $2.576\sigma_{\bar{x}}$	27.5 ±10.018 (±36.43%)	
99.9%, $3.291\sigma_{\bar{x}}$	27.5 ±12.799 (±46.54%)	
99.99%, $3.891\sigma_{\bar{x}}$	27.5 ±15.132 (±55.03%)	
99.999%, $4.417\sigma_{\bar{x}}$	27.5 ±17.178 (±62.47%)	
99.9999%, $4.892\sigma_{\bar{x}}$	27.5 ±19.025 (±69.18%)	

Room 233
Standard Deviation, σ: **1.5**
Count, N: 2
Sum, Σx: 203
Mean, μ: 101.5
Variance, σ^2: 2.25

Steps

$$\sigma = \sqrt{\frac{1}{N}\sum_{i=1}^{N}(x_i - \mu)^2}.$$

$$\sigma^2 = \frac{\Sigma(x_i - \mu)^2}{N}$$

$$= \frac{(103 - 101.5)^2 + \ldots + (100 - 101.5)^2}{2}$$

$$= \frac{4.5}{2}$$

$$= 2.25$$

$$\sigma = \sqrt{2.25}$$

$$= 1.5$$

Margin of Error (Confidence Interval)
The sampling mean most likely follows a normal distribution. In this case, the standard error of the mean (SEM) can be calculated using the following equation:

$$\sigma_{\bar{x}} = \frac{\sigma}{\sqrt{N}} = 1.0606601717798$$

Based on the SEM, the following are the margins of error (or confidence intervals) at different confidence levels. Depending on the field of study, a confidence level of 95% (or statistical significance of 5%) is typically used for data representation.

Confidence Level	Margin of Error	Error Bar
68.3%, $\sigma_{\bar{x}}$	101.5 ±1.061 (±1.04%)	
90%, $1.645\sigma_{\bar{x}}$	101.5 ±1.745 (±1.72%)	
95%, $1.960\sigma_{\bar{x}}$	101.5 ±2.079 (±2.05%)	
99%, $2.576\sigma_{\bar{x}}$	101.5 ±2.732 (±2.69%)	
99.9%, $3.291\sigma_{\bar{x}}$	101.5 ±3.491 (±3.44%)	
99.99%, $3.891\sigma_{\bar{x}}$	101.5 ±4.127 (±4.07%)	
99.999%, $4.417\sigma_{\bar{x}}$	101.5 ±4.685 (±4.62%)	
99.9999%, $4.892\sigma_{\bar{x}}$	101.5 ±5.189 (±5.11%)	

Room 237
Standard Deviation, σ: **0.5**
Count, N: 2
Sum, Σx: 131
Mean, μ: 65.5
Variance, σ^2: 0.25
Steps

$$\sigma = \sqrt{\frac{1}{N}\sum_{i=1}^{N}(x_i - \mu)^2}.$$

$$\sigma^2 = \frac{\Sigma(x_i - \mu)^2}{N}$$

$$= \frac{(66 - 65.5)^2 + \ldots + (65 - 65.5)^2}{2}$$

$$= \frac{0.5}{2}$$

$$= 0.25$$

$$\sigma = \sqrt{0.25}$$

$$= 0.5$$

Margin of Error (Confidence Interval)

The sampling mean most likely follows a normal distribution. In this case, the standard error of the mean (SEM) can be calculated using the following equation:

$$\sigma_{\bar{x}} = \frac{\sigma}{\sqrt{N}} = 0.35355339059327$$

Based on the SEM, the following are the margins of error (or confidence intervals) at different confidence levels. Depending on the field of study, a confidence level of 95% (or statistical significance of 5%) is typically used for data representation.

Confidence Level	Margin of Error	Error Bar
68.3%, $\sigma_{\bar{x}}$	65.5 ±0.354 (±0.54%)	
90%, $1.645\sigma_{\bar{x}}$	65.5 ±0.582 (±0.89%)	
95%, $1.960\sigma_{\bar{x}}$	65.5 ±0.693 (±1.06%)	
99%, $2.576\sigma_{\bar{x}}$	65.5 ±0.911 (±1.39%)	
99.9%, $3.291\sigma_{\bar{x}}$	65.5 ±1.164 (±1.78%)	
99.99%, $3.891\sigma_{\bar{x}}$	65.5 ±1.376 (±2.10%)	
99.999%, $4.417\sigma_{\bar{x}}$	65.5 ±1.562 (±2.38%)	
99.9999%, $4.892\sigma_{\bar{x}}$	65.5 ±1.73 (±2.64%)	

Room 303
Standard Deviation, σ: **1**
Count, N: 2
Sum, Σx: 4
Mean, μ: 2
Variance, σ^2: 1

Steps

$$\sigma = \sqrt{\frac{1}{N}\sum_{i=1}^{N}(x_i - \mu)^2}.$$

$$\sigma^2 = \frac{\Sigma(x_i - \mu)^2}{N}$$

$$= \frac{(1 - 2)^2 + \ldots + (3 - 2)^2}{2}$$

$$= \frac{2}{2}$$

$$= 1$$

$$\sigma = \sqrt{1}$$

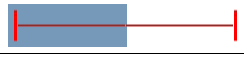
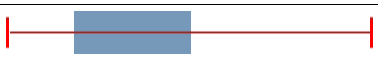
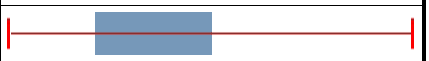
$$= 1$$

Margin of Error (Confidence Interval)

The sampling mean most likely follows a normal distribution. In this case, the standard error of the mean (SEM) can be calculated using the following equation:

$$\sigma_{\bar{x}} = \frac{\sigma}{\sqrt{N}} = 0.70710678118655$$

Based on the SEM, the following are the margins of error (or confidence intervals) at different confidence levels. Depending on the field of study, a confidence level of 95% (or statistical significance of 5%) is typically used for data representation.

Confidence Level	Margin of Error	Error Bar
68.3%, $\sigma_{\bar{x}}$	2 ±0.707 (±35.36%)	
90%, $1.645\sigma_{\bar{x}}$	2 ±1.163 (±58.16%)	
95%, $1.960\sigma_{\bar{x}}$	2 ±1.386 (±69.30%)	
99%, $2.576\sigma_{\bar{x}}$	2 ±1.822 (±91.08%)	
99.9%, $3.291\sigma_{\bar{x}}$	2 ±2.327 (±116.35%)	
99.99%, $3.891\sigma_{\bar{x}}$	2 ±2.751 (±137.57%)	
99.999%, $4.417\sigma_{\bar{x}}$	2 ±3.123 (±156.16%)	
99.9999%, $4.892\sigma_{\bar{x}}$	2 ±3.459 (±172.96%)	

Room 307
Standard Deviation, σ: **0.5**
Count, N: 2
Sum, Σx: 41
Mean, μ: 20.5
Variance, σ^2: 0.25

Steps

$$\sigma = \sqrt{\frac{1}{N}\sum_{i=1}^{N}(x_i - \mu)^2}.$$

$$\sigma^2 = \frac{\Sigma(x_i - \mu)^2}{N}$$

$$= \frac{(21 - 20.5)^2 + \ldots + (20 - 20.5)^2}{2}$$

$$= \frac{0.5}{2}$$

$$= 0.25$$

$$\sigma = \sqrt{0.25}$$

$$= 0.5$$

Margin of Error (Confidence Interval)
The sampling mean most likely follows a normal distribution. In this case, the standard error of the mean (SEM) can be calculated using the following equation:

$$\sigma_{\bar{x}} = \frac{\sigma}{\sqrt{N}} = 0.35355339059327$$

Based on the SEM, the following are the margins of error (or confidence intervals) at different confidence levels. Depending on the field of study, a confidence level of 95% (or statistical significance of 5%) is typically used for data representation.

Confidence Level	Margin of Error	Error Bar
68.3%, $\sigma_{\bar{x}}$	20.5 ±0.354 (±1.72%)	
90%, $1.645\sigma_{\bar{x}}$	20.5 ±0.582 (±2.84%)	
95%, $1.960\sigma_{\bar{x}}$	20.5 ±0.693 (±3.38%)	
99%, $2.576\sigma_{\bar{x}}$	20.5 ±0.911 (±4.44%)	
99.9%, $3.291\sigma_{\bar{x}}$	20.5 ±1.164 (±5.68%)	
99.99%, $3.891\sigma_{\bar{x}}$	20.5 ±1.376 (±6.71%)	
99.999%, $4.417\sigma_{\bar{x}}$	20.5 ±1.562 (±7.62%)	
99.9999%, $4.892\sigma_{\bar{x}}$	20.5 ±1.73 (±8.44%)	

September, 2019
Room 104
Standard Deviation, σ: **2**
Count, N: 2
Sum, Σx: 160
Mean, μ: 80
Variance, σ^2: 4

Steps

$$\sigma = \sqrt{\frac{1}{N}\sum_{i=1}^{N}(x_i - \mu)^2}.$$

$$\sigma^2 = \frac{\Sigma(x_i - \mu)^2}{N}$$

$$= \frac{(82 - 80)^2 + \ldots + (78 - 80)^2}{2}$$

$$= \frac{8}{2}$$

$$= 4$$

$$\sigma = \sqrt{4}$$

$$= 2$$

Margin of Error (Confidence Interval)
The sampling mean most likely follows a normal distribution. In this case, the standard error of the mean (SEM) can be calculated using the following equation:

$$\sigma_{\bar{x}} = \frac{\sigma}{\sqrt{N}} = 1.4142135623731$$

Based on the SEM, the following are the margins of error (or confidence intervals) at different confidence levels. Depending on the field of study, a confidence level of 95% (or statistical significance of 5%) is typically used for data representation.

Confidence Level	Margin of Error	Error Bar
68.3%, $\sigma_{\bar{x}}$	80 ±1.414 (±1.77%)	
90%, $1.645\sigma_{\bar{x}}$	80 ±2.326 (±2.91%)	
95%, $1.960\sigma_{\bar{x}}$	80 ±2.772 (±3.46%)	
99%, $2.576\sigma_{\bar{x}}$	80 ±3.643 (±4.55%)	
99.9%, $3.291\sigma_{\bar{x}}$	80 ±4.654 (±5.82%)	
99.99%, $3.891\sigma_{\bar{x}}$	80 ±5.503 (±6.88%)	
99.999%, $4.417\sigma_{\bar{x}}$	80 ±6.247 (±7.81%)	
99.9999%, $4.892\sigma_{\bar{x}}$	80 ±6.918 (±8.65%)	

Room 107
Standard Deviation, σ: **6**
Count, N: 2
Sum, Σx: 132
Mean, μ: 66
Variance, σ^2: 36

Steps

$$\sigma = \sqrt{\frac{1}{N}\sum_{i=1}^{N}(x_i - \mu)^2}.$$

$$\sigma^2 = \frac{\Sigma(x_i - \mu)^2}{N}$$

$$= \frac{(60 - 66)^2 + \ldots + (72 - 66)^2}{2}$$

$$= \frac{72}{2}$$

$$= 36$$

$$\sigma = \sqrt{36}$$

$$= 6$$

Margin of Error (Confidence Interval)
The sampling mean most likely follows a normal distribution. In this case, the standard error of the mean (SEM) can be calculated using the following equation:

$$\sigma_{\bar{x}} = \frac{\sigma}{\sqrt{N}} = 4.2426406871193$$

Based on the SEM, the following are the margins of error (or confidence intervals) at different confidence levels. Depending on the field of study, a confidence level of 95% (or statistical significance of 5%) is typically used for data representation.

Confidence Level	Margin of Error	Error Bar
68.3%, $\sigma_{\bar{x}}$	66 ±4.243 (±6.43%)	
90%, $1.645\sigma_{\bar{x}}$	66 ±6.979 (±10.57%)	
95%, $1.960\sigma_{\bar{x}}$	66 ±8.316 (±12.60%)	
99%, $2.576\sigma_{\bar{x}}$	66 ±10.929 (±16.56%)	
99.9%, $3.291\sigma_{\bar{x}}$	66 ±13.963 (±21.16%)	
99.99%, $3.891\sigma_{\bar{x}}$	66 ±16.508 (±25.01%)	
99.999%, $4.417\sigma_{\bar{x}}$	66 ±18.74 (±28.39%)	
99.9999%, $4.892\sigma_{\bar{x}}$	66 ±20.755 (±31.45%)	

Room 233
Standard Deviation, σ: **6**
Count, N: 2
Sum, Σx: 68
Mean, μ: 34
Variance, σ^2: 36

Steps

$$\sigma = \sqrt{\frac{1}{N}\sum_{i=1}^{N}(x_i - \mu)^2}.$$

$$\sigma^2 = \frac{\Sigma(x_i - \mu)^2}{N}$$

$$= \frac{(40 - 34)^2 + \ldots + (28 - 34)^2}{2}$$

$$= \frac{72}{2}$$

$$= 36$$

$$\sigma = \sqrt{36}$$

= 6

Margin of Error (Confidence Interval)
The sampling mean most likely follows a normal distribution. In this case, the standard error of the mean (SEM) can be calculated using the following equation:

$$\sigma_{\bar{x}} = \frac{\sigma}{\sqrt{N}} = 4.2426406871193$$

Based on the SEM, the following are the margins of error (or confidence intervals) at different confidence levels. Depending on the field of study, a confidence level of 95% (or statistical significance of 5%) is typically used for data representation.

Confidence Level	Margin of Error	Error Bar
68.3%, $\sigma_{\bar{x}}$	34 ±4.243 (±12.48%)	
90%, $1.645\sigma_{\bar{x}}$	34 ±6.979 (±20.53%)	
95%, $1.960\sigma_{\bar{x}}$	34 ±8.316 (±24.46%)	
99%, $2.576\sigma_{\bar{x}}$	34 ±10.929 (±32.14%)	
99.9%, $3.291\sigma_{\bar{x}}$	34 ±13.963 (±41.07%)	
99.99%, $3.891\sigma_{\bar{x}}$	34 ±16.508 (±48.55%)	
99.999%, $4.417\sigma_{\bar{x}}$	34 ±18.74 (±55.12%)	
99.9999%, $4.892\sigma_{\bar{x}}$	34 ±20.755 (±61.04%)	

Room 237
Standard Deviation, σ: **16**
Count, N: 2
Sum, Σx: 76
Mean, μ: 38
Variance, σ^2: 256

Steps

$$\sigma = \sqrt{\frac{1}{N}\sum_{i=1}^{N}(x_i - \mu)^2}.$$

$$\sigma^2 = \frac{\Sigma(x_i - \mu)^2}{N}$$

$$= \frac{(54 - 38)^2 + \ldots + (22 - 38)^2}{2}$$

$$= \frac{512}{2}$$

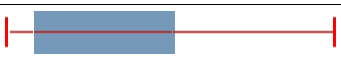
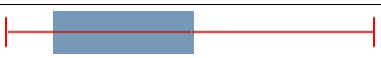
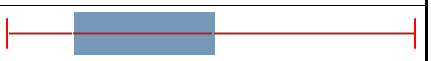
$$= 256$$

$\sigma = \sqrt{256}$
$= 16$

Margin of Error (Confidence Interval)
The sampling mean most likely follows a normal distribution. In this case, the standard error of the mean (SEM) can be calculated using the following equation:

$$\sigma_{\bar{x}} = \frac{\sigma}{\sqrt{N}} = 11.313708498985$$

Based on the SEM, the following are the margins of error (or confidence intervals) at different confidence levels. Depending on the field of study, a confidence level of 95% (or statistical significance of 5%) is typically used for data representation.

Confidence Level	Margin of Error	Error Bar
68.3%, $\sigma_{\bar{x}}$	38 ±11.314 (±29.77%)	
90%, $1.645\sigma_{\bar{x}}$	38 ±18.611 (±48.98%)	
95%, $1.960\sigma_{\bar{x}}$	38 ±22.175 (±58.35%)	
99%, $2.576\sigma_{\bar{x}}$	38 ±29.144 (±76.70%)	
99.9%, $3.291\sigma_{\bar{x}}$	38 ±37.233 (±97.98%)	
99.99%, $3.891\sigma_{\bar{x}}$	38 ±44.022 (±115.85%)	
99.999%, $4.417\sigma_{\bar{x}}$	38 ±49.973 (±131.51%)	
99.9999%, $4.892\sigma_{\bar{x}}$	38 ±55.347 (±145.65%)	

Room 303
Standard Deviation, σ: **2.5**
Count, N: 2
Sum, Σx: 39
Mean, μ: 19.5
Variance, σ^2: 6.25

Steps

$$\sigma = \sqrt{\frac{1}{N}\sum_{i=1}^{N}(x_i - \mu)^2}.$$

$$\sigma^2 = \frac{\Sigma(x_i - \mu)^2}{N}$$

$$= \frac{(17 - 19.5)^2 + \ldots + (22 - 19.5)^2}{2}$$

$$= 12.5$$

$$= 6.25$$

$$\sigma = \sqrt{6.25}$$

$$= 2.5$$

Margin of Error (Confidence Interval)

The sampling mean most likely follows a normal distribution. In this case, the standard error of the mean (SEM) can be calculated using the following equation:

$$\sigma_{\bar{x}} = \frac{\sigma}{\sqrt{N}} = 1.7677669529664$$

Based on the SEM, the following are the margins of error (or confidence intervals) at different confidence levels. Depending on the field of study, a confidence level of 95% (or statistical significance of 5%) is typically used for data representation.

Confidence Level	Margin of Error	Error Bar
68.3%, $\sigma_{\bar{x}}$	19.5 ±1.768 (±9.07%)	
90%, $1.645\sigma_{\bar{x}}$	19.5 ±2.908 (±14.91%)	
95%, $1.960\sigma_{\bar{x}}$	19.5 ±3.465 (±17.77%)	
99%, $2.576\sigma_{\bar{x}}$	19.5 ±4.554 (±23.35%)	
99.9%, $3.291\sigma_{\bar{x}}$	19.5 ±5.818 (±29.83%)	
99.99%, $3.891\sigma_{\bar{x}}$	19.5 ±6.878 (±35.27%)	
99.999%, $4.417\sigma_{\bar{x}}$	19.5 ±7.808 (±40.04%)	
99.9999%, $4.892\sigma_{\bar{x}}$	19.5 ±8.648 (±44.35%)	

Room 307

Standard Deviation, σ: **8.5**
Count, N: 2
Sum, Σx: 37
Mean, μ: 18.5
Variance, σ^2: 72.25

Steps

$$\sigma = \sqrt{\frac{1}{N}\sum_{i=1}^{N}(x_i - \mu)^2}.$$

$$\sigma^2 = \frac{\Sigma(x_i - \mu)^2}{N}$$

$$= \frac{(10 - 18.5)^2 + \ldots + (27 - 18.5)^2}{2}$$

$$= \frac{144.5}{2}$$

$$= 72.25$$

$$\sigma = \sqrt{72.25}$$

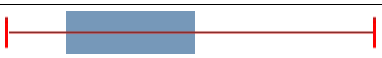
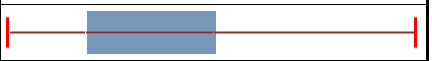
$$= 8.5$$

Margin of Error (Confidence Interval)

The sampling mean most likely follows a normal distribution. In this case, the standard error of the mean (SEM) can be calculated using the following equation:

$$\sigma_{\bar{x}} = \frac{\sigma}{\sqrt{N}} = 6.0104076400857$$

Based on the SEM, the following are the margins of error (or confidence intervals) at different confidence levels. Depending on the field of study, a confidence level of 95% (or statistical significance of 5%) is typically used for data representation.

Confidence Level	Margin of Error	Error Bar
68.3%, $\sigma_{\bar{x}}$	18.5 ±6.01 (±32.49%)	
90%, $1.645\sigma_{\bar{x}}$	18.5 ±9.887 (±53.44%)	
95%, $1.960\sigma_{\bar{x}}$	18.5 ±11.78 (±63.68%)	
99%, $2.576\sigma_{\bar{x}}$	18.5 ±15.483 (±83.69%)	
99.9%, $3.291\sigma_{\bar{x}}$	18.5 ±19.78 (±106.92%)	
99.99%, $3.891\sigma_{\bar{x}}$	18.5 ±23.386 (±126.41%)	
99.999%, $4.417\sigma_{\bar{x}}$	18.5 ±26.548 (±143.50%)	
99.9999%, $4.892\sigma_{\bar{x}}$	18.5 ±29.403 (±158.93%)	

APPENDIX II

Solution A

(Crystal violet (Huckers)

Crystal violet (90 % dye content) 2.0 g

Ethyl alcohol (95 %) 20.0 ml

Solution B

Ammonium oxalate 0.8 g

Distilled water 80.0 ml Mixed solution A and B

Gram's iodine

Iodine 1.0 g

Potassium iodide 2.0 g

Distilled water 300.0 ml

Ethyl alcohol (95 %)

Ethyl alcohol (100 %) 95.0 ml

Distilled water 5.0 ml

Safranine

Safranine 0.25 ml

Ethyl alcohol (95 %) 10.0 ml

Distilled water 100.0 ml

Kovac's reagent for detection of indole

P-Dimethylaminobenzaldehyde 5.0 g

Amyl alcohol 75.0 ml Hydrochloric acid (concentrated) 25.0 ml

Dissolved the p-Dimethylaminobenzaldehyde in the amyl alcohol and added the hydrochloric acid.

Nutrient Agar

Nutrient agar (digest of animal tissue 5.00, beef extract 1.50, yeast extract 1.50, sodium chloride 5.00, agar 15.00 g/l) and final pH (at 25 °C) 7.4 ± 0.2 was prepared by suspending 28.0 g in 1000 ml of distilled water. Heated to boiling to dissolve the medium completely then sterilized by autoclaving at 15 lbs pressure (121 °C) for 15 min.