

1 **Microorganisms Isolated from Hospital**
2 **Environmental Surfaces in Akure Metropolis, Ondo**
3 **State, Nigeria.**

4
5 **ABSTRACT**

6 Nosocomial infection is a rising problem in developing countries. Microorganisms Isolated
7 from three private and public hHospital eEnvironmental sSurfaces in Akure Metropolis,
8 Ondo State, Nigeria were investigated in this study. Bacterial and fungal organisms were
9 isolated and compared among the three hospitals. The study revealed that bacteria were the
10 most predominant microorganisms found in the hospital environmental surfaces than fungi.
11 *Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli, Pseudomonas aeruginosa,*
12 *Klebsiellapneumoniae* and *Bacillus cereus* were the bacterial isolates while fungi include
13 *Aspergillus fumigatus, Aspergillus niger* and *Candida albicans.* *Staphylococcus aureus* was
14 found to be predominant bacteria but *Aspergillus funmigatus* was the predominant fungi. The
15 result showed that the microbial loads of the public hospitals were higher than that of the
16 private hospitals. The bacteria load of the male ward was found to be higher than that of the
17 female ward while the fungal loads of each of the hospital environmental surfaces of female
18 were higher than that of the male. The study revealed that bacteria were the most
19 predominant microorganisms found in the hospital environment than fungi. *Staphylococcus*
20 *aureus, Streptococcus pyogenes, Escherichia coli, Pseudomonas aeruginosa, Klebsiella*
21 *pneumoniae* and *Bacillus cereus* were the bacterial isolates while fungi include *Aspergillu-s*
22 *fumigatus, Aspergillu-s_niger* and *Candida albicans.* *Staphylococcus aureus* was found to be
23 predominant bacteria. All the hospital environmental surfaces were contaminated with one or
24 more microorganisms in the course of the research.

25 Keywords: Hospital; ward; environmental; bacteria; fungi.

26 **Introduction**

27 Nosocomial infection is an infection occurring in a patient in a hospital or other health care
28 facility in whom the infection was not present or incubating at the time of admission. It is
29 estimated that in developing countries, nosocomial infections concern above 25% of
30 hospitalized patients, and in the developed countries from 5 to 10% (Wenzel, 1999). This

31 includes infections acquired in the hospital but appearing after discharge, and also
32 occupational infections among staff of the facility (Benenson, 1995). The sources of
33 infections can be: patients, medical personnel, visitors or parts of the environment: equipment
34 and hospital items, also arthropods inhabiting hospitals.

35 Patient care is provided in facilities which range from highly equipped clinics and
36 technologically advanced university hospitals to front-line units with only basic facilities
37 (World Health Organization, 2002). Despite progress in public health and hospital care,
38 infections continue to develop in hospitalized patients, and may also affect hospital staff.
39 Many factors promote infection among hospitalized patients: decreased immunity among
40 patients; the increasing variety of medical procedures and invasive techniques creating
41 potential routes of infection; and the transmission of drug-resistant bacteria among crowded
42 hospital populations, where poor infection control practices may facilitate transmission
43 (World Health Organization, 2002).

44 Hospital acquired infection is an additional affliction to the patient admitted to the hospital
45 for some serious illness and is caused by pathogens which are prevalent in hospital
46 environment (Davaneet *al.*, 2014). In the hospital, microbes are ubiquitous; and can reach
47 the sick patient through various sources, such as air, water, food, contaminated equipments,
48 linen, catheters, scopes, ventilators, contaminated disinfectants and other preparations used
49 for treatment, visitors, infected patients, etc (Davaneet *al.*, 2014). Recently, the probable
50 involvement of surfaces and equipment from the hospital environment as a disseminating
51 source of pathogens, including resistant bacteria, has been highlighted (Sehulsteret *al.*, 2003).
52 There are no meaningful standards for permissible levels of microbial contamination of
53 inanimate surfaces in hospital environment, but an increased microbial load on surfaces may
54 imply the possibility of finding a pathogen (Dancer, 2004). Microorganisms that are often
55 associated with hospital acquired infections are *Staphylococcus aureus*, *Micrococcus* sp.,
56 *Pseudomonas* sp., *Proteus* sp., *Escherichia coli*, *Enterobacter*, *Bacillus cereus*, *Cladosporium*
57 sp., *Aspergillus* sp., and viruses (Ekhaiseet *al.*, 2008). *Pseudomonas aeruginosa* has been
58 particularly incriminated in nosocomial infection because of its intrinsic resistance to most
59 antibiotics and its ability to survive and multiply at low temperatures and in disinfectant
60 solutions (Ohsakiet *al.*, 2007). This study aimed at isolating and comparing bacterial and
61 fungal microorganisms isolated from the environmental surfaces of three public and private
62 tertiary healthcare institutions in Akure metropolis, Ondo state, Nigeria.

63 The aim of this study was to examine the microorganisms isolated from hospital
64 environmental surfaces in Akure metropolis, Ondo state, Nigeria.
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67

68 **MATERIALS AND METHODS**

69 **Description of study location**

70 This research work was carried out from September 2016 to April, 2017 in Akure metropolis,
71 Ondo state, Nigeria. Akure covers an area of 14,798.8 ,993.7 square kilometers and lies at
72 latitude 7°15'0"N, 7° 11' N 5°11'42"E and longitude 5°11'42"E, 5°35'E. Akure is one of the
73 18 local government areas of Ondo State with a population of 484,798 based on the 2006
74 population census. It is situated in the peripheral zone of the rainforest of Ondo state. Akure
75 is the administrative capital of Ondo state. Akure lies about 70°15' north of the equator and
76 50°15' east of the Meridian. It is about 700 km Southwest of Abuja and 311 km north
77 of Lagos State. The town is situated in the tropic rainforest zone in Nigeria.

78 **Collection of samples**

79 Samples were collected by swab sticks from Male Accident and Emergency Bed, Female
80 Accident and Emergency Bed, Male Toilet, Female Toilet, Male Surgical Ward Chair,
81 Female Surgical Ward Chair, Male Medical Ward Floor, Female Medical Ward Floor, Male
82 Ward Air flora, Female Ward Air flora, Theatre Couch, Injection Room Tables, Neonatal
83 Ward Couch and Maternity Ward Couch from Health Centre FUTA, Don Bosco Catholic
84 Hospital and State Specialist Hospital Akure. The date, time, conditions and sites of sampling
85 were noted. Basically, swabs were used, at least, for each sampling site. For sampling, swabs
86 were moistened in 2 ml sterile saline solution and rolled several times over a surface area of
87 around 25 cm², and the swab sticks were transported to the laboratory. Sampling was always
88 done between 8-10am

89 **Isolation of microorganisms from hospital environment**

90 Isolation of microorganisms from hospital environment were carried out as described
91 by Bakkaliet *al.* (2005) with slight modification. Basically, swabs were used, at least, for
92 each sampling site. For sampling, swabs were moistened in 2 ml sterile saline solution and
93 rolled several times over a surface area of around 25 cm², and the swab sticks were

94 transported to the laboratory. Sampling was always done between 8-10am. A five-fold serial
95 dilution was made and 0.1 ml of the 10^{-3} and 10^{-5} dilutions were uniformly pour-plated onto
96 14 cm diameter wide agar plates and of nutrient agar, Potato dextrose agar, MacConkey agar
97 and EMB agar.

98

99 **Characterization of bacterial isolates**

100 The pure culture of each isolate was examined. Microscopic examination, staining
101 techniques and biochemical tests were carried out on the isolates according to the methods
102 described by Olutiola *et al.* (2000) and Cheesbrough (2010).

103 **Identification of fungal isolates**

104 Fungal isolates were characterized and identified based on macroscopic and
105 microscopic details with reference to Barnett and Hunter (1998).

106 **Statistical analysis of data**

107 All experiments were carried out in triplicate, and data obtained were subjected to one way
108 analysis of variance, while the means were compared by Duncan's New Multiple Range Test
109 at 95 % confidence interval using Statistical Package for Social Sciences version 16.0.
110 Differences were considered significant at $p \leq 0.05$.

111 **RESULTS**

112 **Table 1: Bacterial load of hospital environmental surfaces.**

Study Area(Source)	FUTA Health Centre	State Specialist Hospital (Cfu/ml)	DonBosco Hospital (Cfu/ml)
MAEB	4.1×10^4	4.6×10^4	2.0×10^3
FAEB	2.9×10^4	3.1×10^4	1.0×10^3
MT	TNC	TNC	TNC
FT	TNC	TNC	TNC
MSWC	ND	4.5×10^4	3.0×10^3
FSWC	ND	2.0×10^3	1.0×10^3

MMWF	3.3×10^4	4.1×10^4	3.0×10^3
FMWF	3.0×10^3	3.4×10^4	2.0×10^3
MWA	3.9×10^4	4.5×10^4	3.0×10^3
FWA	3.0×10^3	5.0×10^3	2.0×10^3
TC	ND	2.0×10^3	1.0×10^3
IRT	3.0×10^3	4.0×10^3	1.0×10^3
NWC	3.0×10^3	4.0×10^3	2.0×10^3
MWC	4.0×10^3	5.0×10^3	2.0×10^3

113 **LEGEND:** Not Determine (ND), No Growth (NG), Male Accident and Emergency Bed
 114 (MAEB), Female Accident and Emergency Bed (FAEB), Male Toilet (MT), Female Toilet
 115 (FT), Male Surgical Ward Chair (MSWC), Female Surgical Ward Chair (FSWC), Male
 116 Medical Ward Floor (MMWF), Female Medical Ward Floor (FMWF), Male Ward Air flora
 117 (MWA), Female Ward Air flora (FWA), Theatre Couch (TC), Injection Room Tables (IRT),
 118 Neonatal Ward Couch (NWC) and Maternity Ward Couch (MWC).

119 Table 1: The bacterial load of each of the items isolated from different hospital environmental
 120 surfaces is shown in Table 1, it was observed that bacterial load of the toilet were higher than
 121 other surfaces, while the bacterial load from each of the male hospital environmental
 122 surfaces was higher than that of the female hospital environmental surfaces. It was also
 123 observed that the bacterial loads isolated from government own hospital was higher than
 124 those microorganisms isolated from private hospital

125 **Table 2: Fungal load of hospital environmental surfaces.**

Study Area(Source)	FUTA Health Centre (Sfu/ml)	State Specialist Hospital (Sfu/ml)	Don Bosco Hospital ((Sfu/ml))
MAEB	2.0×10^3	1.5×10^4	1.0×10^3
FAEB	3.0×10^3	3.0×10^3	2.0×10^3
MT	4.0×10^3	4.4×10^4	3.0×10^3
FT	6.0×10^3	7.1×10^4	4.0×10^3
MSWC	ND	NG	NG
FSWC	ND	NG	NG
MMWF	1.0×10^3	2.0×10^3	NG

FMWF	2.0×10^3	4.0×10^3	1.0×10^3
MWA	2.0×10^3	3.0×10^3	NG
FWA	3.0×10^3	4.0×10^3	1.0×10^3
TC	ND	NG	NG
IRT	NG	NG	NG
NWC	2.0×10^3	3.0×10^3	1.0×10^3
MWC	NG	NG	NG

126 **LEGEND:** Not Determine (ND), No Growth (NG), Male Accident and Emergency Bed
127 (MAEB), Female Accident and Emergency Bed (FAEB), Male Toilet (MT), Female Toilet
128 (FT), Male Surgical Ward Chair (MSWC), Female Surgical Ward Chair (FSWC), Male
129 Medical Ward Floor (MMWF), Female Medical Ward Floor (FMWF), Male Ward Air flora
130 (MWA), Female Ward Air flora (FWA), Theatre Couch (TC), Injection Room Tables (IRT),
131 Neonatal Ward Couch (NWC) and Maternity Ward Couch (MWC).

132 Table 2: The fungal load of each of the fungi isolated from different hospital environmental
133 surfaces are shown in Table 2, it was observed that fungal load of the toilet was found to be
134 higher than other surfaces, while the fungal load isolated from each of the female hospital
135 environmental surfaces was higher than that of the male hospital environmental surfaces. It
136 was also observed that the fungal loads isolated from government own hospital was higher
137 than those microorganisms isolated from private hospital

138 **Table 3: Rate of occurrence of different bacteria isolated from FUTA Health**
139 **Centre, State Specialist hospital Akure and Don Bosco Hospital Akure**

Bacteria	Number of surfaces Tested	Percentage positivity (%)
<i>Staphylococcus aureus</i>	39	22.81
<i>Streptococcus pyogenes</i>	24	14.04
<i>Escherichia coli</i>	21	12.28
<i>Pseudomonas aeruginosa</i>	27	15.79
<i>Klebsiella pneumoniae</i>	33	19.30
<i>Bacillus cereus</i>	27	15.79
Total	171	100.01

140 Table 3: The rate of occurrence of different bacteria isolated from different hospital
 141 environmental surfaces is presented in Table 3. It was observed that *Staphylococcus aureus*
 142 had the highest rate of occurrence, while *Escherichia coli* had the lowest rate of occurrence out
 143 of the bacteria isolated for different hospital environment surfaces

144

145

146 **Table 4: Rate of occurrence of different fungi isolated from FUTA Health Centre,**
 147 **State Specialist hospital Akure and Don Bosco Hospital Akure**

Fungi	Number of surfaces Tested	Percentage positivity (%)
<i>Aspergillus fumigatus</i>	21	36.84
<i>Aspergillus flavus</i>	18	31.58
<i>Candida albicans</i>	18	31.58
Total	57	100

148 Table 4: The rate of occurrence of different fungi isolated from different hospital
 149 environmental surfaces is presented in Table 4. It was observed that *Aspergillus fumigatus*
 150 had the highest rate of occurrence followed by *Candida albicans* and *Aspergillus niger* which
 151 share the same number percentage positivity.

152 **DISCUSSION**

153 Hospital associated infections have been linked with many factors among which is the
 154 microbial quality of the indoor air of different wards and units of each hospital (Ekhaise *et*
 155 *al.*, 2010). This type of infection occurs in 5% of all acute care hospitalization in the United
 156 State and has been reported to be responsible for the death of one out of every five thousand
 157 patients attending an American hospital (Putsept, 1981). In Nigeria, the rate of nosocomial
 158 infection ranges between 2.7%-3.8% (Onipede *et al.*, 2004). Hence, ~~this requires calls for~~
 159 ~~looking at~~ every possible measure to control the rise including (among other investigations)
 160 examining the quality of indoor air of the hospital wards and units. Each of the hospital
 161 environmental surfaces was contaminated with microorganisms.

162 Bacteria were found to be more predominant than fungi, the bacteria isolated from the
 163 hospital surfaces were *Bacillus cereus*, *Escherichia coli*, *Klebsiella pneumoniae*.

164 | *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Streptococcus pyogenes* while fungi
165 | include *Aspergillus fumigatus*, *Aspergillus niger* and *Candida albicans*. *Staphylococcus*
166 | *aureus* was found to be predominant bacteria with the occurrence of 22.81%, this correlate
167 | with the report of Awosika *et al.* (2012) who reported *Staphylococcus aureus* as the most
168 | frequently isolated bacterium from hospital surface. *Staphylococcus aureus* as the most
169 | frequently isolated bacterium from hospital surface has been incriminated in various diseases
170 | such as post-operative infections, urinary tract infections, skin infections, respiratory
171 | infections and food poisoning (Murray *et al.*, 1995). Proper control measures, such as
172 | increase in hygiene, are required to combat infections by *Staphylococcus aureus* in these
173 | hospital wards and units (Awosika *et al.*, 2012). The occurrence of bacteria in hospitals
174 | has been commonly related to some possible sources of dissemination: bottle soap
175 | (Buffet-Bataillon *et al.*, 2009), hands of healthcare professionals (Tan *et al.*, 2013), gloves
176 | and gowns (Rock *et al.*, 2014), mobile phones (Ustun and Cihangiroglu, 2012) paper
177 | money and coins (Angelakis *et al.*, 2014). *Aspergillus fumigatus* was found to be
178 | predominant fungi with frequency occurrence of 36.84%, this correlate with the report of
179 | Cagginao *et al.* (2014) who reported that *Aspergillus fumigatus* was the most commonly
180 | isolated (68.5%).

181 | The bacterial load of the male ward was found to be higher than that of the female,
182 | this could be due personal hygiene of the female, this ~~contrary in line~~ with the report of
183 | Ekhaïse *et al.* (2008) who reported that a quantitative study of different hospital units showed
184 | that the children ward and female ward had the highest total bacterial count followed by the
185 | bacteriology laboratory.

186 | The fungal loads of each of the hospital environment surfaces of female were higher
187 | than that of the male. In hospital environments, airborne molds are a potential risk for
188 | patients because of possible inhalation of conidia (Augustowska and Dutkiewicz , 2006).
189 | Because surgical procedures expose patients to infective complications, the operating theater
190 | is considered a complex habitat in which all sources of pollution have to be kept under
191 | control (Partridge-Hinckley *et al.*, 2009; Grossi *et al.*, 2011). In particular, the widespread
192 | presence of *Aspergillus* spp. is the major extrinsic risk factor for invasive aspergillosis,
193 | caused by *A. fumigatus* and other species of *Aspergillus*, such as *A. flavus*, *A. niger*, and *A.*
194 | *terreus*, depending on the local epidemiology (Singh and Paterson, 2005) and according to
195 | the season (Panagopoulou *et al.*, 2007).

196 The microbial load of the public hospital were higher than that of the private, this
197 tallied with the report Ekhaise *et al.* (2008) who reported high microbial counts recorded for
198 the public hospital (Central Hospital) as compared to private hospital (Faith Medical
199 Center), could be due to the subsidized ~~ds~~ user fees rate of the public hospital, ~~thereby~~ ~~as~~
200 accommodatinge more people, as compared to the private hospital, where higher user
201 fees are charged and are not within the reach of the poor people in the society. These findings
202 could be explained by many factors including the number of visitors visiting the children and
203 female wards, which exceeded visitors in other hospital units. It was noted that the amount of
204 materials brought from outside such as personal belongings, food and fruits were more
205 common in children and female wards. These are recognized as sources of hospital
206 contamination. Hospital surfaces are contaminated by factors inherent to the presence of
207 patients, such as biological fluids, sometimes associated ~~with~~ invasive and non-
208 invasive ~~assistance~~ techniques and hygiene. Another contamination factor would be the
209 circulation of vectors as carrier agents for fungi and bacteria resistant to antimicrobials
210 (Prado *et al.*, 2006; Rodovalho *et al.*, 2007)

211 The microbial load of the theater couch and surgical ward were found to be the
212 lowest, this could probably be due to the fact that there is high sanitary standards in this area
213 as compared to other hospital areas and also that the theater is a restricted area, which ~~this~~
214 tallied with the result ~~submission~~ of Ekhaise *et al.* (2008) who reported that the number of
215 microorganisms in the theater was extremely low.

216 Although, surfaces are not directly connected to transmission in most hospital
217 infections, the impact of hygiene and cleaning procedures in microbial control is
218 evident. It is suggested that microorganisms associated to hospital infections are able
219 to survive during large periods of time, thus being a continuous source of
220 contamination in cases where population control is not efficiently conducted (Kramer
221 *et al.*, 2006; Rossi *et al.*, 2008).

222 Regular surveillance, cleaning and restriction of patient's relatives might be among
223 the strict measures necessary to reduce ~~or totally eliminate~~ the microbial load of indoor air
224 ~~in~~ of this hospital wards and units (Awosika *et al.*, 2012)

225 **CONCLUSION**

226 This investigation has been able to identify and prove the sensitivity patterns of
227 microorganism isolated from hospital environment surfaces. The study has shown that all the
228 hospital environmental surfaces examine in the course of the study in Akure, Nigeria were
229 contaminated with one or more microorganisms.

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