

**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  $1.1 \times 10^2$  to  $9.9 \times 10^3$  cfu/ml, while the total fungi count ranged from 0.0 to  $3.6 \times 10^3$  cfu/ml. The bacteria isolated include: *Corynebacterium kutscheri*, *Lactobacillus casei*, *Bacillus sphaerious*, *Staphylococcus aureus*, *Bacillus subtilis*, *Aeromonas spp*, *staphylococcus epidermidis*, *Pseudomonas spp*, *Micococcus varians*, while the fungi isolated include *Rhizopus stolonifer*, *Saccharyomyces cerevisiae*, *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

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

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

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

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

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

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

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

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

## 105 **MATERIALS AND METHODS**

### 106 **2.0 Preparation of Materials**

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

### 112 **2.1 Sterilization of Materials**

113 Before the commencement of any analysis, materials used were sterilized as described. Glass  
114 wares to be used were washed thoroughly with detergent, rinsed, air dried and wrapped with  
115 aluminum foil, then sterilized using an autoclave at 160°C for 1 hour.

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

### 120 **2.2 Sampling Locations and Sites**

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

### 131 **2.3 Collection of Samples**

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

### 139 **2.4 Preparation of Media**

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

#### 143 **2.4.1 Preparation of Nutrient Agar (NA)**

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

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

#### 149 **2.4.2 Preparation of Sabouraud Dextrose Agar (SDA)**

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

#### 155 **2.4.3 Eosin Methylene Blue Agar (EMB)**

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

#### 159 **2.4.4 Mannitol Salt Agar**

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

#### 163 **2.4.5 Peptone Water**

164 Peptone water was prepared by dissolving 15g of the powder in 1litre of distilled water. The  
165 suspension is homogenized and the conical flask was then plugged with cotton wool,  
166 wrapped firmly with aluminum foil and heated on bursen burner for 5minutes.

#### 167 **2.5 Serial Dilution**

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

#### 173 **2.6 Microbiological Analysis**

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

##### 177 **2.6.1 Estimation of Total Viable Microbial Count**

178 Using the pour plate method, 1ml of each sample (from serial dilution of  $10^{-1}$  and  $10^{-2}$ ) was  
179 pipetted into petri-dishes. Sterile molten agar was then poured aseptically into each petri dish  
180 and the plates were swirled gently to mix the agar and inoculum properly. After which the  
181 agar solidifies, the plates were then tapped and labelled and incubated turned upside down at  
182 37°C for 18-24 hours for bacteria and at room temperature for 72hours for fungi.

##### 183 **2.6.2 Isolation and Preservation of Pure Cultures.**

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

##### 193 **2.6.3 Isolation and Maintenance of Pure Culture of Microorganisms**

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

197 were transferred into agar slant in which bacteria were introduced into nutrient agar slants  
198 and fungi were introduced into sabouraud agar slant, nutrient agar slant were incubated at  
199 37°C for 24 hours while sabouraud agar slant were incubated in a dark sterile cupboard at 25°C  
200 for 72 hours. The pure isolates were observed to check for growth and then kept in the  
201 refrigerator as stock cultures (Fawole and Oso,2007).

## 202 **2.7 Characterization and Identification of Bacterial Isolates**

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

### 205 **2.7.1 Colonial Morphology**

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

### 210 **2.7.2 Cellular Characteristics**

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

#### 215 **2.7.2.1 Gram Staining**

216 It was used to differentiate bacterial isolates into Gram negative or Gram positive group. A  
217 thin smear of the bacterial isolate were made from the pure culture on a clean grease free  
218 slide. The smear was air-dried in each case and immediately heat fixed by passing the reverse  
219 side of the slide over flame. Then, the smear were flooded with crystal violet (primary stain)  
220 for 30 seconds drained and flooded with Lugol's iodine (mordant) for 10 seconds and rinsed  
221 with distilled water. The smears were then decolourized with 90% alcohol for 5seconds and  
222 quickly rinsed with distilled water. Then, the slides were then flooded with safranin  
223 (secondary/counter stain) for 30 seconds, rinsed with distilled water and air dried. The slides  
224 were later examined under the oil immersion ( $\times 100$ ) objective lens. The Gram-positive cells  
225 appeared purple to blue while Gram negative cells appeared pink to red (Fawole and Oso,  
226 2007).

#### 227 **2.7.2.2 Spore Staining**

228 A smear of each organism was prepared and heat fixed properly by passing it severally over a  
229 flame. The heat fixed smear were the allowed to cool before staining. The already fixed  
230 smear were then flooded with malachite green stain and heated an steamed over a water bath  
231 for 10 minutes with a constant addition of more malachite green stain to avoid drying out of  
232 the stain. Slide was then washed properly under tap and flooded with safranin for 20 seconds.  
233 This was then washed under a tap and blot dried. It was the examined under the microscope  
234 using oil immersion objective lens ( $\times 100$ ). The spore appears green in colour resting in the  
235 organism while the vegetative portion of the bacterium stains red to pink (Fawole and Oso,  
236 2007).

#### 237 **2.7.2.3 Motility Test**

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

### 245 **2.7.3 Biochemical Tests**

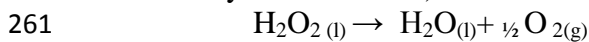
246 The biochemical tests performed include oxidase, citrate utilization, urease, sugar  
247 fermentation etc.

#### 248 **2.7.3.1 Oxidase Test**

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

#### 254 **2.7.3.2 Catalase Test**

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



#### 262 **2.7.3.3 Coagulase Test**

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

#### 268 **2.7.3.4 Triple Sugar Ion Agar Test**

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

275 TSI agar was then inoculated by streaking the surface of each slant with each isolate, then  
276 stabbing deep down into the butt. The tubes were incubated and observed with 18-24 hours of  
277 incubation, in order to detect the presence of sugar fermentation, gas production, and  
278 hydrogen sulphide (H<sub>2</sub>S) production. Colour change from pink to yellow indicated sugar  
279 fermentation and acid production, black colour indicated production of H<sub>2</sub>S, pushing up or  
280 splitting of the agar from the butt indicated gas production (Fawole and Oso, 2007).

#### 281 **2.7.3.5 Indole Test**

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

#### 289 **2.7.3.6 Starch Hydrolysis Test**

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

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

#### 300 **2.7.3.7 Sugar Fermentation Tests**

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

#### 311 **2.7.3.8 Urease Test**

312 Urease test was used to detect the enzyme urease that splits urea to  $\text{NH}_3$  and  $\text{CO}_2$  (Willey *et al.*,  
313 2007). The slanted agar surface was streaked with the isolate using a sterile inoculating loop  
314 and inoculated at 37°C for 5 days. Media turns from orange to pink or red if urease is present  
315 and no colour change if it is absent (Fawole and Oso, 2007).

#### 316 **2.7.3.9 Nitrate Reduction Test**

317 Nitrate Reduction test was used to detect whether an organism can use nitrate as an electron  
318 acceptor (Willey *et al.*, 2007). Nutrient Broth was prepared and 1ml of nitrate ( $\text{NaNO}_3$ ) was  
319 added. It was dispensed into the McCartney bottles and sterilized. After sterilization, it was  
320 allowed to cool and the isolates were inoculated into it and incubated for 48 hours. After  
321 incubation, 0.5ml of 1% sulphanilic acid in 5N acetic acid and 0.5ml of 1% alpha-naphtol was  
322 added. A positive result is indicated by the presence of maroon or pinkish colouration while  
323 brownish colouration indicates a negative result.

#### 324 **2.7.4.0 Haemolysis Test**

325 Haemolysis test was carried out to differentiate some species of bacteria that have the ability to  
326 lyse blood cells. Blood agar was first prepared by preparing Nutrient Agar and autoclaving it at  
327 121°C for 15 minutes. It was allowed to cool after which fresh blood was added into it and  
328 mixed thoroughly before it was poured into sterile Petri-dishes and allowed to solidify. After  
329 solidifying, the plates were inoculated with the isolates by just dropping the isolates at a spot on  
330 the agar and incubated at 37°C for 24 hours. After incubation, haemolysis was observed. Three  
331 haemolytic patterns can be observed: alpha ( $\alpha$ ) haemolysis showed green colouration around  
332 the organism, beta ( $\beta$ ) haemolysis showed a clean zone around the organism and gamma ( $\gamma$ )  
333 haemolysis showed no colour change on the blood agar (Willey *et al.*, 2007).

#### 334 **2.7.4.1 Oxidation-Fermentation Test**

335 This was carried out by dissolving 1.95 grams of Nutrient Broth, 2.25 gram of Agar Agar, 1  
336 gram of glucose and 0.75 gram of NaCl were measured into a conical flask and mixed with  
337 150ml distilled water. Bromothymol blue indicator and 1.5ml of 0.5%  $\text{Na}_2\text{HPO}_4$  was added.  
338 The mixture was heated to melt the agar and then dispensed into McCartney bottles; two bottles  
339 per isolate and autoclaved for 15 minutes at 121°C. After autoclaving, it was allowed to solidify  
340 and the isolate was inoculated into the agar and stabbed to the bottom of the bottle. The bottles  
341 were labelled open and close. The close bottles were covered with candle wax after inoculation.  
342 It was incubated for 7 days. Acid production in the cultures is indicated by a change in colour  
343 of the medium from green to yellow. Acid production in both the open and sealed bottles  
344 suggests a fermentation reaction. Acid formation in the open bottles only suggests an oxidative

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

#### 347 **2.7.4.2 Citrate Test**

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

#### 355 **2.7.4.3 Methyl Red-Voges-Proskauer (MR-VP) Tests**

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

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

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

#### 368 **2.7.4.4 Growth in 6.5% NaCl Broth**

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

### 374 **2.8 Characterization and Identification of Fungal Isolates**

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

#### 379 **2.8.1 Fungal Staining**

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

## 384 **RESULTS**

### 385 **3.1 Enumeration of Bacterial Isolates.**

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

$$\frac{\text{Ncfu/ml of isolate}}{\text{Area of sitesampled (cm}^2\text{)}}$$

389

390



391 **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	$8.0 \times 10^3$	$9.9 \times 10^3$	$1.6 \times 10^3$	$5.1 \times 10^3$	$4.2 \times 10^3$
Floor 2	$7.6 \times 10^3$	$6.4 \times 10^3$	$9.8 \times 10^3$	$4.4 \times 10^3$	$3.0 \times 10^3$
Floor 3	$9.9 \times 10^3$	$9.2 \times 10^3$	$5.9 \times 10^3$	$3.5 \times 10^3$	$5.1 \times 10^3$
Floor 4	$7.2 \times 10^3$	$3.2 \times 10^3$	$7.6 \times 10^3$	$2.5 \times 10^3$	$3.5 \times 10^3$
Chair 1	$6.72 \times 10^3$	$1.1 \times 10^2$	$8.0 \times 10^3$	$2.8 \times 10^3$	$5.1 \times 10^3$
Chair 2	$3.04 \times 10^3$	$1.3 \times 10^2$	$1.2 \times 10^2$	$2.7 \times 10^3$	$4.4 \times 10^3$
Chair 3	$3.2 \times 10^3$	$2.8 \times 10^3$	$4.4 \times 10^3$	$4.1 \times 10^3$	$2.2 \times 10^3$
Chair 4	$1.6 \times 10^3$	$9.6 \times 10^3$	$1.2 \times 10^3$	$3.0 \times 10^3$	$3.2 \times 10^3$
Table 1	$8.2 \times 10^3$	$1.7 \times 10^3$	$2.8 \times 10^3$	$6.7 \times 10^3$	$1.6 \times 10^2$
Table 2	$1.0 \times 10^2$	$2.2 \times 10^3$	$1.4 \times 10^2$	$2.2 \times 10^3$	$1.1 \times 10^2$
Table 3	$1.4 \times 10^2$	$3.2 \times 10^3$	$1.2 \times 10^2$	$3.5 \times 10^3$	$1.0 \times 10^2$
Table 4	$1.0 \times 10^2$	$2.2 \times 10^3$	$4.4 \times 10^3$	$2.2 \times 10^3$	$1.9 \times 10^2$
Toilet doorknob 1	$2.2 \times 10^3$	$3.2 \times 10^3$	$6.4 \times 10^3$	$1.1 \times 10^2$	$1.0 \times 10^2$
Toilet doorknob 2	$2.8 \times 10^3$	$3.5 \times 10^3$	$5.6 \times 10^3$	$1.9 \times 10^3$	$1.4 \times 10^2$
Classroom doorknob 1	$2.2 \times 10^3$	$4.3 \times 10^3$	$1.3 \times 10^2$	$1.1 \times 10^2$	$1.2 \times 10^2$
Classroom doorknob 2	$1.7 \times 10^3$	$4.1 \times 10^3$	$1.6 \times 10^3$	$1.9 \times 10^3$	$1.4 \times 10^2$
Classroom doorknob 3	$2.5 \times 10^3$	$1.1 \times 10^2$	$1.0 \times 10^2$	$1.7 \times 10^3$	$1.1 \times 10^3$
Handrail 1	$2.5 \times 10^3$	$1.1 \times 10^2$	$4.4 \times 10^3$	$1.2 \times 10^2$	$1.0 \times 10^2$
Handrail 2	$3.8 \times 10^3$	$1.6 \times 10^3$	$1.6 \times 10^3$	$3.2 \times 10^3$	$1.6 \times 10^2$
Handrail 3	$4.8 \times 10^3$	$1.1 \times 10^3$	$1.7 \times 10^3$	$3.3 \times 10^3$	$1.2 \times 10^2$

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393 **3.2 Characterization and Identification of Bacterial Isolates**

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

399 **3.3 Occurrence of Bacterial Isolate**

400 The table below shows the incidence of the characterized bacterial isolates in each sampled  
 401 routine i.e. floor, table, chair, doorknobs and handrails. School 1 and School 5 had the highest  
 402 occurrence of all the bacteria characterized (the nine bacteria isolated were present in school  
 403 1 and school 5).

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**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
<i>Corynebacterium kutscheri</i>	+	-	+	-	+
<i>Lactobacillus casei</i>	+	+	+	+	+
<i>Bacillus sphaerious</i>	+	-	-	-	+
<i>Bacillus subtilis</i>	+	+	+	+	+
<i>Aeromonas sp.</i>	+	+	+	+	+
<i>Staphylococcus aureus</i>	+	+	+	+	+
<i>Staphylococcus epidermidis</i>	+	+	+	+	+
<i>Pseudomonas sp.</i>	+	+	+	+	+
<i>Micrococcus sp.</i>	+	+	+	+	+

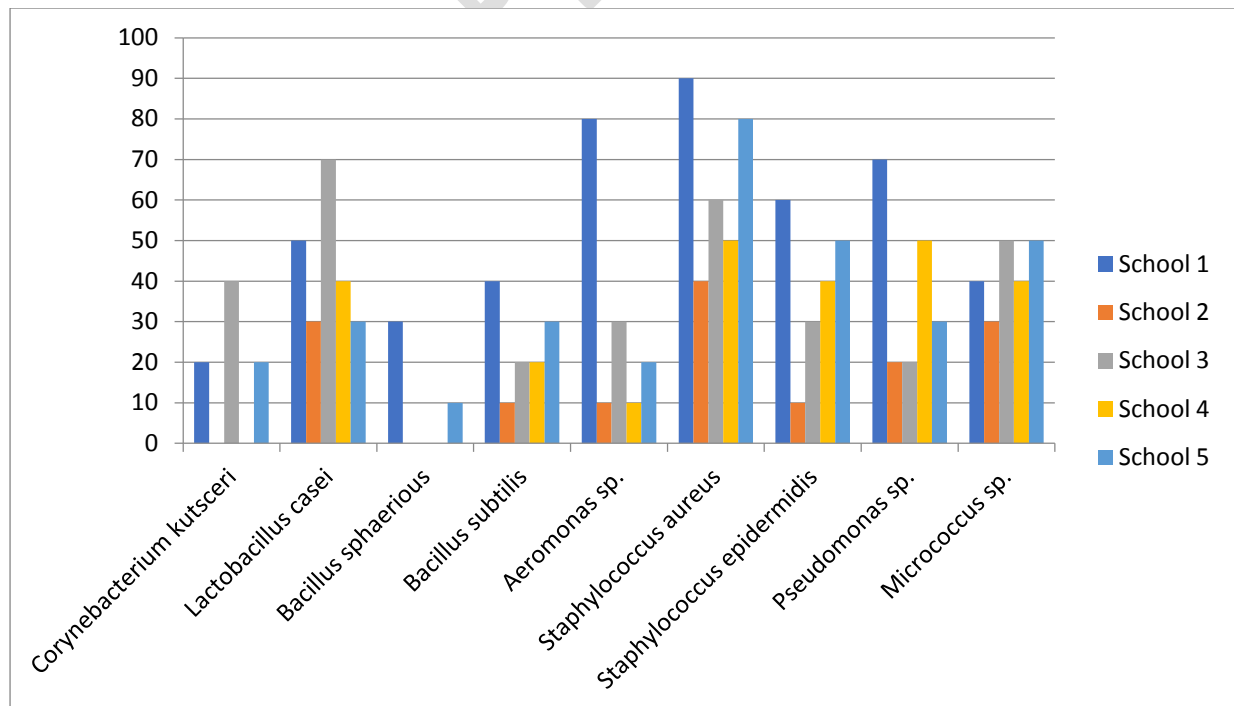
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KEYS:   +: Present            -: Absent

413 **3.3 Frequency of Bacteria Isolates**

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

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

423 **3.4 Fungal Isolates**

424 **Enumeration of Fungal Isolate**

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

$$\frac{\text{Ncfu/ml of isolate}}{\text{Area of sitesampled (cm}^2\text{)}}$$

428 **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 <sup>3</sup>	1.0 × 10 <sup>3</sup>	2.2 × 10 <sup>2</sup>	2.5 × 10 <sup>2</sup>	2.3 × 10 <sup>2</sup>
Floor 2	1.0 × 10 <sup>3</sup>	1.0 × 10 <sup>3</sup>	1.9 × 10 <sup>2</sup>	3.0 × 10 <sup>2</sup>	1.9 × 10 <sup>2</sup>
Floor 3	1.2 × 10 <sup>3</sup>	4.0 × 10 <sup>3</sup>	0	0	0
Floor 4	1.1 × 10 <sup>2</sup>	0	0	0	0
Chair 1	0	1.0 × 10 <sup>2</sup>	0	1.0 × 10 <sup>2</sup>	0
Chair 2	0	1.0 × 10 <sup>2</sup>	0	0	0
Chair 3	0	0	0	1.5 × 10 <sup>2</sup>	0
Chair 4	0	3.3 × 10 <sup>3</sup>	0	1.2 × 10 <sup>2</sup>	0
Table 1	6.4 × 10 <sup>2</sup>	1.4 × 10 <sup>2</sup>	1.3 × 10 <sup>2</sup>	0	0
Table 2	0	1.6 × 10 <sup>3</sup>	2.2 × 10 <sup>2</sup>	0	1.3 × 10 <sup>2</sup>
Table 3	2.4 × 10 <sup>3</sup>	0	2.3 × 10 <sup>2</sup>	1.9 × 10 <sup>2</sup>	1.2 × 10 <sup>2</sup>
Table 4	1.2 × 10 <sup>3</sup>	1.2 × 10 <sup>3</sup>	1.0 × 10 <sup>2</sup>	2.1 × 10 <sup>2</sup>	1.0 × 10 <sup>2</sup>
Toilet doorknob 1	2.7 × 10 <sup>3</sup>	2.4 × 10 <sup>2</sup>	0	4.0 × 10 <sup>2</sup>	0
Toilet doorknob 2	1.6 × 10 <sup>3</sup>	1.2 × 10 <sup>2</sup>	0	2.0 × 10 <sup>2</sup>	0
Classroom doorknob 1	0	2.0 × 10 <sup>2</sup>	2.0 × 10 <sup>2</sup>	2.0 × 10 <sup>2</sup>	0
Classroom doorknob 2	1.6 × 10 <sup>2</sup>	1.2 × 10 <sup>2</sup>	0	1.2 × 10 <sup>2</sup>	0
Classroom doorknob 3	1.2 × 10 <sup>2</sup>	1.4 × 10 <sup>3</sup>	0	0	0
Handrail 1	1.0 × 10 <sup>2</sup>	0	1.0 × 10 <sup>2</sup>	0	0
Handrail 2	2.7 × 10 <sup>3</sup>	0	2.1 × 10 <sup>2</sup>	0	2.0 × 10 <sup>2</sup>
Handrail 3	1.0 × 10 <sup>2</sup>	0	1.5 × 10 <sup>2</sup>	0	0

452 **3.6 Occurrence of Fungal Isolate**

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

456 **Table 4: Occurrence of fungi isolates from the environmental surfaces**

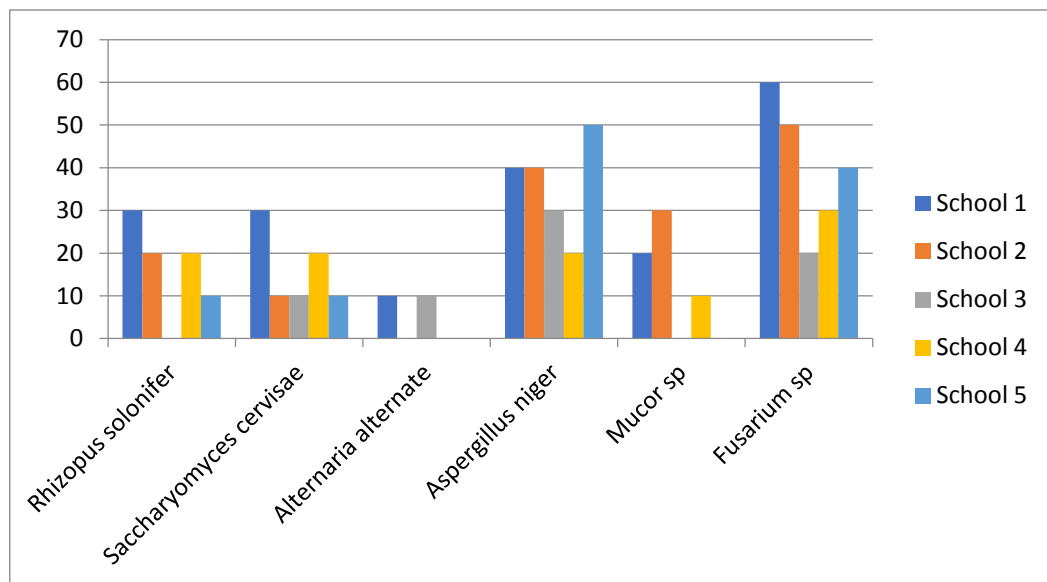
Isolate	School 1	School 2	School 3	School 4	School 5
<i>Rhizopus solonifer</i>	+	+	-	+	+
<i>Saccharyomyces cerevisiae</i>	+	+	+	+	+
<i>Alternaria alternata</i>	+	-	+	-	-
<i>Aspergillus niger</i>	+	+	+	+	+
<i>Mucor</i> sp	+	+	-	+	-
<i>Fusarium</i> sp	+	+	+	+	+

457 KEYS: +; Present -; Absent

458 **3.7 Frequency of fungal isolates**

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

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

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469 **DISCUSSION AND CONCLUSION**

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

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

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

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

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

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

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

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

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

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

542 *Micococcus varians* and *Aeromonas spp*: These organisms are ubiquitous in soil and water.  
 543 However, despite their common occurrence in nature, they only rarely cause infections in  
 544 human (Igari *et al.*, 2003)  
 545 *Mucor spp.*, *Fusarium spp.*, *Rhizopus stolonifer*, *Aspergillus niger* are ubiquitous and isolated  
 546 from indoor air environment, soils and on the skin. They are rarely pathogenic, except in  
 547 immunosuppressed individuals (Ribes *et al.*, 2000).  
 548 *Alternaria alternate* are opportunistic pathogen on numerous hosts causing leaf spots, rots  
 549 and blights on many plant parts. It can also cause upper respiratory tract infections and  
 550 asthma in humans with compromised immunity (Igari *et al.*, 2003).  
 551 In view of the high level of bacterial and fungal contamination as well as the isolation and  
 552 identification of some potential bacterial and fungal pathogens from the contact surfaces of  
 553 some secondary schools in Ilorin, it shows that the students in these schools may easily  
 554 contact these microorganisms and thus become infected. Although not every exposure results  
 555 in infection, a risk assessment for infection based on the host immune system, mechanism of  
 556 exposure, infectious dose of exposure, virulence of the agent is considered.  
 557 The relative high microbial count of the floor can be attributed to the fact that floors and have  
 558 become effective formite and vechiles for harbouring microorganisms that the students come  
 559 in contact with. Surprisingly, there was no record of total coliform, peradventure, due to the  
 560 use of disinfectants in some of the schools or in particular a school (school 1) which was  
 561 observed that there was no or low usage of the toilet due to fear of contacting toilet diseases.

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