Microorganisms Isolated from Hospital Environmental Surfaces in Akure Metropolis, Ondo State, Nigeria.

ABSTRACT

Microorganisms Isolated from Hospital Environmental Surfaces in Akure Metropolis, Ondo State, Nigeria were investigated. The study revealed that bacteria were the most predominant microorganisms found in the hospital environmental surfaces than fungi. *Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli, Pseudomonas aeruginosa, Klebsiellapneumoniae*and *Bacillus cereus* were the bacterial isolates while fungi include *Aspergillus fumigatus, Aspergillus niger* and *Candida albicans. Staphylococcus aureus* was found to be predominant bacteria but *Aspergillus funmigatus* was the predominant fungi. The result showed that the microbial loads of the public hospitals were higher than that of the private hospitals. The bacteria load of the male ward was found to be higher than that of the female ward while the fungal loads of each of the hospital environmental surfaces of female were higher than that of the male. The study revealed that bacteria were the most predominant microorganisms found in the hospital environment than fungi. *Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Bacillus cereus* were the bacterial isolates while fungi include *Aspergillu sfumigatus, Aspergillu sniger* and *Candida albicans. Staphylococcus aureus* was found to be predominant bacteria. All the hospital environmental surfaces were contaminated with one or more microorganisms in the course of the research.

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

Introduction

Nosocomial infection is an infection occurring in a patient in a hospital or other health care facility in whom the infection was not present or incubating at the time of admission. It is estimated that in developing countries, nosocomial infections concern above 25% of hospitalized patients, and in the developed countries from 5 to 10% (Wenzel, 1999). This includes infections acquired in the hospital but appearing after discharge, and also occupational infections among staff of the facility (Benenson, 1995). The sources of

infections can be: patients, medical personnel, visitors or parts of the environment: equipment and hospital items, also arthropods inhabiting hospitals.

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

Hospital acquired infection is an additional affliction to the patient admitted to the hospital for some serious illness and is caused by pathogens which are prevalent in hospital environment (Davane*et al*., 2014). In the hospital, microbes are ubiquitous; and can reach the sick patient through various sources, such as air, water, food, contaminated equipments, linen, catheters, scopes, ventilators, contaminated disinfectants and other preparations used for treatment, visitors, infected patients, etc (Davane*et al*., 2014). Recently, the probable involvement of surfaces and equipment from the hospital environment as a disseminating source of pathogens, including resistant bacteria, has been highlighted (Sehulster*et al*., 2003). There are no meaningful standards for permissible levels of microbial contamination of inanimate surfaces in hospital environment, but an increased microbial load on surfaces may imply the possibility of finding a pathogen (Dancer, 2004).Microorganisms that are often associated with hospital acquired infections are *Staphylococcus aureus, Micrococcus* sp., *Pseudomonas* sp., *Proteus* sp., *Escherichia coli*, *Enterobacter*, *Bacillus cereus*, *Cladosporium* sp., *Aspergillus* sp., and viruses (Ekhaise*et al*., 2008). *Pseudomonas aeruginosa* has been particularly incriminated in nosocomial infection because of its intrinsic resistance to most antibiotics and its ability to survive and multiply at low temperatures and in disinfectant solutions (Ohsaki*et al*., 2007).

MATERIALS AND METHODS

Description of study location

This research work was carried out from September 2016 to April, 2017 in Akure metropolis, Ondo state, Nigeria. Akure covers an area of 14,798.8 ,993.7 square kilometers and lies at latitude 7°15′0″N, 7^0 11′ N 5°11′42″E and longitude 5°11′42″E, 5^O35'E. Akure is one of the 18 local government areas of Ondo State with a population of 484,798 based on the 2006 population census. It is situated in the peripheral zone of the rainforest of Ondo state. Akure is the administrative capital of Ondo state. Akure lies about 70°15' north of the equator and 50°15' east of the Meridian. It is about 700 km Southwest of Abuja and 311 km north of Lagos State. The town is situated in the tropic rainforest zone in Nigeria.

Collection of samples

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

Isolation of microorganisms from hospital environment

Isolation of microorganisms from hospital environment were carried out as described by Bakkali*et al.* (2005) with slight modification. Basically, swabs were used, at least, for each sampling site. For sampling, swabs were moistened in 2 ml sterile saline solution and rolled several times over a surface area of around 25 cm^2, and the swab sticks were transported to the laboratory. Sampling was always done between 8-10am. A five-fold serial dilution was made and 0.1 ml of the 10^{-3} and 10^{-5} dilutions were uniformly pour-plated onto 14 cm diameter wide agar plates and of nutrient agar, Potato dextrose agar, MacConkey agar and EMB agar.

Characterization of bacterial isolates

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

Identification of fungal isolates

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

Statistical analysis of data

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

RESULTS

Table 1: Bacterial load of hospital environmental surfaces.

Study Area(Source)	FUTA Health Centre (Cfu/ml)	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

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

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

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

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

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

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

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

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

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

Fungi	Number of surfaces Tested Positive	Percentage positivity (%)
Aspergillusfumigatus	21	36.84
Aspergillusflavus	18	31.58
Candida albicans	18	31.58
Total	57	100

Table 4: The rate of occurrence of different fungi isolated from different hospital environmentalsurfaces is presented in Table 4. It was observed that*Aspergillusfumigatus had* the highest rate of occurrence followed by *Candida albicans* and *Aspergillusniger* which share the same number percentage positivity.

DISCUSSION

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

Bacteria were found to be more predominant than fungi, the bacteria isolated from the hospital surfaces were *Bacillus cereus, Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Staphylococcus aureus,* and *Streptococcus pyogenes*while fungi include *Aspergillus fumigatus, Aspergillus niger* and *Candida albicans. Staphylococcus aureus* was found to be predominant bacteria with the occurrence of 22.81%, this correlate with the report of Awosika *et al.* (2012) who reported *Staphylococcus aureus*as the most frequently isolated bacterium from hospital surface. *Staphylococcus aureus* as the most frequently isolated bacterium from hospital surface has been incriminated in various diseases such as post-operative infections, urinary tract infections, skin infections, respiratory infections and

food poisoning (Murray *et al*., 1995). Proper control measures, such as increase in hygiene, are required to combat infections by *Staphylococcus aureus* in these hospital wards and units (Awosika *et al.,* 2012). The occurrence of bacteria in hospitals has been commonly related to some possible sources of dissemination: bottle soap (Buffet-Bataillon*et al*., 2009), hands of healthcare professionals (Tan *et al*., 2013), gloves and gowns (Rock *et al*., 2014), mobile phones (Ustun and Cihangiroglu, 2012) paper money and coins (Angelakis*et al*., 2014). *Aspergillus funmigatus* was found to be predominant fungi with frequency occurrence of 36.84%, this correlate with the report of Cagginao*et al*. (2014)who reported that *Aspergillus fumigates* was the most commonly isolated (68.5%).

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

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

The microbial load of the public hospital were higher than that of the private, this tallied with the report Ekhaise*et al*. (2008) who reported high microbial counts recorded for the public hospital (Central Hospital) as compared to private hospital (Faith Medical Center), could be due to the subsidizes rate of the public hospital so as accommodate more people, compared to the private hospital, where high fees are charged and are not within the reach of the poor people in the society. These findings could be explained by many factors including the number of visitors visiting the children and female wards, which exceeded visitors in other hospital units. It was noted that the amount of materials brought

from outside such as personal belongings, food and fruits were more common in children and female wards. These are recognized as sources of hospital contamination. Hospital surfaces are contaminated by factors inherent to the presence of patients, such as biological fluids, sometimes associated to assistance techniques and hygiene. Another contamination factor would be the circulation of vectors as carrier agents for fungi and bacteria resistant to antimicrobials (Prado *et al*., 2006; Rodovalho*et al*., 2007)

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

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

Regular surveillance, cleaning and restriction of patients relative might be among the strict measures necessary to reduce or totally eliminate the microbial load of indoor air of this hospital wards and units (Awosika*et al*., 2012)

CONCLUSION

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

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