

Full Length Research Paper

# Antibiotic sensitivity assay on pathogenic microorganisms isolated from selected areas in some primary health centres in Akure Metropolis, Nigeria

Omoya, Funmilola Ph.D<sup>1\*</sup> and Afolabi, Temitope MBCHB, MPH, FMCPH<sup>2</sup>

<sup>1</sup>Department of Microbiology, School of Sciences, The Federal University of Technology, Akure, Nigeria.

<sup>2</sup>Department of Community Health, Faculty of Clinical Sciences, Obafemi Awolowo University, Ile-Ife, Nigeria.

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Isolation and identification of pathogenic microorganisms present on surface of facilities in wards of selected primary health centres were done. A total of 720 swab specimen from different surfaces (beddings, toilet seats, floor and door handles) were collected in triplicates and analyzed using microbiological standard to evaluate the hygienic status of these wards. Antibiotic sensitivity test was carried out on the pathogenic isolates using selected antibiotics and antifungal agents. The bacterial isolated are *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Proteus mirabilis*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, while the fungi are *Candida albicans* and *Candida dubliensis*. The highest bacterial load of  $53.33 \pm 1.86$  cfu/ml was obtained from maternity ward toilet while the least bacterial load of  $1.67 \pm 0.33$  cfu/ml was obtained from the pillows of pediatric ward. Their sensitivity to commercial antibiotics showed that *Pseudomonas aeruginosa* and *Staphylococcus aureus* were the most resistant bacteria to these antibiotics. Ofloxacin exerted the highest inhibitory effect against all the bacteria. Most of the isolates were resistant to tetracycline and streptomycin. These results inferred that pathogenic microorganisms resistant to some commonly used drugs can be acquired from these health centres. Therefore, adequate ward hygiene is necessary to reduce hospital acquired infections.

**Keywords:** Hospital, wards, acquired, infections, isolation, pathogenic, sensitivity, microorganisms, antibiotics.

## INTRODUCTION

A hospital acquired infection or nosocomial is an infection whose development is favored by a hospital environment. They are usually acquired by either a patient during a hospital visit (or when hospitalized), hospital staff or patients' relatives that visit when the patient is on admission in the hospital (Prescott *et al.*, 2011).

Nosocomial is responsible for 1.7 million hospital-associated infections in the United States and about 25,000 deaths in Europe annually from all types of microorganisms including bacteria (Pollack and Andrew, 2010). Nosocomial infections can cause severe pneumonia and infection of the urinary tract, blood stream

and other parts of the body.

Nosocomial infections are commonly encountered in Africa and in Nigeria in particular. According to Cheesbrough (2010), this is primarily due to factors such as hospital hygiene / cleanliness, personal hygiene of patients, overcrowding hospital wards and illiteracy. These infections are usually difficult to attack with antibiotics (Yusuf *et al.*, 2013). Equally, antibiotics resistance is fast spreading to more Gram positive and Gram negative bacteria that can infect people within the hospital environment (Omoya *et al.*, 2015).

The aim of this research is therefore focused on the isolation and identification of microorganisms from the different wards of some selected health centres in Akure metropolis and their susceptibility to some of the commercially available antibiotics.

\*Corresponding author E-mail: [fomoya@yahoo.com](mailto:fomoya@yahoo.com); +2348033738650

## MATERIALS AND METHODS

### Description of study area

The study area is Akure South Local Government Area. Akure South Local Government Area was carved out of Ondo Municipal Government of Akure central in 1996 after the creation of Ekiti State. It covers a land area of 15, 500 square kilometers. It has a population density of 3, 300 persons per square kilometer (National Population Census, 2006). The Akure South Local Government Area shares boundaries with Akure North Local Government Area and Akure East Local Government Area respectively.

Akure South Local Government Area has a total population of 360, 268; comprising of 173, 153 males and 187, 115 females according to the 2006 national population with 2010 estimated population of 459,164 using a growth rate of 3.2% from 2006 census. It is an urban area and therefore, no major farming activities take place. Yoruba and other tribes dominate the area. The residents are engaged in various economic activities such as trading, transportation business, civil service and education.

The symbol of tradition is evident in Akure South Local Government Area. The official resident of the Oba Adesida is situated in the area. There are twelve (12) primary Health care centres in the area, fifty-nine (59) registered private health facilities, two (2) public secondary health care facilities and no tertiary health facilities in the area. There are four mission (private) hospitals that provide secondary health care for the people. The surrounding Local Government areas have public secondary health centres.

### Study design

This study was a descriptive cross sectional survey.

### Study population and study subjects

All consenting primary health centers' facilities in Akure metropolis of Akure South Local Government area were included in the survey regardless of size and location.

### Sample size determination

A total of seven hundred and twenty (720) swab samples were collected from the surfaces of beddings (pillows and bed sheets), toilet seats, floor and door handles of male, female and children wards from the following primary health centres in Akure metropolis:

1. Primary health centre, Aule;
2. Primary health centre, Ayedun;
3. Primary health centre, Isolo;
4. Primary health centre, Arakale;
5. Primary health centre, Oba-ile and

6. Primary health centre, Orita-Obele.

### Sampling technique

Samples were collected from each surface in triplicates. This was done or repeated two times in two weeks for all the wards of the basic primary health centres selected in Akure metropolis. The samples were analyzed microbiologically to ascertain their hygienic status by isolating bacteria from them following the method of Fawole and Oso, (2004). The different media used (Nutrient agar, Eosin-Methylin Blue agar and McConkeyagar) were prepared according to the manufacturers' instructions before sterilizing at 121°C for 15 minutes in an autoclave. The bacteria isolates were identified using morphological characterization, biochemical characteristics and sugar fermentation reaction tests as outlined by Prescott *et al.*, (2011).

### Preservation of culture

Nutrient agar powder was dissolved in distilled water according to the manufacturer specification to prepare a double strength agar. 10ml of the dissolved agar were dispensed into each bijou bottles and screwed tight for sterilization in an autoclave at 121°C for 15 minutes, after sterilization the agar was allowed to set in a slanting position, with sterile inoculating loop, a loopful of the pure inoculum is streaked on the surface of the slant agar aseptically.

### Antibiotic sensitivity test

The antibiotic sensitivity test was carried out in order to know the sensitivity of the microorganism to the different commercially available antibiotics. These antibiotics discs include: Tetracycline (TET), Gentamicin (GEN), Ofloxacin(OFL), Cotrimoxazole (COT), Chloramphenicol (CHL), Streptomycin (STR), etc. Disc diffusion method was applied to determine the effect of standard antibiotics on the bacterial isolates as described by Oladunmoye *et al.*, (2014). Sterile Petri dishes were seeded aseptically with cultures of the test organisms each while about 15ml of sterilized Muller- Hinton agar was poured aseptically on the seeded plates. The plate were swirled carefully for even distribution and allowed to gel. With the aid of sterile forceps the antibiotics disc were placed firmly on solidified plates and incubated for 24hours at 37°C. After incubation, clear areas around the disc represent the zones of inhibition and the areas without clear zones were also observed. Seeded agar plates without antibiotics disc served as the control experiment. The zones of inhibition were measured in millimeter (mm). The experiment was carried out in triplicate.

### Data analysis

Calculation of occurrence rate of each bacterium was done using the method of Baker *et al.* (2006). All the data obtained were subjected to descriptive one way analysis

of variance, SPSS version 16, Microsoft window 8 with Duncan New Multiple Range Test as follow up test.

### Ethical consideration

Consent: Approval for the study was obtained from the Ondo State Hospital Management Board. Respondents were told of their right to decline the questionnaire without any consequences but appealed to not to decline it.

Confidentiality: Data collected was used only for research purpose and kept confidential on a password protected computer. Names and addresses were not included in the data collection questionnaires and thus data collected cannot be linked with any person.

Beneficence: Data obtained in this study can be useful in policy making and to identify appropriate areas requiring attention to improve health care service delivery in Ondo State.

### RESULTS

The results of bacterial isolation from the various parts of the wards in Arakale basic health centre showed that the highest bacterial load of  $52.00 \pm 3.46$  cfu/ml was obtained from toilet in maternity ward, while the least bacterial load of  $2.67 \pm 0.67$  cfu/ml was obtained from the floor of the male ward. Generally, the toilets of all the wards recorded the highest bacterial load obtained in this work with the male ward's toilet recording the least bacterial load. Comparatively, the floor from all the wards had the least bacterial load. There was no surface from which bacteria was not isolated in this basic health centre. The result of the bacterial load from this basic health centre is displayed in figure 1.

Keys: BDS=Bed sheet; DHD=Door handles; FLO=Floor; PIL=Pillow; TOI=Toilet

Figure 2 shows the mean bacterial load from Aule basic health centre. The bed sheet from this health centre recorded the highest bacterial load of  $28.33 \pm 3.18$  cfu/ml. the pillow from the paediatric ward had the least bacterial load of  $1.67 \pm 0.33$  cfu/ml. generally, the bacterial load from the paediatric ward of this health centre was low for all the surfaces evaluated.

Keys: BDS=Bed sheet; DHD=Door handles; FLO=Floor; PIL=Pillow; TOI=Toilet

The same pattern of bacterial load was recorded for Ayedun basic health centre, except that the toilet recorded the highest bacterial load of  $19.00 \pm 2.65$  cfu/ml. The bacterial load was least for all the surfaces evaluated in the paediatric ward of this health centre, an indication that the paediatric ward of this health centre was kept very clean. This result is shown in figure 3.

Keys: BDS=Bed sheet; DHD=Door handles; FLO=Floor; PIL=Pillow; TOI=Toilet

Isolo basic health centre showed that the highest bacterial load of  $53.33 \pm 1.86$  cfu/ml was obtained from

toilet in maternity ward, while the least bacterial load of  $3.33 \pm 1.33$  cfu/ml was obtained from the bed sheet of the paediatric ward. Generally, the toilets of all the wards recorded the highest bacterial load obtained in this work with the male ward's toilet recording the least bacterial load. Comparatively, the floor from all the wards had the least bacterial load. There was no surface from which bacteria was not isolated in this basic health centre. The result of the bacterial load from this basic health centre is displayed in figure 4.

Keys: BDS=Bed sheet; DHD=Door handles; FLO=Floor; PIL=Pillow; TOI=Toilet

Figures 5 and 6 shows the bacterial loads for the Oba-Ile and Orita-Obele health centres respectively. In both health centres, the toilets recorded the highest bacterial loads of  $32.67 \pm 3.48$  cfu/ml and  $26.00 \pm 3.51$  cfu/ml respectively. Equally, the least bacterial load was from the paediatric and male wards for the two health centres. The results equally showed that all the surfaces analyzed had bacteria in them with varying bacterial load from them.

A comparative evaluation of the different surfaces from which these bacteria were isolated as well as the bacterial load of the various wards evaluated is shown in figures 7-10 respectively.

Table 1 shows the total number of isolates from each health centre. A total number of eight (8) isolates were obtained from Arakale and Isolo basic health centres respectively. This number represents the highest number of isolates from all the health centres. The least number of isolates of five (5) was obtained for Aule and Ayedun basic health centres respectively.

The isolates were subjected to different identification processes. Tables 6 a, b and c shows the morphological characteristics, biochemical reactions and sugar fermentation tests results used for the identification of the isolates.

Table 2 shows the distribution of isolates from various basic health centres evaluated. Worthy of note is the fact that *Pseudomonas* species was isolated from four of the centres while *Staphylococcus aureus* and *Staphylococcus epidermidis* were isolated from all the basic health centres.

The distribution table showed that the toilets and the bed sheet housed most of the isolates in this work. All the isolates were present in the toilets and only two out of the eight were not isolated from the bed sheets. This is shown in Table 3.

Figure 11 shows the result of the sensitivity test of the bacterial isolates identified from the various surfaces. Of all the isolates, *Pseudomonas aeruginosa* and *Staphylococcus saprophiticus* are the most resistant bacteria to the antibiotics they were subjected to. Generally, ofloxacin exert the highest inhibitory effect against all the bacterial isolates, while most of the bacteria resisted tetracycline and streptomycin.

Two species of candida were isolated during this work

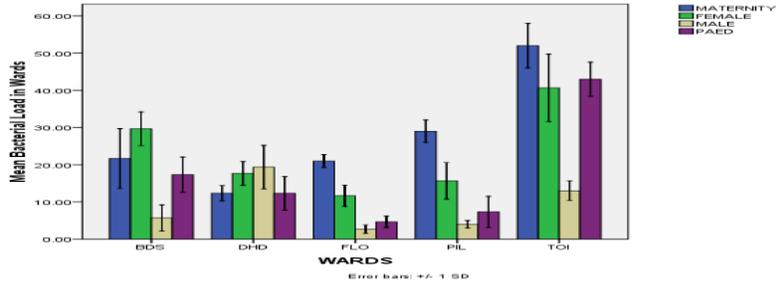


Figure 1. The mean bacterial load (cfu/ml) from Arakale Basic Health Centre.

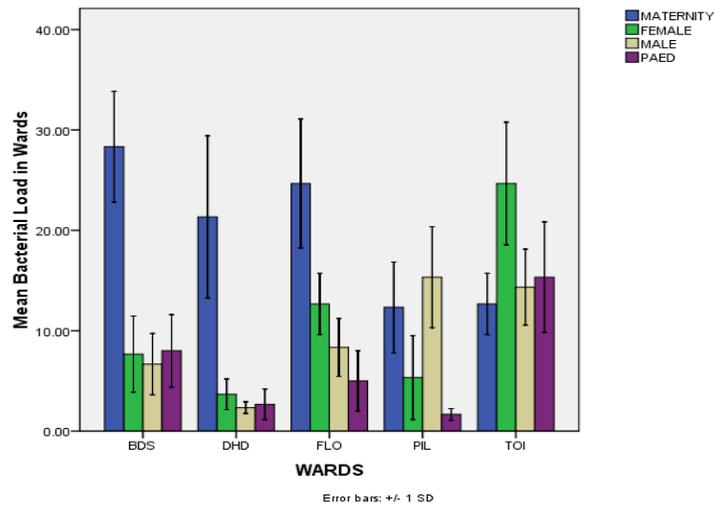


Figure 2. The mean bacterial load (cfu/ml) from Aule Basic Health Centre.

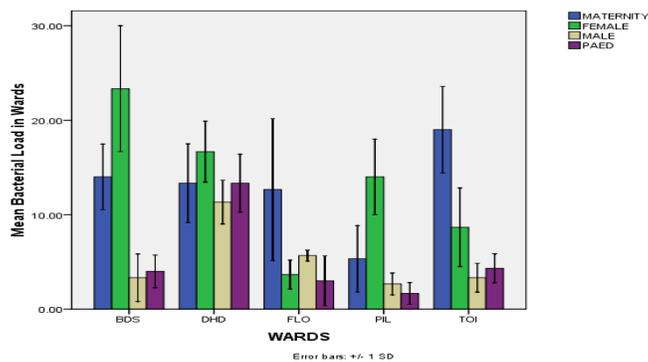


Figure 3. The mean bacterial load (cfu/ml) from Ayedun Basic Health Centre.

which is *Candida albicans* and *Candida dubliensis*. These two species of *Candida* were subjected to three specific antifungal agents which are nystatin, ketocunazole and fulcin respectively. The results showed that the two yeasts were most susceptible to nystatin and least

susceptible to ketocunazole. *Candida albicans* was however more susceptible to nystatin than *Candida dubliensis* with a mean zone of inhibition of  $22.50 \pm 1.25$  mm against  $18.95 \pm 1.05$  mm for *Candida dubliensis*. This result is shown in figure 12.

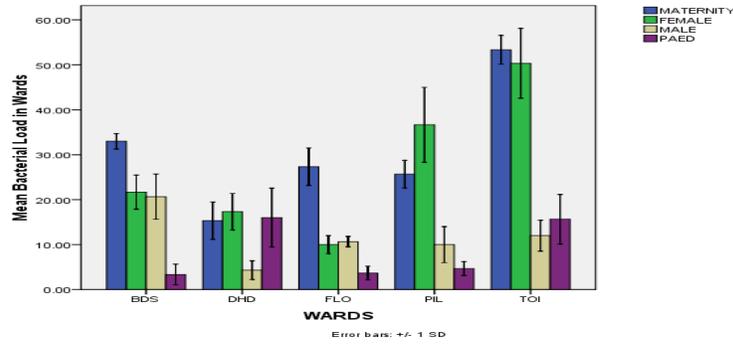


Figure 4. The mean bacterial load (cfu/ml) from Isole Basic Health Centre.

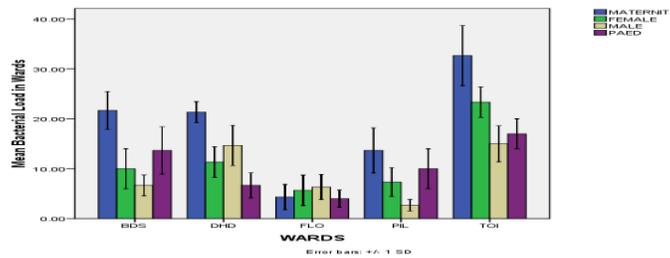


Figure 5. The mean bacterial load (cfu/ml) from Oba-Ile Basic Health Centre  
 Keys: BDS=Bed sheet; DHD=Door handles; FLO=Floor; PIL=Pillow; TOI=Toilet.

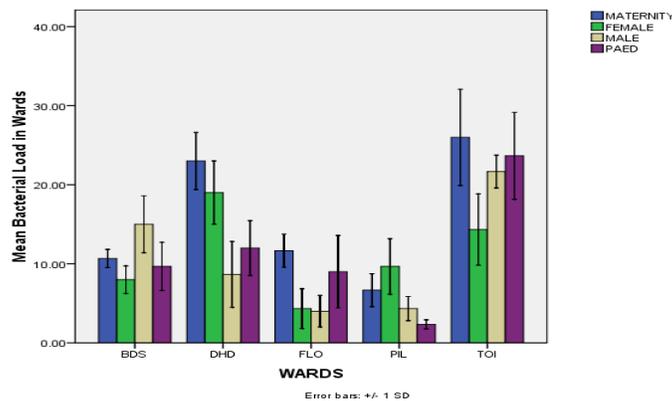
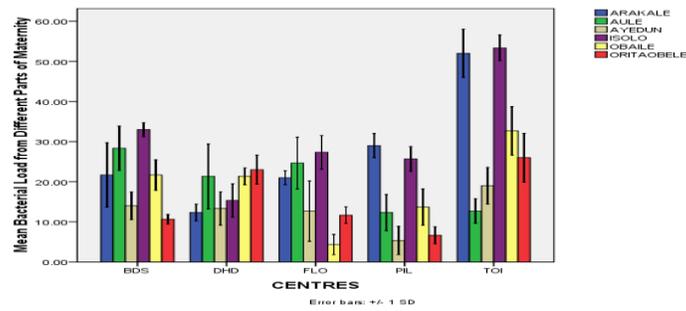


Figure 6. The mean bacterial load (cfu/ml) from Orita-Obele Basic Health Centres  
 Keys: BDS=Bed sheet; DHD=Door handles; FLO=Floor; PIL=Pillow; TOI=Toilet.

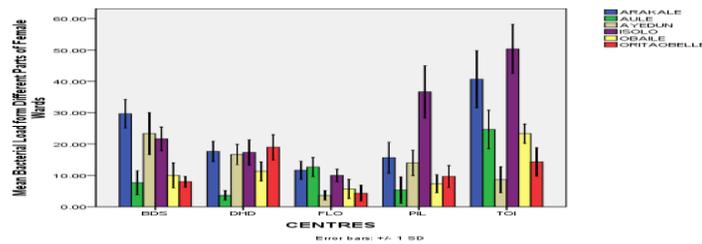
**DISCUSSION**

The results on the bacterial load of the pillows from different wards in the different basic health centres is similar to the results obtained by Benenson, (2005) who recorded a sudden increase in the microbial load of pillows in hospital were children were admitted as a result of communicable diseases in a hospital. According to Plowman, (2009), the major reason for the high bacterial load of pillows and beddings in the hospitals is basically due to the fact that patients under critical conditions may

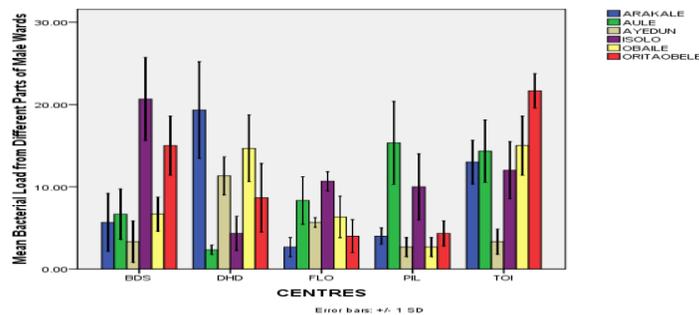
not be able to bath for days and most hospitals in developing countries may not have enough of these beddings for daily change. The toilets which contain the highest bacterial load from all the basic health centres may require special attention too. The toilets however are not expected to be sterile since waste products are emptied there. However, according to Mayon-White (2008), the admitted patients who may be on antibiotics visit the toilets frequently, therefore, the toilets must be kept clean always to prevent nosocomial infections. Equally, visitors to the



**Figure 7.** Comparative chart of the microbial load (cfu/ml) of all the Maternity wards in all the Basic Health Centres Analyzed  
 Keys: BDS=Bed sheet; DHD=Door handles; FLO=Floor; PIL=Pillow; TOI=Toilet



**Figure 8.** Comparative chart of the microbial load (cfu/ml) of all the Female wards in all the Basic Health Centres Analyzed  
 Keys: BDS=Bed sheet; DHD=Door handles; FLO=Floor; PIL=Pillow; TOI=Toilet.

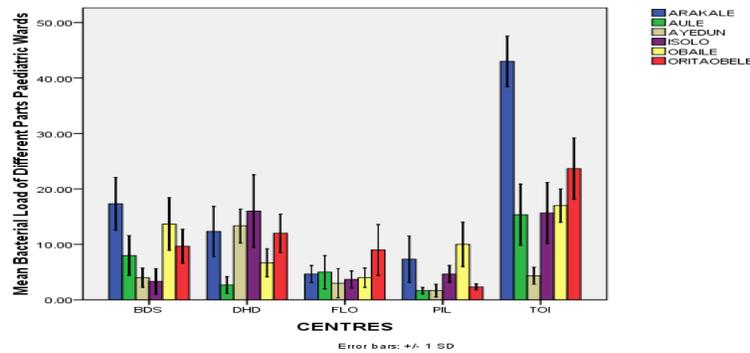


**Figure 9.** Comparative chart of the microbial load (cfu/ml) of all the Male wards in all the Basic Health Centres Analyzed  
 Keys: BDS=Bed sheet; DHD=Door handles; FLO=Floor; PIL=Pillow; TOI=Toilet

hospitals who may carriers of pathogenic microorganisms may also visit the toilets and may contribute to the bacterial load of such (Wenzel, 2005). Tikhomirov(2007) is of the opinion that the laboratory in the basic health centres should carry out routine bacterial isolation, bacterial load assessment of the toilets as well as assessment of the sterilizing agents as to whether they

are effective against the pathogens found in such toilets or not.

The isolation of bacteria from the door handles of these basic health centres have shown the need for constant hand washing before entrance into the various wards of the basic health centres and hand washing when leaving. This research has shown that the door handle is a major



**Figure 10.** Comparative chart of the microbial load (cfu/ml) of all the Paediatric wards in all the Basic Health Centres Analyzed  
Keys: BDS=Bed sheet; DHD=Door handles; FLO=Floor; PIL=Pillow; TOI=Toilet.

**Table 1.** Total number of isolates identified from each basic health centre.

S/N	Health Centre	No. of isolates
1	Arakale basic health centre	8
2	Aule basic health centre	5
3	Ayedun basic health centre	5
4	Isolo basic health centre	8
5	Oba-Ile basic health centre	6
6	Orita_Obele basic health centre	7

**Table 2.** Distribution of all the isolates from various health centres.

Centre	1	2	3	4	5	6	7	8
Arakale	+	+	+	+	+	+	+	+
Aule	+	+	+	+	+	-	-	-
Ayedun	+	+	+	+	+	-	-	-
Isolo	+	+	+	+	+	+	+	+
Oba-Ile	+	+	+	+	+	+	-	-
Orita-Obele	+	+	+	+	+	+	+	-

Keys: +=Present / Isolated; -=Absent / Not isolated

1=*Escherichia coli*; 2=*Staphylococcus aureus*; 3=*Staphylococcus epidermidis*;  
4=*Proteus mirabilis*; 5=*Bacillus subtilis*; 6=*Pseudomonas aeruginosa*;  
7=*Klebsiellapneumoniae*; 8=*Candida albicans*

way nosocomial infections can spread like wide fire. Emmerson, (2006) in his work survey of infection in hospitals showed that 40% of nosocomial infections may be obtained from door handles. There is therefore need for constant swabbing of the door handle to reduce the microbial load per time(Poole, 2015).

The bacterial load results obtained from the floor of the wards are similar to the result obtained by Gikas, (2009) when he isolated from the floor of the maternity wards of selected hospitals in Greece. The result is also in accordance with the result obtained by Raymond and

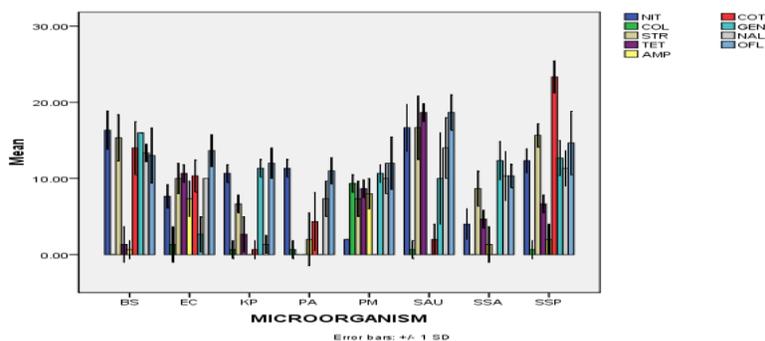
Aujard, (2010) when they isolated bacteria from the pediatric wards of selected hospitals in Greece. They concluded that since the wards are not restricted to certain people of high hygienic status, many of them would be a source of the bacteria coming from either hands or bodies.

Many factors contribute to the frequency of nosocomial infections: hospitalized patients are often immune-compromised, they undergo invasive examinations and treatments, and patient care practices and the hospital environment may facilitate the transmission of microorga-

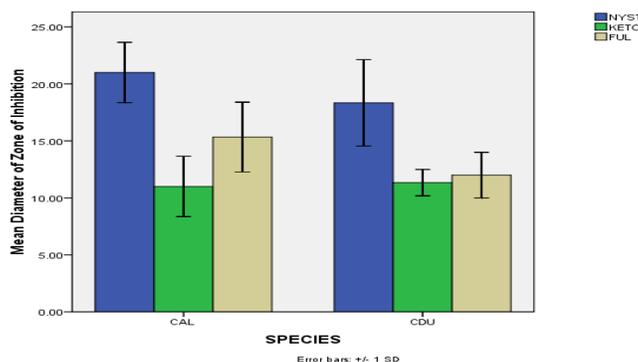
**Table 3.** Distribution of isolates in surfaces swabbed.

S/N	Surface	1	2	3	4	5	6	7	8
1	Bed sheet	-	+	+	+	-	+	+	-
2	Door handles	+	+	+	-	+	-	-	-
3	Floor	+	+	-	-	+	+	+	-
4	Pillow	-	+	+	-	-	-	+	-
5	Toilets	+	+	+	+	+	+	+	+

Keys. 1=*Escherichia coli*; 2=*Staphylococcus aureus*; 3=*Staphylococcus epidermidis*; 4=*Proteus mirabilis*; 5=*Bacillus subtilis*; 6=*Pseudomonas aeruginosa*; 7=*Klebsiellapneumoniae*; 8=*Candida albicans*.  
 +=Present / Isolated; -=Absent / Not isolated



**Figure 11.** Antibiotic sensitivity test on isolates  
 Keys: NIT=Nitrofurantoin; COL=Chloramphenicol; STR=Streptomycin; TET=Tetracyclin; AMP=Ampicillin; COT=Coltrimoxazole; GEN=Gentamycin; NAL=Nalidixic acid; OFL=Ofloxacin.  
 BS=*Bacillus subtilis*; EC=*Escherichiacoli*; KP=*Klebsiellapneumoniae*; PA=*Pseudomonas aeruginosa*; PM=*Proteus mirabilis*; SAU=*Staphylococcus aureus*; SSA=*Staphylococcusepidermidis*; SSP=*Staphylococcus species*.



**Figure 12.** Antifungal sensitivity test of *Candida* species  
 Keys: Nyst= Nystatin; Keto=Ketocunazole; Ful=fulcin, CAL=*Candida albicans*, CDU= *Candida dubliensis*

nisms among patients. The selective pressure of intense antibiotic use promotes antibiotic resistance (Poole, 2015). While progress in the prevention of nosocomial infections has been made, changes in medical practice

continually present new opportunities for development of infection (Poole, 2015). According to Prescott *et al.*, (2011), the number of patients visiting a hospital is a pointer to the number of

visitors that will visit such hospital. Benenson (2005) states that this point well abused in developing countries were a patient has multiple visitors coming daily when they are on hospital admission.

## CONCLUSION

The health care facilities studied are highly contaminated with microorganisms that are pathogenic. Hence the need for adequate monitoring of hygienic status of health facilities to reduce or prevent hospital acquired infections.

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