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Bacteria Agents of Respiratory Tract Infection Among Sanitary Worker in Uturu, Abia State

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Abstract:- Micro-organisms associated with respiratory exudate (sputum) from sanitary workers in Uturu, Abia state were investigated using sputum culture on different culture media. The bacteria isolated were *Bacillus cereus, Staphylococcus aureus, Pseudomonas aeruginosa, Corynebacterium diphtheriae, Mycobacterium tuberculosis* and *Streptococcus pneumonia* species. The bacterium most prevalent was *C. diphtheriae* species (69%). The followed by *S.aureus* (65%). The latest prevalent was *P. aeruginosa* (12%). Individuals of 20 to 40 years were more infected than the other age groups. The use of masks was found to reduce the inhalation of the organisms from air. The full time sanitary workers were more significantly infected than the part time ones. Seasonal influence also played significant role as the frequency of infection was higher in the dry season than the rainy season via drop-let air infection and equally via food-borne infection.

Keywords:- *Bacteria; Exudates; Respiratory Tract; Sanitary Workers; Isolates; Infection.*

I. INTRODUCTION

The respiratory system is a system concerned with respiration in living organisms. Respiration is the process by which substances are broken down and oxidized in the living cells of organisms of release energy while carbon (iv) oxide and water vapour are released as waste products.

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6H_2O + \text{Energy}$$

Respiration tract is the rout via the nostrils that opens to the external surface down to the alveoli.

➢ *The Normal Characteristics of the Respiratory Tract are:*

- Flat surface to increase area/ volume ratio
- Large surface are for gaseous exchange
- Moist surface that permit gases to diffuse in solution
- Thin cell covering to permit for mobility of dissolved gases
- Rich supply of blood
- Highly vascularized lungs and internal gills.

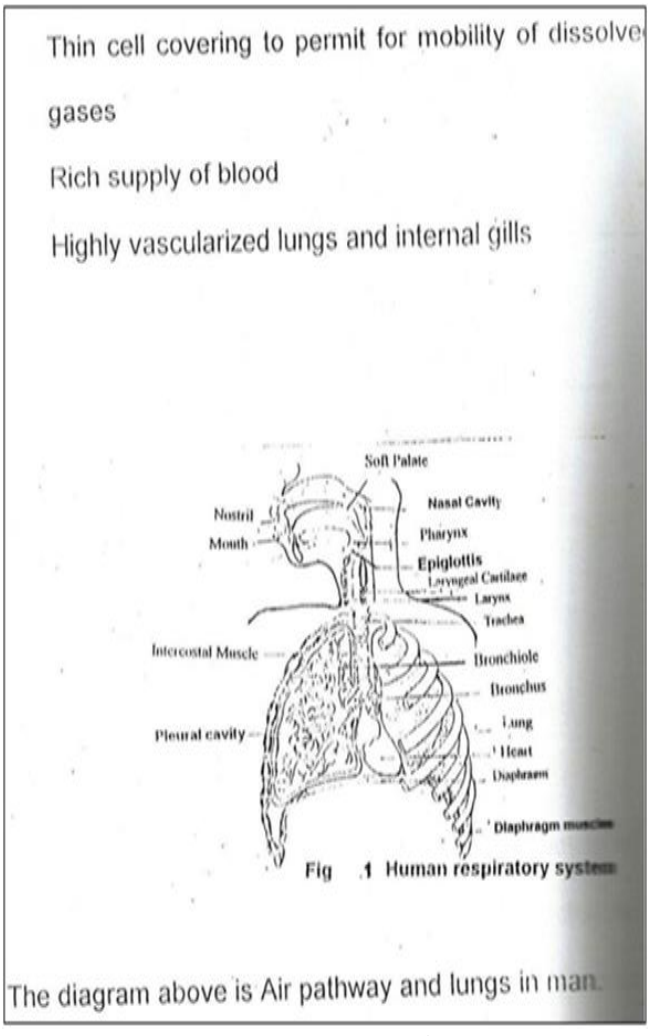

Fig 1 Human Respiratory Systems

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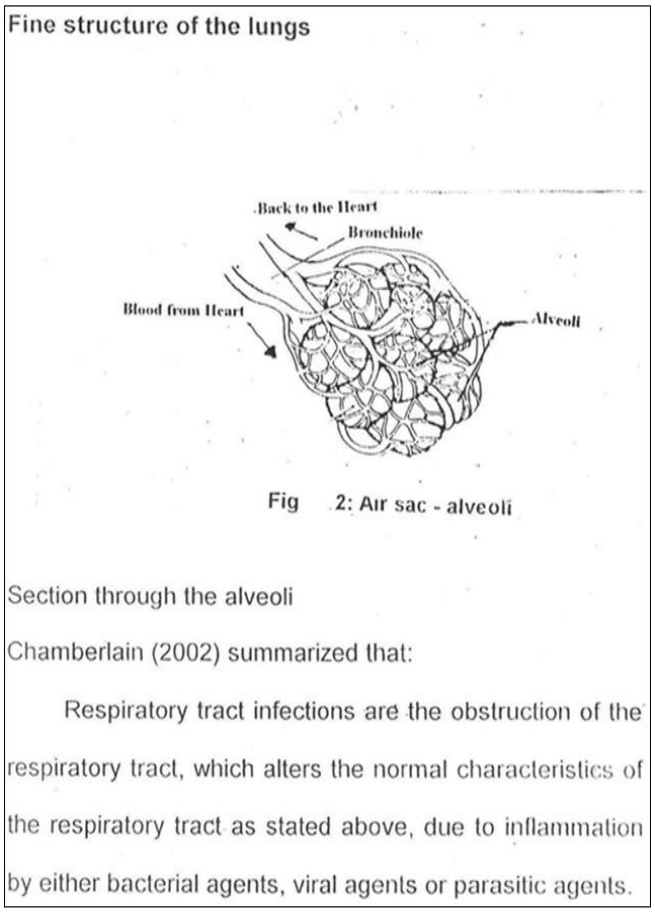

Fig 2 Air Sac-alveoli

Precisely in this research, major effort is channeled toward bacterial agents of the respiratory tract infection in Uturu Abia State Nigeria. The pathogenesis of this infection arise from the inhalation of contaminated air from person to person, from infected soil, through poor hygiene, consumption of baked food that has been stored for long, consumption of tuberculous infected pork and beef meat that are not well cooked as well as non-adherence to immunosuppressive and debilitated patients.

The transient variable of air microbial flora depends on a number of factors. Species of microbes in any given environment is partly constant to vehicle of transmission such as person to person and partly varies with the source of contamination, extrinsic factors and the nature of the organisms themselves (Pelczar *et al*., 1993). The number of microorganisms in the air usually directly varies with the amount of dust. Since most of this sanitary workers irrespective of the level of their health, respire or work around dust particles, though in Brownian motion but do not multiply in this medium also these sanitary waste in wet form also emit malodorous fumes which act as a chemoattractant to these microbes thus multiplying profusely around these waste products.

Some of these bacteria agents are carried via the smoke and soot. Chamberlain (2002) argues that the average individual ingests/ inhales about 8 microorganisms per minute or 10,000 per day.

While some of these aerial organisms survive for a few seconds, others stay alive for weeks or months. (Jawetz *et al*., 2001, Pelczar *et al*.,1993) the extensive factors for the bacteria survival in air range from atmosphere conditions such as humidity, temperature, the microbe and composition of the dust particles carrying the organism. Although, it has not yet been proved of having a known indigenous microorganism in air rather various types of microorganisms (bacteria) find themselves being mobilized by air currents. Most of these organisms are translocate into the air through direct and indirect human activities.

With the recent rapid air pollution due to human activities such as indiscriminate dumping of refuse, deposition of faecal matters on the top soil, use of power generating sets, car exhausts, smoke inhalation, inhalation of malodourous fumes from indiscriminate dumped refuse, allow an easier translocation of the bacteria agents to a great distance.

Infectious diseases are spread by way of droplets in the air (air propagules), a route which gives rise chiefly respiratory tract diseases. Sneezing, coughing, laughing and talking releases microbial droplets, which get dry and are carried wind. The wind lifts light sand or dust particle and leaves the heavy dried ones. (Pelczar *et al*.,1993) argues that these microorganisms which find themselves in the air constitute the microorganisms of the air in the immediate vicinity.

Bacteria agents of diverse numbers and species have been isolated from the air like *Corynebacterium species, Mycobacteria species , Pseudomonas species and bacillus species*.

Among the molds, most are found in air composition especially their spore. Since these organisms are mainly soil inhabiting and requires open environment for natural growth and multiplication (Pelczar *et al*., 1993). Therefore they have high probability of being carried into the air by wind.

The coatings found in spores and mold enable them exist in some adverse environmental conditions thereby prolonging their survival while being carried over a distance. Such as molds, and yeast organisms isolated by various researchers from air. (Thomas *et al*., 1992, Pelczar *et al*., 1993). Another transient particle associated with sanitary workers are the viruses such as measles virus, polio virus, influenza viruses have also been isolated from the air (Greenwood *et al*., 1992).

Though not all the bacteria agents of respiratory tract infection are constantly found in air despite the significant role played by air disease transmission.

These bacteria agents are inhaled along with air during respiration. A large number of human infections are air borne which are usually disease of the respiratory tract. The prominent among them are Diphtheria, influenza, tuberculosis, cough, pneumonia, sore throat and common cold.

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Respiratory diseases have the characteristics of occurring in epidemic forms, attacking large number of persons in a short time, in a particular environment and seasonally too.

They also spread profusely in crowded area with a lot of human activities.

A respiratory discharge of exudate by single cough or sneeze from an infected person can introduce up to 10000 infection doses of pathogens into the air, and unto eating utensils, bed linen, clothing and fingers.

Bacteria agents that pass the larynx are caught in the bronchi thereby making the inspired air free of organisms. The moist films that cover the mucosa of the upper respiratory tract and in which bacteria remove from inspired air are embedded, consist of mucus (thin viscid substances), former notion is useless (Brook and Madigan 1992). In addition it has been argued that the normal secretion of the respiratory from lysozymes.

Though from observation, the outline defence mechanism of the respiratory tract, there are mechanical expulsion, phagocytic effect and others. Some of the organisms still cause respiratory tract infection. Therefore, it is noteworthy to say that it is only those organisms that circumvent the host defence system that will cause the respiratory tract infection at a given condition (depressed), which now raise the case of technique, experience, of medical importance to respiratory pathogens from the commensals of the normal flora of the respiratory system. Such flora include staphylococcus specie,alpha hemolytic streptococci or viridans streptococci, Moraxella (formerly Brahamella) caterrhalis (Chamberlain 2002).

Some of these microorganisms may cause disease under certain circumstances, either due to previous damage by viral infection, loss of immunity or because of physical damage to respiratory tract epithelium especially from smoking this could predispose the respiratory tract to infection by some of these normal flora.

The present of *Corynebacterium* species in the respiratory tract of healthy humans has been reported by various authors. For instance (Dublanchet *et al.,* 1988) cited that approximately 59% of normal healthy adults carry these organisms in their anterior naves.

Hospital personnel anterior often have higher carrier rates than the human population at large. These bacteria agents are often resistant to one or more antibiotics in use. However, there are two types of normal body flora, the resident flora and the transient relatively fixed types of microorganism regularly found in a given area at a given resident flora and the transient relatively fixed types of microorganism regularly found in a given area at a given age, when disturbed it promptly re-establishes itself. While the later consists of non-pathogenic or potentially pathogenic microorganisms that inhabit the skin or mucus membranes for hours, days or weeks. It is derived from the environment and does not establish itself permanently on the surface. The transient flora is of little significance as long as the normal resident flora remains intact, but if the resident flora is disturbed, the transient microorganisms (Bacteria) may colonize, proliferate and produce diseases.

Inhalation of malodourous fumes from decaying taste produce is encouraged by certain human occupation, while the normal inspiration of air is a natural process. The fast vibration against the line of air current forces particles in the air into the respiratory tract, the speed often reduces the efficiency of the natural absorbing function of the mucosa, resulting in the passage of bacteria and other microorganisms the nasal mucosa surface (Brook and Madigan 1991). The situation has led to the use of facial, nasal or mouth, masks by professional sanitary workers to reduce the force with which air currents and some free radicals are being projected toward the respiratory tract.

However, the lack of job opportunities and the urge to seek of white cola job in addition to harsh economic conditions in Nigeria and some other parts of the under developed and developing African Countries has forced some individuals to seek for some of the ill health job such as daily cleaning of the toilets and bathrooms disposing faecal matters and other decaying waste products, and daily sweeping and cleaning of the roads as a means of daily living.

These commercial sanitary workers. In Igbo speaking areas of Nigeria are known and called “Ndi na edota obodo ocha” most of them do not use mask while cleaning up surrounding in a haste to speed up and cover a large area and to make more money per day.

These results in inhalation of sorts of particles microorganisms, giving rise to various form of respiratory tract infection. The early morning and the afternoon shift without adequate body covering also exposes or predisposes these health workers to cold, pneumonia, very high temperature and other forms of air borne infections.

Uturu is one of such areas that make use of, and employ health workers in the form of sanitary workers in and outside the University premises.

Uturu is a very dusty area and since the number of microorganisms in the air directly varies as the amount of dust particle in the air. It is assumed that the air within Uturu is filled with lots of microbes (Bacteria) and other free radicals.

In the light of the above, the research work is aimed at isolating and identifying the bacteria agents found in the respiratory track of the Commercial Sanitary Workers. In Uturu: The data obtained will be analyzed in relation to:

- Age of operation
- Time of operation
- Period of involvement
- Use of masks

- Seasonal influence

The information obtained will be disseminated on the target population through public enlightenment campaigns and primary health care programs.

II. SAMPLE COLLECTION

Approved Ethical clearance permission was first obtained from the Head of Department Microbiology, Abia State University, Uturu, thereafter, the purpose of the study was explained to the sanitary workers and their consents were also obtained for their inclusion in this study.

Questionnaire was used in selecting the workers mainly of (18 – 50) years.

Table 1 a List of Districts for Study Groups of Sanitary Workers Suspected of Respiratory Infection.

Group		Population
A	Ukwu Nwangwu	35
B	Mary bus stop	10
C	Administrative block	20
D	ABSU work ways	20
E	Hostels (ABSU)	25
F	Class room (ABSU)	

- Since the groups were ethically and socioeconomically balanced.
- Four groups with the largest population of sanitary workers with respiratory tract infections were chosen and designed as follows.

Table 1 (b) List of 4 Selected for the Study

Study Group	Controls
Group A = 35	Group D= 20
Group C = 20	Group E = 25
55	45

➢ *Area of Study*

The study area is Uturu, a town at the southern apex of Abia State at boundary Imo State. Most of its residents are Civil servants, traders and farmers.

The use of mechanized instruments in disposing these waste products were few while the use of hand rake, long brooms, cutlasses and waste baskets were extensively used as materials or equipment for sanitary workers.

Most of the streets and surrounding roads are either untarred of roughly graded hence dust. In the University compound the water system of the toilet rooms are dilapidated even the bathrooms. The water run ways (gutter) were littered with refuse and waste water that percolate on the surface thus effusing malodorous substance and eventually trapping highly virulent Microorganism around them.

➢ *Subject of Study*

One hundred of the fully registered Commercial sanitary workers were randomly chosen for this work. Individual between the ages of 18 – 50 were screened in the work.

➢ *Population Sample and Sampling*

Technique among all samples, the one selected at random as study subject are used for designing this research project, are called sampling.

The study subject within the area of study are designate as “odd” numbered (from the alphabetical list of the people with respiratory tract infection) and the controls as “even” numbered

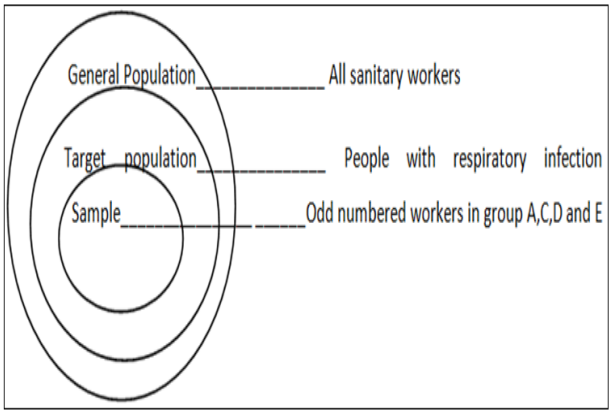

Fig 3 Procedure for Selection of Sample, it is Derived from Above

➢ *Instrument for Data Collection*

Structural personal interview question were set out and focus group interviews the multiple choice items was encouraged. See Appendix 1.

➢ *Validity and Reliability of the Instrument.*

The validation of this instrument dealt with the description of the procedure adopted as stated above which ensured that the instrument measured what it was designed to assess and measure.

In this case the question asked at random made it possible that the inference were ascertained either by deduction or by induction method.

Deduction in the sense that after responding “I do smoke, I do have chest pain, I vomit blood sometime, sore throat.

Inference = respiratory tract infection. Every smoker and sanitary worker malnourished is infested with respiratory tract disease; Onuoha Friday is a sanitary worker with poor nutrient.

Onuoha Friday is infested with respiratory tract disease. In addition, the test-retest also applied.

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➢ *Specimen Collection and Handling*

The following guidelines were designed to be used by the laboratory personnel that help for collecting and transporting the respiratory exudates specimens to the bacteria laboratory.

The clinical microbiology laboratory played a critical role during patient care, but the value of its results is dependent upon specimen handling specimen handling involves proper selection. Appropriate collection and timely transportation of the specimen to microbiology laboratory which were strictly adhered to microbiology laboratory can be of little value to the physician and thus offer only minimal service to patient care if the specimens are improperly handled.

➢ *Principles of Specimen Collection*

It is obviously a truism that the test be no better than the specimen on which it was made. Therefore, the laboratory was relied on the careful collection of the specimen in an accurate and standardize manner. The welfare of the patient rests not only on the laboratory analysis and the physician's interpretation, but also on the way in which the specimen was obtained and transmitted to the laboratory. There are many variables that were encountered and adhered to for adequate handling of the specimens and all were considered separately to avoid critical errors. The factors that were considered are moisture, time of collection, labeling, handling container transportation, temperature and atmosphere.

➢ *Moisture*

The sputum specimen collected were submitted collected were submitted moist to the laboratory.

Most bacteria cannot survive in a dry environment especially the pathogenic ones e.g. *Corynebacterium* species and *Mycobacterium* species. Therefore dry swabs were of no value, the throat swab specimens collected were delivered at most and immediately to the laboratory before it dries out.

➢ *Time of Collection*

All the specimens collected, were taken before antibiotics are administered. But if already antibiotics have been stated, then the requisition sheets were marked, so that everyone will be aware.

The timing of sputum specimens was very important, detection of pathogenic process of the organism with some disease, the bacteremia occurs only in the early stages of the infection, while in other cases there were continuous presences of bacteria. In many cases the presence of bacteria in sputum is transient and can be best found after a chill when patient is spiked with fever. During a chill the bactericidal properties of sputum are accentuated; the alveoli and the micro-vessels constrict. And become clogged with cells and during the chill.

➢ *Labeling and Handling Containers*

All containers used for the specimen collection were sterile. The patient was instructed to handle the container as aseptically as possible i.e. not to touch the inside of the container, laying lid down in such a way to contaminate it, leaving the lid off for an excessive amount of time etc.

Whenever any of the specimen spills on the outside it was immediately cleaned with disinfectant. The lid was secured tightly and the container transported with care to insure against spillage.

- All containers were labeled clearly with patient's ID Group number and source of specimen. (sputum)
- All specimens from Uturu were sent in zip-lock bags to microbiology laboratory (ABSU) Abia State University Uturu.
- All specimens were accompanied by a requisition sheet completely filled out. The requisition sheet includes information on the specimen container as well as my supervisors, examination requested, time specimen was collected, laboratory diagnosis.

➢ *Effect of Temperature*

Most of the microorganisms found in the clinical specimens have range of temperature of 37°c and most have a broad range of temperature tolerance, however respiratory pathogens die rapidly when subjected to temperatures below or above their optimal requirements. No specimen collected (sputum) were refrigerated, rather, were delivered immediately to the laboratory after collection.

➢ *Effect of Atmosphere*

The atmosphere plays a very important role in isolation and identifying pathogenic bacteria. The two principal gases that effect metabolism of the bacteria are oxygen and carbon dioxide. Most sputum specimen require small amount of varying concentration of $C0_2$.

In terms anaerobic organisms, the specimen was placed in an oxygen-free environment with 30 minutes after collection. Port-A-cul vials and vacutainer brand anaerobic swabs were meant to be used.

III. PRINCIPLES FOR THE DIAGNOSIS OF THESE BACTERIA ISOLATES FROM THE HUMAN RESPIRATORY EXUDATES

A tentative diagnosis of respiratory diseases are made by using different stains on the direct smear of sputum each etiologic agent has its particular stains for identification.

Also the cultural methods are employed and are considered more reliable than staining direct smears, because only few organisms are necessary for growth and finally the sensitivity test which indicates the most effective drug for the disease treatment. And to differentiate respiratory normal flora from pathogenic isolates.

A. Sterilization of Wares

Mostly petri-dishes (glass) bottles and other glass wares are washed thoroughly with detergent dried on the racks and packed into the hot air oven. Then they allowed to cool at room temperature before use.

➢ *Preparation of Media*

Commercial available dehydrated preparations were used for all studies. All the agar media used for this research were dissolved in distilled water after weighing in a calibrated scale, then steamed for 15minutes and autoclaved at 121° C (at 1.05kg/Cm^3 pressure) for 15minutes. All the media used were dispensed per sterilized 9cm diameter petri-dish.

Culture media used are namely Blood agar. Nutrient Algar and Mac Conkey agar, Agar Slant preparation.

- *Nutrient Agar*

Nutrient Agar powder was obtained commercially its composition includes meat extract, peptone from meat and agar. This medium is a general purpose media as it supports the growth of major bacteria. Twenty-eight grams of the agar powder was dissolved in 1 litre of distilled water and is sterilized by autoclaving at 121°C 15minutes. The sterile media is allowed to cool to 45°C and and poured into sterile Petri-dishes in about 20ml aliquots the plates were allowed to set after which they were kept in the inoculation chamber ready for use.

- *Blood Agar*

After the preparation of blood agar base using the same method as in nutrient agar preparation but now twenty-five grams in 1 litre of distilled water mixed, and sterilized.

It was liquefied by warming in an autoclave at 50^0c. The sterile defibrinated blood 10ml was added aseptically and thoroughly mixed by rotation (of tube) with the blood agar base. The sterile agar was poured into the sterile petri-dishes and was left to solidify. After which it was kept in the inoculation chamber ready for use.

- *Mac Conkey Agar*

This medium was obtained in powdered form. This is a differential medium primarily meant to differentiate lactose fermenters, which show pale colonies.

It is composed of peptone, lactose, bile salt, sodium chloride, metal pod indicator and agar. Following the manufacturer's instruction: Fifty-two grams of the agar powder was suspended in 1 litre of distilled water.

The mixture was sterilized by autoclaving at 121^0c for i5minutes. Then plates were allowed to set after which they were stored at 4^0C in the refrigerator or inoculation chamber ready for use.

- *Agar Slants Preparation*

All the above mentioned medium were prepared by the following methods. Each 32.5grams of Blood agar, 14 grams of nutrient agar were weighed out and added to four separate conical flasks containing 500mls of distilled water each they were thoroughly mixed and the powder well dissolved.

Three millitres of already boiled agar medium were poured into clean bijoux bottles and covered (not too tightly). The bijoux bottles and their contents were placed in an autoclave and sterilized at 121^0C at 15 psi for 15minutes.

After sterilization the bijoux bottles were placed in a slanting position on a disinfected table. The agar medium solidifies into slants; which can be used for isolate preservation.

B. Culturing Specimen

The throat swabs collected were streaked on the media plates within 30 minutes of collection, the plates inoculated were Blood agar, Nutrients agar, MacConkey agar. The method streaking was by rolling swaps firmly but gently over a small portion of the agar surface. For the Blood agar, MacConkey agar and nutrient agar plates, a wire loop sterilized by flaming was used in spreading the inoculums by streaking over the rest of the plate to obtain well isolate colonies. The plates were inoculated at 37^0C for 24-48 hours in an incubator. After 24 hours, the plates were examined for the presence of colonies of bacteria.

Smears of selected colonies were made on slides which were subsequently gram stained, Albert stained and acid fast stained and examined microscopically.

- Gram stain = *P. aeruginosa, B. cereus. S. aureus, S. pneumoniae,*
- Albert stain = *C. diphtheriae*
- Acid fast stain = *M. tubercuosis*

➢ *Staining Techniques.*

Different stains were used depending on the Bacteria in check out for.

➢ *Gram Staining*

It was the preliminary staining reaction used to characterize the different isolates from the Nutrient agar, Blood agar and MacConkey agar plates.

According to the different Gram stain reaction, easy identification of Gram positive and gram negative microorganisms were made the following reagents were used.

Crystal violet, Gram iodine, ethanol and carbolfulschin.

Individual smears were made on grease free slides, allowed to dry and heat fixed by passing it over a Bunsen flame severally. The smears were flooded with Gram's iodine for 30 seconds, after which they were rinsed with water and decolorized with ethanol slides were rinsed with water and counter stained with carbolfulscin for 30 seconds, the counter stained were rinsed off with water, blot dried

and examined microscopically using oil immersion objective lens.

➢ *Acid Staining*

A differential staining to distinguish acid fast bacteria, because of their relatively impermeable and resistant to simple stains except in the presence of hot strong reagents. Materials: Mycobacterium spp. from sputum 5-day culture on nutrient agar slant. Zeihl-Nilsens Carbolfulscin, Acid alcohol (3% conc. Hcl in 95% ethyle alcohol), Loeffler's methylene blue, Beaker, tripod stand wire–guaze, slides, loop, Bunsen burner, compound light microscope, immersion oil, supporting glass rods (2 rods taped at both ends about 4cm apart).

➢ *Procedure*

- A beaker of boiling water was set
- The supporting glass rods were then placed on the beaker
- The beaker and rods were adjusted to ensure that the rods were level
- A heat-fixed smear of the bacterium culture was prepared
- The slide was flood with strong carbolfuchsin stain. Allowed to steam for 5minutes.
- The slide now rinsed with tap water until no colour comes off the smear.
- The slide was decolourized with 3% acid alchohol until the red colour disappears.
- The slide washed slowly in running tap
- Then counter stain with the loefflers methyl lens blue for 1minute.
- Using oil immersion lens, the slide was examined, observations were made.

Alberts' stain (*Corynebacterium diphtheria*) the original stain produced by Albert used a mixture of toluidine blue and methylene green in glacial acetic acid and ethanol. But laybourne substituted malachite green for methyl green because it enhances the colour of the final preparation.

Materials: Touidine blue, malachite green, glacial acetic acid, ethanol, iodine.

➢ *Procedure*

- A smear of dilute culture was made and heat-fixed.
- The smear was flood with Albert's stain for 3minutes – 5minutes.
- The slide was washed under slow running tap.
- Air-dried
- The preparation was flood with a dilute solution of lugol's dined for 1minute.
- Washed in water and dried.
- The slide was examined under oil immersion lens (x100).

Result: Volutin granules appear blue black in colour while the cytoplasm appears blue.

C. Characterization of Bacteria

➢ *Purification of Isolates in Culture Media.*

Isolates were purified by repeatedly sub-culturing on agar medium. Bacteria Colonies on those agar media were sub-cultures, on fresh agar. After obtaining pore cultures, the isolates were studied for their microscopy, morphology, staining reaction and various differentiating Biochemical tests were carried out.

➢ *Maintainance and Preservation of Isolates*

Pure colonies of the isolates were transferred into already prepared agar slants in bijoux labeled and preserved for further use. The preserved culture were kept at temperature for further use. The preserved cultures were kept at temperature of $28+2^0$c and were categorized a 5 gram- negative and gram positive bacteria.

➢ *Characterization of Bacteria*

- *Isolates from the used Agar media.*
- *Using Biochemical test*
- *Coagulase Test*

✓ This test detects the presence of coagulase an enzyme which causes Coagulation of blood plasma.
✓ Coagulase is produced by all *S.aureus* (Duguid et al., 1978)
✓ Two forms of coagulase occur, a "free" Coagulase which was tested for on the slides.

- *Methyl Red*

This test was used to detect organisms that produced sufficient acid during the fermentation of glucose and maintain a low PH value enough to change the colour of the methyl red indicator. Glucose phosphate broth added about 5 drops of the methyl red indicator and thoroughly mixed and sterilized.

The medium was then inoculated at 37^0C for 24hours. A red colour indicates a positive result while a yellow colour donates a negative result.

- *Urease Test:*

This test was carried out using urea medium (modification of Christensen's test medium). The test was aimed at observing the ability of the organisms to split urea by means of ureas (an enzyme) produced by microorganism, the medium was dissever in 100ml of distilled water and 5ml of phenol red solution was added, the medium was dispensed into bijoux bottles and sterilized using an autoclave.

The bottles after sterilization were allowed to cool and solidify. The isolates were maculated by stabbing the solid medium with sterile inoculating needles. The culture plates were then incubated at 37^0c for 3-5 days. A change to pink colour is positive indicating urea hydrolysis.

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- *Motility Test*

This test for microorganisms was done using the hanging drop method. A drop of the test microorganism added into water suspension to make a homogenous mixture. The mixture was placed on a cover-slip. A sharp dashing movement in different direction across the field of view of compound microscope indicate a positive result.

- *Catalses Test*

Some species of microorganisms (bacteria) are able to produce the enzyme catalase, which oxidizes hydrogen peroxide to water and oxygen.

In this test, a few drops of 3% hydrogen peroxide were added to a lapful of bacteria suspension on a slide – a positive test is indicated by the gas bubbles production.

- *Indole Test*

This test was carried out as described by Harrgan and Maclan (1979). This test demonstrates the ability of certain bacteria to breakdown an amino acid, trypdophan to indole. Each of this test organism was cultured in peptone water to which Kovac's reagent was added, sterilized and incubated at 37^0C for 48hours a colour change to Pink by the medium was indicated as positive result, but when no colour change was observed, the result was negative.

- *Vogues Proskauer*

This test was carried out using MRVP medium as described by Cruickshank et al (1982). To a 2-day old MRVP medium culture incubated at 37^0C, 10% potassium hydroxide (KOH) was added gently down the tube side, so as to stand for an hour. Bacteria that produce a neutral end product from glucose or dextrose fermentation gives a pink colour at the interphase (VP positive) where as a negative result was observed when no colour formation occurred at the interphase.

IV. RESULTS

The bacterial agents isolated from the respiratory exudate of patient in *Uturu* were *C. diphtheriae, S. aureus, B. cereus, M. tuberculosis, S. Pneumoniae, P. aeruginosa*..

Table 1 shows the different organisms and their characteristics.

The most prevalent organism was *C. diphtheriae* (69%) followed by *S. aureus* (65%) and while *B. cereus* (18%) and *P. aeruginosa* (12%) were the lowest occurring bacterial from Table 2.

From Table 3, age played a significant role in the rate of occurrence of the bacteria agents.

There was gradual rise in the frequency of occurrence of the bacteria agents with age which peaked at 30-40 years old group.

Individual of below 20 years of age were infected lowest followed by those of age greater than 40 years old.

Table 4: there was a significant difference between sanitary workers that were mask and those that do not. There was a higher risk of being unmasked while working, their chances of being infected = 0.25 which means whether masked or unmasked the chances of being infected with pathogenic bacteria is partly constant and partly varies with protection of prevention measures.

From table 5: the period of involvement in the sanitary work also played a role. There was an increase in the rate of bacterial infection with increase in the period of occupation with highest infection found in period of 7-8 years then decrease at longer period because the host immune system can now form antibodies that combat and phagocytes these bacteria agents, the highest infected organism observed was *C. diphtheriae* followed by *S. aureus.*

From table 6: professional influence also played a significant role in this research fulltime sanitary worker were infected highest with *C. diphtheriae* (70%) while least infected with *P. aeruginosa* (67%). Part time sanitary workers were also most infected with *C. diphtheriae* (67.1%) followed by *S. aureus* (65.7%) while least infected with *S.pneumonia* (38.6%).

From table 7: seasonal distribution played role in the research. Both in rainy and dry season *C. diphtheriae* was the highest in occurrence in dry season(90%) while in wet season = 62% thus rainy season enhance their virulence and survival chances, this was followed by *B. cereus* with (76%) in dry season and lower during wet season (40%).

The least was *P. aeruginosa* both in dry and wet season 8% respectively.

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Table 1 Bacteria Isolated and their Characteristics

	Organism	Colony morphology	Gram reaction	Indole	Methyl red	Vogues proskavar	Citrate	Catalase	Urease	Coagulase	Motility
1	*C. diphtheriae*	Opaque gray pleomorphic shape	Refractive granules curved shaped rods	+	+	+	+	+	-	ND	-
2	*M. tuberculosis*	Beaded or granular hydrophobic rough	Retain red colour origin, thin rods chains, clump, AF+Ve acid fast+ve, oxidase+ve	-	+	+	-	+	+	ND	-
3	*S. pneumonia*	Smooth edges, raised and moist	G+, cocci in chain	-	-	+	+	-	-	ND	-
4	*B. cereus*	Gram raised colonies with entire edges	G+rods in chains of 3 and 4	-	+	-	-	+	+	ND	-
5	*P. aeruginosa*	Cream colonies spreading widely, turns yellowish green and finally brown	G-rods with polar flagella in chains and singles	-	-	-	+	+	+	ND	-
6	*S.aureus*	Opaque smooth edges concave	G+cocci in custers	-	+	-	-	+	+	+	-

G+ = Gram positive Key ND – Not Determined G-= Gram negative.

Table 2 Frequency and Percentage Frequency of Bacteria Isolated

BACTERIA	**Experiment**			**Control**		
	NE	**NP**	**%**	**NE**	**NP**	**%**
S. pneumoniae	100	47	47	10	2	20
C. diphtheria	100	69	60	10	3	30
B. cereus	100	18	18	10	1	10
S. aureus	100	65	65	10	3	30
M. tuberculosis	100	34	34	10	1	10
Pseudomonas	100	12	12	10	1	10

Key:- NE – Number Examined; NP – Number positive % - Percentage

Table 3 Frequency and Percentage Frequency of Positive Isolates with Respect to age Differences

BACTERIA	**AGE <20 NE**	**NP**	**%**	**AGE 21 -30 NE**	**NP**	**%**	**AGE 31-40 NE**	**NP**	**%**	**AGE >40 NE**	**NP**	**%**
S. pneumoniae	15	7	46.7	35	22	62.9	32	13	40.6	18	6	33.3
C. diphtheriae	15	10	66.7	35	28	80.0	32	20	62.3	18	10	35.6
B. cereus	15	3	20	35	10	28.6	32	5	15.6	18	2	11.1
S. aurues	15	8	-3	35	28	78.1	32	17	53.1	18	7	38.9
P. aeruginosa	15	2	13.3	35	7	20	32	3	9.3	18	1	5.6
M. tuberculosis	15	5	33.3	35	16	45.7	32	8	25.0	18	4	22.2

Key:- NE – Number Examined, NP – Number positive, % - Percentage

Table 4 Frequency and Percentage Frequency of Positive Isolates with Respect to the use of Mask

BACTERIA	**MASKED**			**UNMASKED**		
	NE	**NP**	**%**	**NE**	**NP**	**%**
S. Peumoniae	27	7	25.9	63	42	66.0
C. diphtheriae	27	10	17.0	63	50	79.4
B. cereus	27	11	40.0	63	53	84.1
P. aeruginosa	27	2	7.4	63	9	14.3
S. aureus	27	8	29.0	63	48	76.0
M.tuberculosis	27	3	11.1	63	43	68.0

Key NE – Number Examined, NP – Number Positive, % - Percentage

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Table 5 Frequency and Percentage Frequency of Positive Isolates with Respect to Period of Involvement in the Profession (Years)

Bacteria	**NE**	**NP**	**%**	**NE**	**NP**	**%**	**NE**	**NP**	**%**	**NE**	**NP**	**%**	**NE**	**NP**	**%**
S. Pneumoniae	35	3	8.6	37	8	21.6	15	5	33.0	8	2	25.0	5	1	20.0
B. cereus	35	14	40.0	37	20	54.1	15	8	53.0	8	3	37.5	5	1	20.0
C. diphtheriae	35	22	62.9	37	25	67.6	15	10	66.0	8	3	37.5	5	2	40.0
P. aeruginosa	35	1	2.9	37	3	8.1	15	1	6.0	8	2	25.0	5	1	20.0
S. aureus	35	16	45.7	37	23	62.2	15	10	66.0	8	4	50.0	5	1	20.0
M.tuberculosis	35	6	17.1	37	17	45.9	15	6	40.0	8	2	25.0	5	2	40.0

Key: NE – Number Examined, NP – Number positive, % - Percentage

Table 6 The Effect of Professional Influence on the Frequency of Isolation

BACTERIA	**PART TIME**					
	NE	**NP**	**%**	**NE**	**NP**	**%**
S. pneumoniae	30	8	26.0	70	27	38.6
B. cereus	30	10	33.3	70	42	60.0
C. diphtheriae	30	21	70.0	70	47	67.1
S. aureus	30	12	40.0	70	46	65.7
P. aeruginosa	30	2	6.7	70	7	10.0
M. tuberculosis	30	5	16.0	70	15	21.0

Key NE – Number Examined, NP – Number positive, % - Percentage

Table 7 The Frequency and Percentage Frequency of Bacteria Isolated with Respect to Seasonal Distribution

BACTERIA	**DRY SEASON**			**WET SEASON**		
	NE	**NP**	**%**	**NE**	**NP**	**%**
S. aureus	50	30	60	50	28	56
S. diptheriae	50	27	54	50	22	44
B. cereus	50	38	76	50	22	40
P. aeruginosa	50	5	10	50	10	20
C. diphtheriae	50	45	90	50	31	62
M. tuberculosis	50	6	12	50	9	18

Key NE – Number Examined, NP – Number positive, % - Percentage

V. DISCUSSION

Some Bacteria Isolated from the respiratory tract among sanitary workers in Uturu, have either been implicated as natural inhabitants of nose, bronchi, trachea; air contaminants and a few are associated with the upper respiratory tract infections.

C. diphtheriae occurs as the flora of the low respiratory tract and skin of healthy individuals other flora include *S. aureus s. epidermidis* and *S. diphtheria* also occur as commensals. (Jawetz *et al*., 2001).

Noting the fact that the asymptomatic carrier can be a source of *Corynebacterium* disease to himself as well as others.

C. diphtheria was isolated from the respiratory tract of 69% of sanitary workers examined. This frequency is still high but lower than observed by Wren and Shety (2001) who observed that *C. diphtheriae* occurs in approximately 75% of people with respiratory tract infection.

This observation indicates that the profession exposes the sanitary workers to more *C. diphtheria* infection (diphtheria).

S. aureus is also another Commensal which is normal inhabitant of the throat, nose and skin (Jawetz *et, al.*, 2001, Cheesbrough, 2000) reported the isolation of *S. aureus* from the upper respiratory tract of healthy people is due to inhalation of dust. *S. diphtheriae* is also a commensals which on habits the nose and skin (Jawetz *et al*., 2001: Cheesbrough, 2000) who reported the isolation of cellular immune response to the organism and their products (Saito, *et al*., 2005).

This accounts for its high frequency of occurrence in the throat of these sanitary workers.

B. cereus are established spore formers, whose spores could be carried about by wind. They are very ubiquitous hence could be found in the respiratory tract due to inhalation of air. This accounts for its high frequency of occurrence in the throats of these sanitary agencies.

P.aeruginosa have been implicated in several human infections including respiratory infection (Jawetz *et al*., 2001). Continuous exposure of the body to different weather and atmosphere predisposes one to pseudomonas infection as the bacteria occur widely in soil, water, animals and plant (Jawetz *et al*., 2001). Those sanitary workers are erroneously exposed to harsh weather conditions and they do not dress on protective clothing like rain coats, mouth and nose masks and sweaters.

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Age played a very significant role in the situation of the various bacteria. This is because age influenced the number of people engaged in the profession. Workers at the two extremes (<20 and >40) years old were not involved much. Workers under 20 years of age are engaged in other professions more compared to sanitary workers. Easily engaged in waste disposals due to scarcity of jobs or as a supplement to their jobs. Invariably, only individuals who actively engaged in this profession will be actively infected.

The length of time of involvement in the profession also played an important role in the isolation of these bacteria. The gradual increase in the prevalence of the organisms show that long exposure or involvement predisposes the sanitary workers to infection by the bacteria. Workers who are new in the system were not significantly infected. This trend shows that time is important.

However, the absence of some bacteria in sanitary workers of longer experience could be attributed to preparation or learning on the work.

All this level of experience, most sanitary workers now know the economic importance of dressing on protective clothing such as mouth and nose masks, raincoats, sweater etc. The use of masks, while sanitizing the environment was quite significant. The unmasked sanitary workers were significantly infected because they were no screens to prevent the entrance of the bacteria into their respiratory tracts. There is free passage of air as there were no barriers. Though, not all those who claim to dress on masks did so always, some used their masks only when they felt it was necessary like in the mask user to be infected too. These circumstances have been more felt in the *C. diphtheria* infestation.

Also other bacteria species had sanitary workers. This shows that they were more of commensals and natural inhabitants of human respiratory tracts. It was observed in full time and part time sanitary workers. In reality, it was quite difficult distinguishing the full time sanitary agencies from part time ones, engaged quite regularly in the profession: that it was not easy classifying them.

The part-time sanitary workers also had some bacteria that are more prevalent to them than the full time ones. This could be due to ill preparation for the job. It was also noted that most of those who did not use masks were part-time ones, they failed to be well equipped for the job, hence were more prevalence to some bacteria than the full timers.

The seasonal distribution in the town played a little role in the frequency of organisms isolated from the sanitary workers. The slight difference observed in the occurrence of the bacteria species was not statistically significant. This shows that some of them are natural inhabitants of the respiratory tract. Especially *C. diphtheriae* and *S. aureus* also *S. pneumoniae*. Only *B. cereus* are spore formers and are very prevalent in dry conditions, this therefore makes their inhabitants easy.

VI. CONCLUSION

Microorganisms can be easily isolated from the respiratory tract of healthy people. The frequency of occurrence of these microorganisms from these sanitary workers is high. Some of these isolates are mere commensals while some are pathogenic. The use of masks reduces the frequency at which these sanitary workers get in can get in contact with microorganisms.

Therefore, sanitary workers are advised to always dress on nose and mouth masks, protect clothing and rain-coats aseptically during harsh weather conditions.

This helps to reduce the rate at which they become infected by microorganisms especially those that causes respiratory tract infections.

In this light, public health workers can help by enlightenment campaign on those sanitary workers on the health hazards, which they expose themselves to, by not dressing on protective clothing.

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APPENDIX 1

➢ *Questionnaire*

a. Name

b. Age

c. Occupation

d. Location of occupation

e. Period of Involvement (in years)

f. Do you enjoy smoking ☐ or any herbal concussion ☐

g. When were you vaccinated last?

h. Do you wear protective clothing sweaters or rain coats? Yes ☐ No ☐

i. Do you ever experience chest pain while coughing? Yes ☐ No ☐

j. If yes how frequent?

k. Do you frequently suffer cold? Yes ☐ No ☐

l. Have you ever had pneumonia? Yes ☐ No ☐

m. Do any of your closest relationssuffer from any respiratory tract diseases? Yes ☐ No ☐