Original Research Article

Incidence of Microorganisms on Environmental Surfaces in Some Secondary Schools in Birnin Kebbi, Kebbi State, Nigeria

ABSTRACT

Due to the ubiquitous nature of bacteria and fungi, the microbiological analysis of environmental surfaces (chairs, tables, floors, hand rails, toilet door knobs and class door knobs) of five (5) secondary schools in Birnin Kebbi metropolis was carried out. Surface swabbing method was used for the collection of a representative sample on the surfaces. Media such as Nutrient agar, Eosin methylene blue, Sabouraud dextrose agar, and Mannitol salt agar were used for the isolation of the organisms. The total bacterial count ranged from 1.1×10^2 to 9.9×10^3 cfu/ml, while the total fungi count ranged from 0.0 to 3.6×10^3 cfu/ml. The bacteria isolated include: *Corynebacterium kutsceri, Lactobacillus casei, Bacillus sphaerious, Staphlococcus aureus, Bacillus subtilis, Aeromonas spp, staphylococcus epidermidis, Pseudomonas spp, Micococcus varians,* while the fungi isolated include *Rhizopus stolonifer, Saccharyomyces cerevisae, Alternaria alternate, Aspergillus niger, Mucor spp., Fusarium spp.*

The isolation of these organisms followed series of procedures, starting with samples collected with swab sticks emulsified with peptone water, followed by the sterile dilution of each sample to a factor of 10^{-1} for chairs, tables, hand rail and door knobs while floor was diluted to a factor of 10^{-2}. One millilitre (1ml) aliquots of which was used as a representative sample used for isolation of pure colonies was followed by series of biochemical test to confirm the identification of each isolate. Despite the routine cleaning practiced in secondary school setting, isolation of microorganisms from secondary school setting is inevitable.

Keywords: Bacterial isolate, Fungal isolate, Microbial characterization, Microorganisms, Microbial ecology

INTRODUCTION

Microorganisms are living things ordinarily too small to be seen without magnification, in terms of numbers and range of distribution, microbes are the abundant organisms on earth (Kathleen and Arthur, 2002). Microbes can be found nearly everywhere, ranging from deep in the earth's crust, to the polar ice and oceans, and to bodies of plants and animals. Being mostly invisible, the actions of microorganisms are usually not as obvious or familiar as those of larger plants and animals. Microorganisms are introduced to human due to the human activities i.e. level of hygiene, level of sanitation, exposure to infected animal etc (Kathleen and Arthur, 2002).

Some microorganisms colonize part of the body without causing infection i.e. the common *Staphylococcus aureus*, which are called 'normal body flora' but can cause infection when found in another site, for example culture of *Staphylococcus aureus,* a harmless commensal on human skin but pathogenic if isolated from a blood sample may represent skin contamination at the time of phlebotomy. Also, the flora of gastro-intestinal tract i.e. *E. coli* which are passed out through excreta, is a serious source and reservoirs from which they are transferred to the susceptible host who then becomes possible source of infection, hence making the control of the infection easy (Francesco Zinzaro, 2010). Faecal matter remains a major reservoir source of human pathogens, which in adverse situation may bring about outbreaks of infection example shigellosis (Francesco Zinzaro, 2010). The incidence of this infection may be attributed to the unhygienic use of the toilet facilities, which results to the

gross contamination of the place including door-handle, which individuals are less likely to see as contaminated (Francesco, 2010).

In as much as these microorganisms are liable to cause infections, the following are the primary beneficial effects of the normal flora that are derived, which are ability to synthesize and secrete vitamins, prevent colonization by pathogen, antagonize other bacteria, stimulate the development of certain tissues, as well as stimulate the production of cross-reactive antibodies (Hara and Shanahan, 2006).

As a result of the increasing number of students in most secondary schools in some areas of Kebbi State, northcentral Nigeria, available sanitary facilities cannot sustain the population and this leads to contamination of surfaces with faecal and other contaminating materials either directly or indirectly. It has been reported that noticeable problems in the metropolis especially in the densely-populated areas of schools lack safe water sources, drainage systems are characterised by heaps of domestic waste materials, which increase the chance of contamination (Calamari *et al.*,1994)

The secondary school students also interact with the surrounding environments and surfaces, among which are materials and humans. Contact surfaces such as doors, toilets, boards, computers and furniture are all potential sources of spread of infections (WHO, 1980; Anson *et al*., 1988; Mohammed *et al*., 2006; Kawo and Rogo, 2008). Thomas and Tillet (1973) reported that enteric pathogens associated with diarrhoea in secondary school students are spread by the faeca-oral route. Microorganisms may survive on environmental surfaces and may subsequently be transferred to a person's hands on contact. Microbial survival on inanimate surfaces as (fomites) depends on a variety of factors including the species, the relative humidity or moisture content, the temperature, the surface materials and its properties (Bloomfield*et al*., 2001).

Pathogenic microorganisms are serious concerns in schools, where contact with various bacterial strains and other microorganisms occur frequently throughout the school day (Whitaker, 2005). Unlike non-pathogens, pathogens can cause disease in humans, whether bacterial or non-bacterial. Though only a small fraction of the thousands of species of bacteria and fungi are pathogenic, serious diseases can result if proper prevention and treatment do not take place. The occurrence of this may be attributed to the unhygienic use of the toilet facilities, picking up of objects on the floor without washing of hands, picking of nose or sneezing on the palm and placing on the table or rail etc which results to the gross contamination of surfaces which individuals are less likely to see as contaminated (Francesco, 2010). These surfaces once contaminated become vehicles for transmission of infection, such that the user may succeed in picking these pathogens on their way out even after washing of hands. The organisms picked in this manner can introduce infection to such individual either orally or topically or can be transferred to another person. The people exposed to this risk factor are the students and school workers who may be unfortunate to come down with the infection. (Francesco, 2010).

Therefore, schools should ensure strict surveillance of microorganisms counts in order to prevent serious outbreaks. Of concern to this study are two categories of microorganisms: bacterial pathogens (*Staphylococcus aureus* and aerobic bacteria) and fungi (molds and yeasts).

Bacterial pathogens include *Staphylococcus aureus* and some species of aerobic bacteria. Commonly found in air and water and on human skin, *S. aureu*s known to cause pneumonia, septicemia, and toxic shock syndrome, as well as wound infections and food poisoning. Non-bacterial pathogens include species of mold and yeast (fungi). Molds tend to be external parasites of humans, causing ringworm, athlete's foot, and jock itch, while yeasts invade internal tissues, infecting the genital tract or activating allergies and other respiratory diseases. Commonly found in moist and dark areas, mold and yeast proliferate in entrances

around the school: to hallways, lavatories, and classrooms. According to a hypothetical "safe [mold] contamination remediation project," contamination by fungi through airways and entranceway surfaces are highlighted as two of the most prevalent forms of transmission (Wayne, 2006). Also to be closely monitored, molds and yeasts make up much of the remaining percentage of pathogenic microorganisms. This study was carried out to determine the microbial load of five secondary schools in Birnin Kebbi, in terms of the bacterial and fungal count of the environmental surfaces that most of the children make easy contact with, so as to know their risk of exposure to infected organisms.

MATERIALS AND METHODS

2.0 Preparation of Materials

The materials used include glass wares such as conical flasks, MacCartney bottles, petri dishes, glass slides, test tubes, cavity slides, cover slips, Durham tubes, swab sticks, micro pipette. Other materials include 70% solution of ethanol, culture media, chemical reagents like 1% sulphanilic acid,1% alpha-naphtol etc for biochemical tests, inoculating loop, filter papers, white polythene bags, cotton wool, aluminum foil, maker, and paper tape.

2.1 Sterilization of Materials

Before the commencement of any analysis, materials used were sterilized as described. Glass wares to be used were washed thoroughly with detergent, rinsed, air dried and wrapped with aluminum foil, then sterilized using an autoclave at 160^{o}c for 1hour.

The work bench was also disinfected by swabbing with 70% solution of ethanol. Also, inoculating loops used were sterilized by flaming till red hot in the blue zone of the fire from the Bunsen burner and cooled by waving before usage. The media used were also sterilized by autoclaving at 121^{o}c for 15 minutes (Fawole and Oso, 2007).

2.2 Sampling Locations and Sites

The sampling sites used were different secondary school within Birnin Kebbi metropolis, Kebbi State. These were: Basaura Institute of Comprehensive Education (BICE), Joda International School, Government Day Girls Secondary School, Nagari College and Salamatu Hussaini Girls Secondary School, which shall be referred to as SCH 1, SCH 2, SCH 3, SCH 4 and SCH 5 respectively. The classrooms seemed apparently clean, some floors were tiled while others were ceramic and the chairs and table were wood, also the door knob were made of iron likewise the hand rails. The environmental surfaces from which samples were taken include the floor, table, chair, toilet door knob, classroom door knob, and hand rails. The choice of surfaces was picked due to the fact that the students have high contact with these surfaces daily and also it is a basic surface found in every individual school.

2.3 Collection of Samples

All samples were collected with a sterile swab stick, into a sterile peptone water. Using a measuring ruler, an area of about 25cm by 25cm was marked out on the table, chair, and floor and swabbed with a sterile swab stick, while the door knobs and hand rails were swabbed all over and the swab was immediately placed into the sterile peptone water and closed to prevent interference with air microbes. Each swab stick was labelled accordingly and taken immediately to the laboratory for analysis. Five batches of samples were taken from five different schools over a period of five months intervals.

2.4 Preparation of Media

The culture media mostly used during the analysis were nutrient agar and sabouraud dextrose agar, Eosin methylene blue agar, Mannitol salt agar. These culture media were prepared from dehydrated commercial powder as follows;

2.4.1 Preparation of Nutrient Agar (NA)

Nutrient agar was used for the isolation and enumeration of heterotrophic bacteria from each sample collected. This was prepared by dissolving 28g of nutrient agar in 1litre of distilled water in a clean conical flask, which was the plugged with cotton wool and sealed with paper

tape, it was the heated for few minutes on a bursen burner in order to ensure proper homogenization before sterilization by autoclaving at 121ºC for 15minutes.

2.4.2 Preparation of Sabouraud Dextrose Agar (SDA)

Sabouraud Dextrose agar was prepared by dissolving 65g of the powder in 1litre of distilled water a clean conical flask, which was the plugged with cotton wool and sealed with paper tape. The mixture was then heated to make the powder dissolve completely in the water before sterilizing at 121ºc for 15minutes. Sabouraud Dextrose agar is used for the isolation of fungi.

2.4.3 Eosin Methylene Blue Agar (EMB)

Eosin Methylene Blue agar was prepared by dissolving 28 g of the powder in 1litre of distilled water. The suspension is homogenized and the conical flask was then plugged with cotton wool, wrapped firmly with aluminum foil and autoclaved at 121ºc for 15 minutes.

2.4.4 Mannitol Salt Agar

Mannitol Salt agar was prepared by dissolving 111g of the powder in 1litre of distilled water. The suspension is homogenized and the conical flask was then plugged with cotton wool, wrapped firmly with aluminum foil and autoclaved at 121ºc for 15minutes.

2.4.5 Peptone Water

Peptone water was prepared by dissolving15g of the powder in 1litre of distilled water. The suspension is homogenized and the conical flask was then plugged with cotton wool, wrapped firmly with aluminum foil and heated on bursen burner for 5minutes.

2.5 Serial Dilution

The peptone water containing each sample from floor, chair, table, door knob, and hand rail, correctly labelled respectively and was thoroughly shaken to ensure a uniform mixture. Using sterile micropipettes calibrated of One millilitre (1ml) of each sample was added to nine milliliter (9ml) of sterile distilled water, thus making a serial dilution factor of 10^{-1} and repeated twice for floor sample, thus making a serial dilution factor of 10^{-2}.

2.6 Microbiological Analysis

Aliquots of 10^{-1} and 10^{-2} serial dilution factor were used for analyzing the constituent microorganisms, using Nutrient agar, Sabouraud Dextrose agar, Eosin Methylene Blue agar, and Mannitol Salt agar

2.6.1 Estimation of Total Viable Microbial Count

Using the pour plate method, 1ml of each sample (from serial dilution of 10^{-1} and 10^{-2}) was pipetted into petri-dishes. Sterile molten agar was then poured aseptically into each petri dish and the plates were swirled gently to mix the agar and inoculum properly. After which the agar solidifies, the plates were then tapped and labelled and incubated turned upside down at 37ºc for 18-24 hours for bacteria and at room temperature for 72hours for fungi.

2.6.2 Isolation and Preservation of Pure Cultures.

For bacteria isolation, a sterile micropipette was used to introduce 1ml from 10^{-1} and 10^{-2} dilution into another 9ml of sterile distilled water to give 10^{-2} and 10^{-3}respectively. In between each dilution, the diluents where shaken thoroughly. After the serial dilution sterile micropipette were used to dispense 0.1ml of the inoculum from the dilution into a sterile Petri dish. After which sterile media to be used was poured aseptically using pour plate method, the plate was then swirled gently for even distribution and the allowed to solidify. The sabouraud dextrose agar plate was incubated at room temperature (25ºc) for 72 hours while other agar was incubated at 37ºc for 24hours. The different colonies obtained on each plate were counted and recorded respectively.

2.6.3 Isolation and Maintenance of Pure Culture of Microorganisms

Based on colonial morphology, representatives of different typical colonies were sub-cultured from the agar plate used for isolation to another solidified agar to obtain a pure culture of an individual organisms for both fungi and bacteria. After the pure cultures were obtained, they

were transferred into agar slant in which bacteria were introduced into nutrient agar slants and fungi were introduced into sabouraud agar slant, nutrient agar slant were incubated at 37ºc for 24 hours while sabouraud agar slant were incubated in a dark sterile cupboard at 25ºc for 72 hours. The pure isolates were observed to check for growth and then kept in the refrigerator as stock cultures (Fawole and Oso,2007).

2.7 Characterization and Identification of Bacterial Isolates

Bacterial isolates were identified based on their colonial morphology, cellular characteristics and biochemical characteristics.

2.7.1 Colonial Morphology

The colonial morphology used in the identification of bacterial isolates include the colony colour, colony shape, colony size, optical characteristics of the colonies, colonial edges, elevation and consistency, which were all observed directly on the plates after appropriate incubation.

2.7.2 Cellular Characteristics

The cellular characteristics of each bacterial isolates, observed under light microscope, were also used in the identification of the isolates. These cellular characteristics include; Gram reaction, cell shape, cell arrangement, motility test, spore production, and possession of capsules.

2.7.2.1 Gram Staining

It was used to differentiate bacterial isolates into Gram negative or Gram positive group. A thin smear of the bacterial isolate were made from the pure culture on a clean grease free slide. The smear was air-dried in each case and immediately heat fixed by passing the reverse side of the slide over flame. Then, the smear were flooded with crystal violet (primary stain) for 30 seconds drained and flooded with Lugol's iodine (mordant) for 10 seconds and rinsed with distilled water. The smears were then decolourized with 90% alcohol for 5seconds and quickly rinsed with distilled water. Then, the slides were then flooded with safranin (secondary/counter stain) for 30 seconds, rinsed with distilled water and air dried. The slides were later examined under the oil immersion (×100) objective lens. The Gram-positive cells appeared purple to blue while Gram negative cells appeared pink to red (Fawole and Oso, 2007).

2.7.2.2 Spore Staining

A smear of each organism was prepared and heat fixed properly by passing it severally over a flame. The heat fixed smear were the allowed to cool before staining. The already fixed smear were then flooded with malachite green stain and heated an steamed over a water bath for 10 minutes with a constant addition of more malachite green stain to avoid drying out of the stain. Slide was then washed properly under tap and flooded with safranin for 20 seconds. This was then washed under a tap and blot dried. It was the examined under the microscope using oil immersion objective lens (×100). The spore appears green in colour resting in the organism while the vegetative portion of the bacterium stains red to pink (Fawole and Oso, 2007).

2.7.2.3 Motility Test

This was done by 'handing drop' technique using 24hours old broth culture of the isolates. A drop of immersion oil was placed round the edge of the depression of the cavity slide. Inoculum of each bacterial isolate were then transferred to the drop of water on the cover slip. The cavity slide was then inverted over the coverslip such that the culture drop is in the centre of the depression. Press the slide down carefully but firmly so that the oil seals the coverslip in position. The slides were then observed under the microscope using ×4 objective lens for motility (Fawole and Oso, 2007).

2.7.3 Biochemical Tests

The biochemical tests performed include oxidase, citrate utilization, urease, sugar fermentation etc.

2.7.3.1 Oxidase Test

This test is used in the identification of organisms that produce the enzyme oxidase. A loopful of each solate was rubbed onto filter paper. Then, a drop of 1% of tetramethyl - p - phenylenediaminedihydrochloride (oxidase reagent) solution was placed on the oxidase strip, the result was determined by formulation of purple colour within 15-30 seconds (John and Lansing, 2002).

2.7.3.2 Catalase Test

Most aerobic microorganisms are capable of producing catalase enzymes although to different extents. The enzyme catalase releases oxygen and water from hydrogen peroxide. A drop of freshly prepared 3% hydrogen perioxide was placed on a clean slide and a pure colony of the organism was picked with a sterile wire loop and placed on it. The preparation was observed for immediate effervescence which indicates positive reaction (production of catalase by the bacterium).

$$H_2O_{2\,(l)} \rightarrow H_2O_{(l)} + \tfrac{1}{2} O_{2(g)}$$

2.7.3.3 Coagulase Test

The test is used to demonstrate the ability of certain species of bacterial isolate to produce coagulase, an enzyme capable of clotting plasma. Slide coagulase test was done by emulsifying the organism in a drop of water on the slide. A drop of the human plasma was added to the suspension of each isolate and stirred for few minutes. Agglutination indicates a positive result and a clear solution shows (Gurgaon and Ashuosh, 2000).

2.7.3.4 Triple Sugar Ion Agar Test

The triple sugar ion (TSI) agar test is generally used for the identification of enteric bacteria. It is also used to distinguish the enterobacteriaceae from other gram –negative intestinal bacilli by their ability to catabolise glucose, lactose, or sucrose, and to liberate sulphides from ferrous ammonium sulphate or sodium thiosulfate. Triple sugar ion agar was prepared according to the manufacturer's specification and dispensed into test tubes, autoclaved and slightly slanted.

TSI agar was then inoculated by streaking the surface of each slant with each isolate, then stabbing deep down into the butt. The tubes were incubated and observed with 18-24 hours of incubation, in order to detect the presence of sugar fermentation, gas production, and hydrogen sulphide (H_2S) production. Colour change from pink to yellow indicated sugar fermentation and acid production, black colour indicated production of H_2S, pushing up or splitting of the agar from the butt indicated gas production (Fawole and Oso, 2007).

2.7.3.5 Indole Test

This test was used to detect bacteria that can break down amino acid to tryptophan to release indole, pyruvic acid and ammonia, catalyzed by tryptophanase. Tryptone soy broth was prepared in test tubes and autoclaved according to manufacturer's specification. The broth was then inoculated with the isolate; it was then incubated at 37ºc for 48 hours. After incubation, 2ml of chloroform and 2ml of Kovac's reagent were added respectively to each tube. After 20 minutes, bacteria producing a red ring in the tube were considered indole positive (Fawole and Oso, 2007).

2.7.3.6 Starch Hydrolysis Test

This test was done to detect bacteria that produce amylase, an enzyme that can hydrolyse starch. The medium was prepared by dissolving 0.5g of starch in 100ml of nutrient broth, after which the medium was sterilized at 121ºc, 15psi for 15minutes. The starch-nutrient agar was poured aseptically into sterile petri-dishes and allowed to solidify. Each isolate was then streaked aseptically on each of the plates and incubated upside down at 37ºc for 24 hours. The appearance of zone of clearance around colonies of isolates after pouring Gram's iodine,

indicates the absence of starch, which must have been hydrolysed by amylase, such organisms test positive for the presence of amylase. But, the appearance of blue- black zones around colonies indicates the presence of starch, such organisms test negative for the production of amylase (Fawole and Oso, 2007).

2.7.3.7 Sugar Fermentation Tests

This was used to determined ability of bacterial isolates to reduce sulphur and ferment certain carbohydrates to produce acidic products and gas. The sugar tested were lactose, glucose, sucrose, fructose, maltose, mannitol. To every 100ml of nutrient broth in different conical flask for each sugar, 0.5g of each sugar was added respectively. The broth was then boiled and phenol red indicator was added. The mixture was dispensed into different MacCartney bottles and Durham tubes were inverted into each bottle. The broth was then inoculated into different broth for all the sugar and incubated at 37ºc for 24hours. Colour change from red to yellow after incubation showed positive result; the sugar was fermented. Displacement of broth in durham tubes showed gas production. No colour change indicates a negative result (Fawole and Oso, 2007).

2.7.3.8 Urease Test

Urease test was used to detect the enzyme urease that splits urea to NH_3 and CO_2 (Willey *et al*., 2007). The slanted agar surface was streaked with the isolate using a sterile inoculating loop and inoculated at 37^{o}C for 5 days. Media turns from orange to pink or red if urease is present and no colour change if it is absent (Fawole and Ọsọ, 2007).

2.7.3.9 Nitrate Reduction Test

Nitrate Reduction test was used to detect whether an organism can use nitrate as an electron acceptor (Willey *et al*., 2007). Nutrient Broth was prepared and 1ml of nitrate ($NaNO_3$) was added. It was dispensed into the McCartney bottles and sterilized. After sterilization, it was allowed to cool and the isolates were inoculated into it and incubated for 48 hours. After incubation, 0.5ml of 1% sulphanilic acid in 5N acetic acid and 0.5ml of 1% alpha-naphtol was added. A positive result is indicated by the presence of maroon or pinkish colouration while brownish colouration indicates a negative result.

2.7.4.0 Haemolysis Test

Haemolysis test was carried out to differentiate some species of bacteria that have the ability to lyse blood cells. Blood agar was first prepared by preparing Nutrient Agar and autoclaving it at 121^{o}C for 15 minutes. It was allowed to cool after which fresh blood was added into it and mixed thoroughly before it was poured into sterile Petri-dishes and allowed to solidify. After solidifying, the plates were inoculated with the isolates by just dropping the isolates at a spot on the agar and incubated at 37^{o}C for 24 hours. After incubation, haemolysis was observed. Three haemolytic patterns can be observed: alpha (α) haemolysis showed green colouration around the organism, beta (β) haemolysis showed a clean zone around the organism and gamma (γ) haemolysis showed no colour change on the blood agar (Willey *et al*., 2007).

2.7.4.1 Oxidation-Fermentation Test

This was carried out by dissolving 1.95 grams of Nutrient Broth, 2.25 gram of Agar Agar, 1 gram of glucose and 0.75 gram of NaCl were measured into a conical flask and mixed with 150ml distilled water. Bromothymol blue indicator and 1.5ml of 0.5% Na_2HPO_4 was added. The mixture was heated to melt the agar and then dispensed into McCartney bottles; two bottles per isolate and autoclaved for 15 minutes at 121^{o}C. After autoclaving, it was allowed to solidify and the isolate was inoculated into the agar and stabbed to the bottom of the bottle. The bottles were labelled open and close. The close bottles were covered with candle wax after inoculation. It was incubated for 7 days. Acid production in the cultures is indicated by a change in colour of the medium from green to yellow. Acid production in both the open and sealed bottles suggests a fermentation reaction. Acid formation in the open bottles only suggests an oxidative

utilization of sugar while no colouration in both open and close tubes indicates non-utilization of sugar or inert or negative reaction (Fawole and Ọsọ, 2007).

2.7.4.2 Citrate Test

Citrate agar contains sodium citrate which serves as the sole source of carbon and ammonium phosphate as the sole source of nitrogen. When citrate is used, it results in alkalinization of the medium. Citrate agar was used to differentiate enteric bacteria on the basis of citrate utilization (Willey *et al*., 2007). Sterile Simmon's Citrate Agar was dispensed into McCartney bottles and allowed to solidify in a slanted position and then inoculated with the isolates, after which it was incubated at 37^{o}C for 5 days. A colour change from green to blue indicates a positive result due to citrate utilization while no colour change indicates a negative result.

2.7.4.3 Methyl Red-Voges-Proskauer (MR-VP) Tests

Methyl Red which is a pH indicator was used to determine whether the isolates carry out mixed acid fermentation while Voges-Proskauer was used to detect the production of acetoin by organisms (Willey *et al*., 2007). Sterile MR-VP broth was dispensed into McCartney bottles and isolates were inoculated into it. Two bottles were prepared for each isolate i.e. one for MR and the other for VP. The isolates were incubated at 37^{o}C for 72 hours.

After incubation, for Methyl Red test – 2 drops of Methyl Red indicator were added to one batch of the isolates. A positive result showed a red colouration while a negative result showed yellow colouration.

For Voges-Proskauer test, 0.5ml of 5% alpha-naphtol reagent and 0.5ml of 40% NaOH containing creatinine was added and allowed to stand for 1 hour. A positive result is indicated by pink to red colour while a negative result is indicated by reddish-brown colour on the surface of the broth (Fawole and Ọsọ, 2007).

2.7.4.4 Growth in 6.5% NaCl Broth

Some amount of Sodium chloride was measured into a conical flask containing 1.95 grams of Nutrient Broth powder and mixed with 150ml distilled water. It was then dispensed into McCartney bottles and autoclaved at 121^{o}C for 15 minutes. The bottles were inoculated with the isolates and incubated at 37^{o}C for 5 days. A turbid broth indicates utilization of the salt while a non-turbid indicates no utilization.

2.8 Characterization and Identification of Fungal Isolates

Fungal isolates were identified based on the gross observation of their colonial morphology, and detailed microscopic examination of each pure isolate characteristics such as the nature of the hypha, colour of the fungi, reverse colour of each colony at the back of the plate and the reproductive structure.

2.8.1 Fungal Staining

A drop of lactophenol in cotton blue reagent was placed on a slide. Using a sterile wire-loop, fungi is properly teased out, picked and spread out in the fungal reagent. The wet slide was carefully covered with coverslip, avoiding the formation of air bubbles. Each fungal isolate was viewed under the microscope, using the ×40 objective lens.

RESULTS

3.1 Enumeration of Bacterial Isolates.

Microbial load per area of site sample (cfu/ml/cm^2). The density of total viable microbial count per area of the site marked out for sampling is determined by dividing the total viable microbial count with the area of sampling site, using the formula

$$\frac{\text{Ncfu/ml of isolate}}{Areaofsitesampled\ (cm\ 2)}$$

3.1 Table 1 : Enumeration of bacterial isolates.

Sampling routines	SCH 1 (cfu/ml)	SCH 2 (cfu/ml)	SCH 3 (cfu/ml)	SCH 4 (cfu/ml)	SCH 5 (cfu/ml)
Floor 1	8.0×10^3	9.9×10^3	1.6×10^3	5.1×10^3	4.2×10^3
Floor 2	7.6×10^3	6.4×10^3	9.8×10^3	4.4×10^3	3.0×10^3
Floor 3	9.9×10^3	9.2×10^3	5.9×10^3	3.5×10^3	5.1×10^3
Floor 4	7.2×10^3	3.2×10^3	7.6×10^3	2.5×10^3	3.5×10^3
Chair 1	6.72×10^3	1.1×10^2	8.0×10^3	2.8×10^3	5.1×10^3
Chair 2	3.04×10^3	1.3×10^2	1.2×10^2	2.7×10^3	4.4×10^3
Chair 3	3.2×10^3	2.8×10^3	4.4×10^3	4.1×10^3	2.2×10^3
Chair 4	1.6×10^3	9.6×10^3	1.2×10^3	3.0×10^3	3.2×10^3
Table 1	8.2×10^3	1.7×10^3	2.8×10^3	6.7×10^3	1.6×10^2
Table 2	1.0×10^2	2.2×10^3	1.4×10^2	2.2×10^3	1.1×10^2
Table 3	1.4×10^2	3.2×10^3	1.2×10^2	3.5×10^3	1.0×10^2
Table 4	1.0×10^2	2.2×10^3	4.4×10^3	2.2×10^3	1.9×10^2
Toilet doorknob 1	2.2×10^3	3.2×10^3	6.4×10^3	1.1×10^2	1.0×10^2
Toilet doorknob 2	2.8×10^3	3.5×10^3	5.6×10^3	1.9×10^3	1.4×10^2
Classroonbm doorknob 1	2.2×10^3	4.3×10^3	1.3×10^2	1.1×10^2	1.2×10^2
Classroom doorknob 2	1.7×10^3	4.1×10^3	1.6×10^3	1.9×10^3	1.4×10^2
Classroom doorknob 3	2.5×10^3	1.1×10^2	1.0×10^2	1.7×10^3	1.1×10^3
Handrail 1	2.5×10^3	1.1×10^2	4.4×10^3	1.2×10^2	1.0×10^2
Handrail 2	3.8×10^3	1.6×10^3	1.6×10^3	3.2×10^3	1.6×10^2
Handrail 3	4.8×10^3	1.1×10^3	1.7×10^3	3.3×10^3	1.2×10^2

3.2 Characterization and Identification of Bacterial Isolates

A total of nine bacteria were characterized (Table 2) and identified during the course of study. The bacterial isolates comprise of both Gram positive and Gram negative bacteria which includes: *Corynebacterium kutsceri, Lactobacillus casei, Bacillus sphaerious, Staphlococcus aureus, Bacillus subtilis, Aeromonas spp, staphylococcus epidermidis, Pseudomonas spp, Micococcus varians.*

3.3 Occurrence of Bacterial Isolate

The table below shows the incidence of the characterized bacterial isolates in each sampled routine i.e. floor, table, chair, doorknobs and handrails. School 1 and School 5 had the highest occurrence of all the bacteria characterized (the nine bacteria isolated were present in school 1 and school 5).

Table 2: Occurrence of bacterial isolates from the environmental surface of the secondary school.

Isolates	School 1	School 2	School 3	School 4	School 5
Corynebacterium kutsceri	+	-	+	-	+
Lactobacillus casei	+	+	+	+	+
Bacillus sphaerious	+	-	-	-	+
Bacillus subtilis	+	+	+	+	+
Aeromonas sp.	+	+	+	+	+
Staphylococcus aureus	+	+	+	+	+
Staphylococcus epidermidis	+	+	+	+	+
Pseudomonas sp.	+	+	+	+	+
Micrococcus sp.	+	+	+	+	+

KEYS: +: Present -: Absent

3.3 Frequency of Bacteria Isolates

The figure below shows the number of occurrence of each bacterial isolate in each school in percentage (%). School 1 had the highest frequency of *Staphylococcus aures, Staphylococcus epidermidis, Bacillus subtilis, Bacillus sphaerious, Aeromonas, Pseudomonas spp*, while School 3 had the highest frequency of *Corynebacterium kutscheri, Micococcus spp* and *Lactobacillus casei* and also School 5 had the highest frequency of *Micococcus spp.*

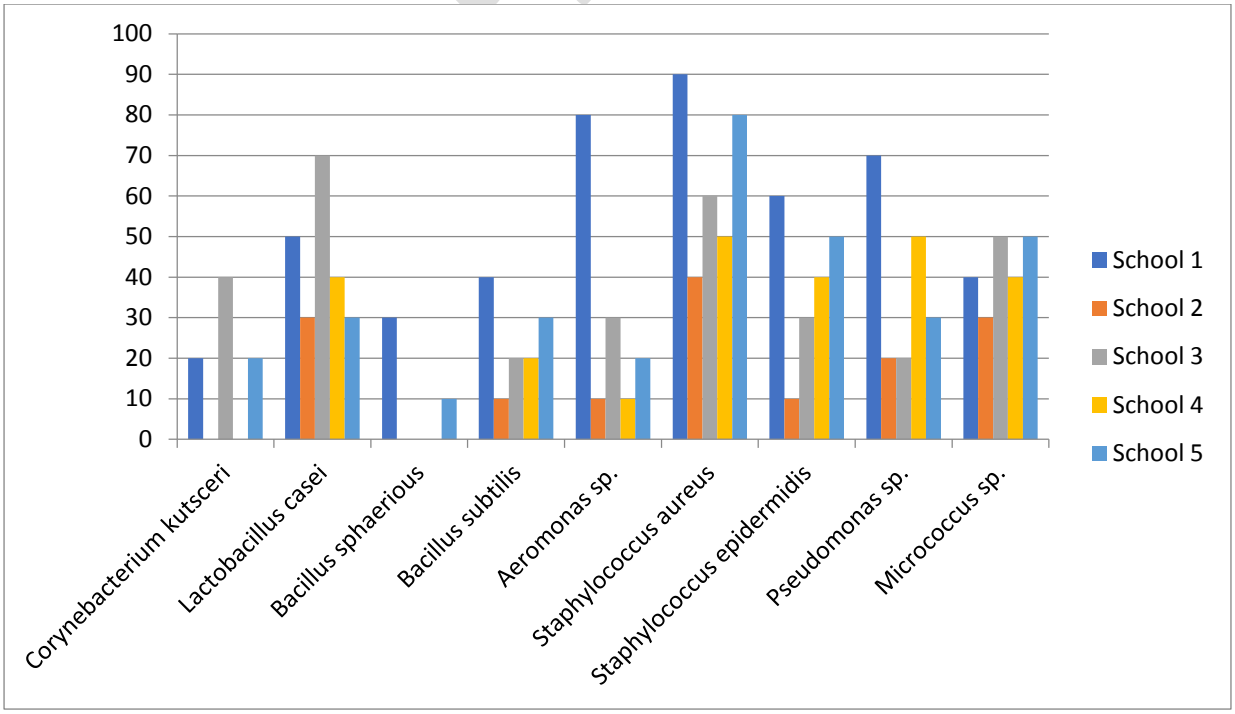

Fig 1: Frequency of bacteria isolates on environmental surfaces in secondary school

3.4 Fungal Isolates

Enumeration of Fungal Isolate

Microbial load per area of site sample (cfu/ml/cm^2). The density of total viable microbial count per area of the site marked out for sampling is determined by dividing the total viable microbial count with the area of sampling site, using the formula

$$\frac{\text{Ncfu/ml of isolate}}{Areaofsitesampled\ (cm\ 2)}$$

Table 3: Enumeration of Fungal Isolate

Location	SCH 1 (cfu/ml)	SCH 2 (cfu/ml)	SCH 3 (cfu/ml)	SCH 4 (cfu/ml)	SCH 5 (cfu/ml)
Floor 1	3.6×10^3	1.0×10^3	2.2×10^2	2.5×10^2	2.3×10^2
Floor 2	1.0×10^3	1.0×10^3	1.9×10^2	3.0×10^2	1.9×10^2
Floor 3	1.2×10^3	4.0×10^3	0	0	0
Floor 4	1.1×10^2	0	0	0	0
Chair 1	0	1.0×10^2	0	1.0×10^2	0
Chair 2	0	1.0×10^2	0	0	0
Chair 3	0	0	0	1.5×10^2	0
Chair 4	0	3.3×10^3	0	1.2×10^2	0
Table 1	6.4×10^2	1.4×10^2	1.3×10^2	0	0
Table 2	0	1.6×10^3	2.2×10^2	0	1.3×10^2
Table 3	2.4×10^3	0	2.3×10^2	1.9×10^2	1.2×10^2
Table 4	1.2×10^3	1.2×10^3	1.0×10^2	2.1×10^2	1.0×10^2
Toilet doorknob 1	2.7×10^3	2.4×10^2	0	4.0×10^2	0
Toilet doorknob 2	1.6×10^3	1.2×10^2	0	2.0×10^2	0
Classroom doorknob 1	0	2.0×10^2	2.0×10^2	2.0×10^2	0
Classroom doorknob 2	1.6×10^2	1.2×10^2	0	1.2×10^2	0
Classroom doorknob 3	1.2×10^2	1.4×10^3	0	0	0
Handrail 1	1.0×10^2	0	1.0×10^2	0	0
Handrail 2	2.7×10^3	0	2.1×10^2	0	2.0×10^2
Handrail 3	1.0×10^2	0	1.5×10^2	0	0

3.6 Occurrence of Fungal Isolate

The table below shows the incidence of the characterized fungal isolates in each sampled routine i.e. floor, table, chair, doorknobs and handrails. School 1 had the highest occurrence of all the fungi characterized (the six fungi isolated were present in school 1).

Table 4: Occurrence of fungi isolates from the environmental surfaces

Isolate	School 1	School 2	School 3	School 4	School 5
Rhizopus solonifer	+	+	-	+	+
Saccharyomy cescervisae	+	+	+	+	+
Alternaria alternate	+	-	+	-	-
Aspergillus niger	+	+	+	+	+
Mucor sp	+	+	-	+	-
Fusarium sp	+	+	+	+	+

KEYS: +; Present -; Absent

3.7 Frequency of fungal isolates

The figure below shows the number of occurrence of each bacterial isolate in each school in percentage (%). School 1 had the highest frequency of *Rhizopus stolonifer, Saccharomyces cerevisae, Fusarium spp, and Alternaria alternate.* while School 2 had the highest frequency of *Mucor spp* while School 3had the highest frequency of *Alternaria alternate* and School 5 had the highest frequency of *Fusarium spp*.

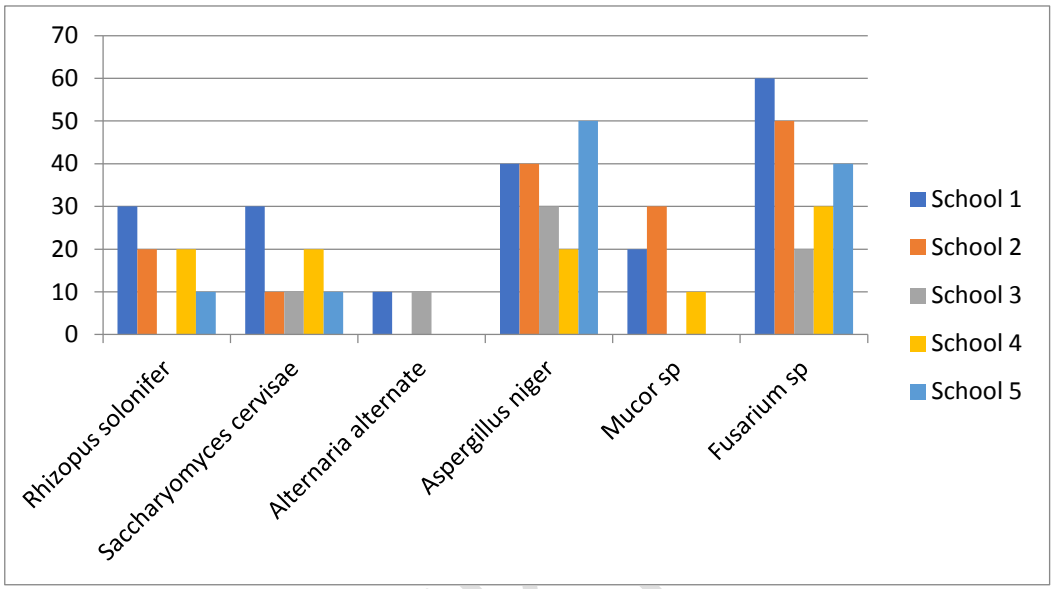

Fig 2: Frequency of fungal isolates on environmental surfaces in secondary schools

DISCUSSION AND CONCLUSION

This study is hinged on microbial ecology, a field of microbiology which seeks to understand how microbes interact with other organisms and with the environment. Traditionally, the study of microbes on surfaces has focused on known pathogens or on microbes that can degrade materials commonly used in fabricating equipment and furniture such as wood. Highest microbial load was obtained from school 1 due to the nature of the school. High population and human activities in the school 1 allows for raising of dust and introduction of many microbes into surfaces, the activities including coughing, sneezing and other sporting event. This was in agreement with Kawo *et al.* (2012), who reported high microbial load on surfaces in some schools in Sokoto, Nigeria. He reported the reason was due to heavy human activities in the schools.

In this study, the bacteria isolated from the secondary schools were *Corynebacterium kutsceri, Lactobacillus casei, Bacillus sphaerious, Staphlococcus aureus, Bacillus subtilis, Aeromonas spp, staphylococcus epidermidis, Pseudomonas spp, Micococcus varians,* while the fungal isolates were *Rhizopus stolonifer, Saccharyomyces cerevisae, Alternaria alternate, Aspergillus niger, Mucor spp., Fusarium spp,* some of which were in accordance with the findings of Kawo (2012), who isolated a total of six (6) different bacteria from eight secondary schools in Sokoto state the organisms isolated which are similar to this study include *Staphylococcus aureus, Micococcus spp and Pseudomonas aeruginosa*. Aderibigbe *et al*. (2014) also supported this with his research which he isolated ten (10) different bacteria from floors and other environmental surfaces in Kwara. Organisms similar to this study were *Staphylococcus aureus, Micococcus varians, Corynebacterium kutscheri.*

High prevalence of *Staphylococcus aureus* in school 1 surfaces could be as a result of the nature of the organisms which mostly can be found on the skin. Skin contact with the

surfaces is most likely the source of the organism. According to Keens *et al.* (2008), he isolated organisms from a secluded area and crowded area in a popular park surface in hong kong, he reported the presence of *Staphylococcus aureus* from only the crowded area. He concluded it was as a result of human activities in the area. *Fusarium spp* also had the highest frequency in School 1 especially on floors, it was observed that students of this school were in contact soils (i.e. students were exposed to tilling of weeds around the school premises with bare hands, picking objects from floors of classes and environment etc). Therefore, the students were liable to come in contact with *Fusarium spp* accidentally. According to Amoo *et al.* (2003), she isolated *Fusarium spp* from floors of Providence crèche centre in Fagba Lagos, State, she reported high amount the presence of *Fusarium* sp from the floors.

A variety of other microorganisms were also observed from the studied locations. Even though these locations are routinely cleaned, the occurrence of microorganisms is assumed to be part of normal microbial flora. However, the unanticipated number of microorganisms at the various surfaces of the secondary schools is a major concern.

The microbial flora observed from the surfaces of various schools can be considered as non-pathogenic microbial biota. However, this study did not attempt to extend its study on microbial specificity towards pathogenic aspects. It is important to point out that the non-pathogenic microorganisms can however mutate into a virulent strain, liberating toxins and causing illness. The distribution of microbes reflects the population and sanitary conditions of the school. The more populated a class, the more likely the children will come down with illness as they are exposed to a variety of infectious organisms amongst themselves. Disease outbreaks are common in conditions of overcrowding coupled with poor hygiene practices. The student are liable to transmit diseases and contract microbes from one another through sneezing, picking object from the floor, use of toilet without proper washing etc.

Below are the implications of some probable organisms isolated, and their degree of infectivity:

Staphylococcus aureus: *Staphylococcus aureus* is frequently found in the nose, respiratory tract, and on the skin. Although *S. aureus* is not always pathogenic, it is a common cause of skin infections, such as abscesses, respiratory infections, such as sinusitis, and food poisoning. The occurrence of *S. aureus* and *S. epidermidis* under these circumstances does not always indicate infection and therefore does not always require treatment (indeed, treatment may be ineffective and re-colonization may occur). The spectrum of disease caused by *S. aureus* ranges from mild skin infections to serious systematic diseases. *Staphylococcus aureus* can infect other tissues when barriers have been breached (eg skin or mucosal lining). In which this leads to furuncles (boils) and carbuncles (a collection of furuncles). Deeply penetrating *S. aureus* infections can be severe, its most commonly seen in wounds, pneumonia, bacteremia (Cosgrove, 2009).

Staphylococcus epidermidis; It is part of the normal human flora, typically the skin flora, and less commonly the mucosal flora. Although *S. epidermidis* is not usually pathogenic, patients with compromised immune systems are at risk of developing infection. These infections are generally hospital-acquired. It is now the most frequent cause of nosocomial infections, at a rate about as high as that due to its more virulent cousin *Staphylococcus aureus*. Interestingly, many of these determinants are believed to have original functions in the non-infectious lifestyle of this microorganism, emphasizing the accidental nature of *S. epidermidis* infections (Olson, 2010).

Corynbacterium kutscheri: This organism is a commonly isolated from the oral cavity of healthy mice and rats. Well documented cases of *C. kutscheri* human infection usually follow a rat bite. C. kutscheri causing infection in humans is notably a soft tissue infection occurring after a recent bite (Amao *et al*.,2002).

Micococcus varians and *Aeromonas spp:* These organisms are ubiquitous in soil and water. However, despite their common occurrence in nature, they only rarely cause infections in human (Igari *et al*., 2003)

Mucor spp., Fusarium spp., Rhizopus stolonifer, Aspergillus niger are ubiquitous and isolated from indoor air environment, soils and on the skin. They are rarely pathogenic, except in immunosuppressed individuals (Ribes *et al*., 2000).

Alternaria alternate are opportunistic pathogen on numerous hosts causing leaf spots, rots and blights on many plant parts. It can also cause upper respiratory tract infections and asthma in humans with compromised immunity (Igari *et al*., 2003).

In view of the high level of bacterial and fungal contamination as well as the isolation and identification of some potential bacterial and fungal pathogens from the contact surfaces of some secondary schools in Ilorin, it shows that the students in these schools may easily contact these microorganisms and thus become infected. Although not every exposure results in infection, a risk assessment for infection based on the host immune system, mechanism of exposure, infectious dose of exposure, virulence of the agent is considered.

The relative high microbial count of the floor can be attributed to the fact that floors and have become effective formite and vechiles for harbouring microorganisms that the students come in contact with. Surprisingly, there was no record of total coliform, peradventure, due to the use of disinfectants in some of the schools or in particular a school (school 1) which was observed that there was no or low usage of the toilet due to fear of contacting toilet diseases.

REFERENCES

Abdulhadi, S.K., Hassan, A.H., Da'u A., (2008).Nasal carriage of *Staphylococcus aureus* among students in Kano, Nigeria *International Journal of Biomedicals*, and Health Sciences. 4(4):151-154.

Buchanan, K..E., and Gibbons, N., (1974) Bergey's Manual of Determinative Microbiology. 8thEdition, the Williams and Wilkins Co., Baltimore.

Center for Disease Control and Prevention (1992). Public health focus: Surveillance, prevention and control of nosocomial infections. MMWR411:783-787.

Chesebrough, M., (2000). Medical laboratory manual for tropical countries– Volume II.ELBS edition.Tropical health technology publications, United Kingdom.2-392.

Choi, S., K., Park, S., Y., Kim, R., Lee, C., Kim, J., F., and Park, S., H. (2008) 'Identification and functional analysis of the fusaricidin biosynthetic gene of *Paenibacillus polymyxa* E681'.*Biochemical and Biophysical Research Communication*. 365(1): 89-95.

Collen, M.F. (2005). History of medical informatics in the United States(1950-1990).American Medical Informatics Association, Washington DC, USA.

Cogen, A., L., Nizet, V., and Gallo, R., L. (2008). 'Skin microbiota: a source of disease or defence?' *Bristish Journal of Dermatology*. 158(3):442-455.

Cruickshank, R., Duguid, P., Marmion BP, Swain RHA (1980). MedicalMicrobiology.The practice of medical microbiology. Churchill Livingstone publishers, UK. 111-449.

Fawole, M., O., and Oso, B., A. (2007) Laboratory Manual of Microbiology. Reprint of the first Public Spectrum Books Limited, Ibadan. 15-33.

Ferson, M.J. (1997). Infection control in child-care setting communicablediseases intelligence. CDI 2122 Australia 21(22):1-23.

Garba, C. (2002). Attachment of *Staphylococcus aureus* to different plastic tubes *in-vitro*. Journal. Medical. Microbiology. 40(1):37-42.

Kawo, A.H, Adam, M.S., Abdullahi, B.A., Sani, N.M., (2009).Prevalence and public health implications of the microbial load of Naira notes. Bayero Journal. Pure and Appl. Sci. 2(1):52-57.

Mayo Clinic Staff. (2006, Aug 1). Children's illness: Top 5 causes of missed school. Retrieved January 6, 2007, from http://www.mayoclinic.com/health/childrens-conditions/CC00059

McGinnis, M.R. 1980. Laboratory handbook of medical mycology. Academic Press, London, UK.156.

Michael, B. (2002). Cross-contamination: Commercial facilities as overloadedbreeding grounds for diseases. The information resources for the cleaning industry. Floor KENT commercial care products, UK.1-7.

Olayemi, A.B., Adebayo, O.O., (1991). Incidence of coliform bacteria on environmental surfaces in some day-care center and nursery schools in Ilorin, Nigeria. Nig. Journal. Medical. Science. 2:57.

Onukwubiri, N., (2005). Incidence of Staphylococcus aureus among apparently healthy students in Bayero University, Kano, Nigeria.B.Scthesis, Department of Biological Sciences, Bayero University, Kano, Nigeria. 38.

Rogo, L.D., Kawo, A.H., (2005). Isolation and characterization of bacteria associated with computer keyboards: A case study of Bayero University, Kano, Nigeria. A paper presented at the 29th annual conference and general meeting of the Nigerian Society forMicrobiology held at the University of Agriculture, Abeokuta, Ogun State, Nigeria between 6th and 10th November, 2005.

Singleton, P., (1999). Bacteria in Biology, Biotechnology and Medicine (5^{th}ed.). Wiley pp.444-454.ISBN 0-47198880-4.

Todar, K. Normal bacterial flora of humans. Todar's Online Textbook of Bacteriology.

Whitaker, E.,(2005). Recommendations for the prevention of Staphylococcal infections for schools. Retrieved December 9, 2006, from http://www.idph.state.il.us/health/infect/schoolstaphrecs.htm

Wiest, Peter., Wiese, Kurt; Jacobs, Michael R.; Morrissey, Anne B.; Abelson, Tom I.; Witt, William; Lederman, Michael M. (1987). Alternaria Infection in a Patient with Acquired Immunodeficiency Syndrome: Case Report and Review of Invasive Alternaria Infections. *Reviews of Infectious Diseases*, **9** (4): 799–803.