

Bacteriological Assessment of Hospital Air and Selected Surfaces in Three Referral Hospitals in Makurdi Middle Belt Nigeria

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ABSTRACT

Hospital environment needs more attention because of the influx of people into the environment for medical checkup and other services. This study unveils the incidence of bacteria isolated from air and selected surfaces in three referral hospitals (City hospital, Federal Medical Centre, Wadata and Saint Theresa Hospital) in Makurdi Middle Belt Nigeria. Fifty (50) bacteria were isolated; hospital air (26%), bed rails (16%), door knobs (2%), floor (4%), nurse table top (10%), operation table (4%), sink (18%), stretchers (2%) and toilet seat (12%). In City Hospital, the bacteria isolated include *Escherichia coli* (6%), *Pseudomonas aeruginosa* (2%), *Staphylococcus aureus* and *Staphylococcus spp* (2%), from Federal Medical Centre, bacteria isolated were *Klebsiella spp* (10%), *Staphylococcus aureus* (4%), *Staphylococcus spp* (8%), *Pseudomonas aeruginosa* (12%) and *E. coli* (6%). In STH, bacteria isolated include *E. coli* (8%), *Pseudomonas aeruginosa* (4%), *Staphylococcus spp* (8%) and *Staphylococcus aureus* (14%). The invitro antibiotics susceptibility pattern shows that *Pseudomonas aeruginosa* showed highest resistant to the antibiotics while *Klebsiella spp* was susceptible to majority of the antibiotics but resistant to cloxacillin and erythromycin. The study reveals *Klebsiella spp*, *Escherichia coli*, *Pseudomonas aeruginosa*, *S. aureus* and other *Staphylococcus spp* as bacteria commonly associated with hospital environment. This study affirms the presence of resistant bacteria strains and highlighted world-wide problem of hospital borne infections as it concerns the study area and population. This report will create awareness and be a good guide to health care workers, patients and the public about the likeliness of contracting nosocomial infection and how to treat such infection. Major recommendations offered suggests that, healthcare workers should be more careful in carrying out their duty to avoid chance of being infected in the course of their work. Also, the in-vitro antibiotics susceptibility testing on the bacterial pathogens in the study will assist the clinicians in making improvement on the management of nosocomial infections.

Keywords: surface swabs, hospital environments, pathogens, in-vitro, antibiotics

INTRODUCTION

The microbes operate in different ecosystem because in their world they are exceedingly diverse in their appearance, metabolism, physiology, and genetics. The incredible biodiversity bacteria range from extreme environment in water even unto the atmosphere in air. This variety of species is as well present in a particular environment like hospitals air and some surfaces in hospitals (Prescott, Harley, and Klein, 2008).

Hospital environments are places that need more attention because of the influx of people coming for medical checkup and other services in the environment. Patients and hospital staff get in contact with this environment every day and some of these patients may have little or no knowledge about the bacteriology of the hospital air and various surfaces that are potential source of infections. Coronavirus disease (COVID-19) pandemic has provided a lot of information about air borne infection, there is need to also shed light on bacteria that are capable of causing infection from hospital premises also known as nosocomial infection. These infections often manifested within forty-eight hours after a patient is being

admitted or thirty days after being discharge from the hospital (Anusree *et al.*, 2015; WHO, 2002). Therefore, bacteria from air can cause infection on patients with compromised immunity, especially when the patients come close a very close range (that is, neglecting physical distancing) to another individual who is a carrier of bacteria that are potential hospital acquired pathogens. So, exchange of gas due to respiration during conversation or exchange of pleasantries will pave way to inhale potential pathogen breathe out by another person at closed range.

Therefore, there is likeliness of contracting nosocomial infection by patient and, or healthcare staff from the hospital environment which is a major route of nosocomial bacterial pathogens (Tambekar, Gulhane, and Bhokare, 2007). At Kenyatta National Hospital, Kenya, *P. aeruginosa*, *Klebsiella*, *Citrobacter*, *S. aureus*, *S. pneumoniae*, *Acinetobacter* and *E. coli* were isolated from tracheal aspirate, urine, blood and pus swabs from the intensive care unit (Peter *et al.*, 2017). Some researchers have highlighted a host of nosocomial bacteria which include *Streptococcus* spp, *Acinetobacter* spp, *Enterococci*, *Pseudomonas aeruginosa*, coagulase-negative *Staphylococcus*, *Staphylococcus aureus*, *Bacillus cereus*, *Legionella*, *Proteus mirabilis*, *Klebsiella pneumoniae*, *E. coli*, and *Serratia marcescens* (Bereket *et al.*, 2012; Drach, 2005; Hassan, Aftab, and Riffat, 2015; Stamm, 2001). Even though, efforts are made in Nigeria through awareness of the general public on airborne infection of COVID -19 which is a viral disease, but there is little awareness on airborne infection caused by bacteria. Hence, the microbiological quality assessment of indoor air is one of the most vital investigations to determine the microbial indoor air pollution. The information on the indoor microbial concentrations of airborne bacteria is necessary both to estimate the health hazard and to create standards for indoor air-quality control (Arzu *et al.*, 2019).

Fu and Wang (2016) reported nosocomial infection control in health care settings with respect to protection against emerging infectious diseases; the report revealed that proper control measure should be taken to ensure that nosocomial infections do not occur in designated healthcare settings that accommodate suspected cases suffering from emerging infectious diseases and a comprehensive and detailed evaluation of nosocomial infection control should be conducted in each designated healthcare setting.

Moreover, hospital environment and its air are inseparable from health care worker and patients, bacteriological assessments of the environment such as indoor air quality cannot be underestimated when it comes to human health. The bacteria that live in troposphere, stratosphere and even ionosphere are capable of causing indoor air pollution (Awosika, Olajubu, and Amusa, 2012; Tambekar, Gulhane, and Bhokare, 2007).

Favorable temperature and poor air ventilation of indoors coupled with poorly designed structure gives an ample opportunity for microbial growth. It has been observed that *Bacillus*, *Clostridium* and *Staphylococci* are the most common bacteria isolated from indoor surfaces (Hayleeyesus and Manaye, 2014). Tambekar, Gulhane, and Bhokare (2007) report showed that bacteria can survive in both indoors and outdoors and the maternity and children hospitals had the highest

bacterial isolates. It is easier to avoid infection transmitted from formites than that of airborne which is often difficult.

However, lithosphere is conducive for a lot of mixed microbes which may become airborne and exposure to this may cause an epidemic infection of a particular environment because contaminated air is significant in transmission of communicable airborne diseases (Klevens *et al.*, 2007; Tambekar, Gulhane, and Bhokare, 2007). It is obvious that hospital acquired infection is on the increase and the rate of antibiotic resistance of bacterial pathogens associated with these infections is alarming in the public health (Wondemagegn *et al.*, 2012).

Consequently, to avoid the risk of infection by nosocomial bacteria, negative-pressure quarantine ICUs and wards should be built in accordance with the demand in the area. Regulations, standards, procedures, and operational instructions on protection against infections from emerging respiratory, gastrointestinal, body fluid, and insect-borne infectious diseases should be established. Periodical regular trainings for the knowledge of prevention and control emerging or unknown infectious diseases, and emergency exercises regarding nosocomial infection events among medics can also help to strengthen the infection control system (Fu and Wang, 2016).

According to previous investigations, the individual and nations economy do suffer loss due to the menace of this infections thereby causing financial burden, disability of patients, morbidity and eventual death also known as mortality of the hospitalized victims. The victim may be infected via surgical wound, blood stream, respiratory, urinary tract infections (Endalafer, Gebre-Selassie, and Kotisso, 2010;; Wondemagegn *et al.*, 2012). Wondemagegn *et al.* (2012) isolated 26.2% *S. aureus* as the leading bacterial isolates followed by *E. coli* and coagulase negative *Staphylococcus* spp. which consist of 21.4% each. Wondemagegn *et al.* (2012) further revealed that almost all the bacteria in the study were resistant to at least two of the antibiotics employed.

In general, bacteriological study of hospital environment has revealed the incidence of some bacteria associated with the environment. This research is aimed at bacteriological assessment of hospital air and selected surfaces in three referral hospitals in Makurdi, middle belt, Nigeria.

MATERIALS AND METHODS

The Study Area and Population

The study areas of the research are three referral hospitals which include: City Hospital (CH), Federal Medical Centre Wadata (FMCW) and Saint Theresa Hospital (STH) in Makurdi, Nigeria. One hundred and forty-three (143) samples which include 104 surface swabs and 39 samples from air were collected from the referral hospitals for laboratory analysis within a period of five months.

Ethical Clearance

The consent of the administrative authorities of Federal Medical Centre Wadata, Saint Theresa Hospital and City

Hospital were obtained before commencement of the research in the hospitals.

Collection of Samples

Air samples were taken by use of plate exposure methods while the surface swabs of the hospitals environments were collected aseptically by the use of sterile swabs sticks by trained personnel and researchers in the morning before commencement of work and transported immediately to the Microbiological Laboratory of Federal University of Agriculture, Makurdi. The parts of the hospital environment where patients, hospital staff and even visitors do have contact with almost every time were used as sample site due to likelihood of being infected. Thus, the analyses of samples collected are as follows:

- (i) 40 samples were taken from City Hospital (C): Thirty (30) samples from selected surfaces and 10 from hospital air. The samples collected from the surfaces include nurse table tops (4), bed rails (3), door knobs (4), from stretchers (3), sinks (4), operation table (4), hospital floor (4), and toilet seats (4), while 10 samples were collected from the hospital air.
- (ii) 47 samples were collected from Saint Theresa Hospital (S): 32 surface swabs which include nurse table tops (4), bed rails (4), door knobs (4), stretchers (4), sinks (4), operation table (4), hospital floor (4), toilet seats (4) and 15 samples were collected from the air of maternity ward (labour room), female medical ward, male medical ward and pediatrics ward.
- (iii) A total of 56 were collected from Federal Medical Centre Wadata (F): 42 samples were collected from the surfaces and 14 samples from the hospital air. The samples collected from the surface swabs include nurse table tops (6), bed rails (6), door knobs (6), stretchers (6), sinks (6), hospital floor (6) and toilet seats (6) and air samples (14) were obtained from Federal Medical Centre.

Materials Used

The materials used for the study include: Bacteriological Media (Nutrient agar, Chocolate agar, Blood agar and MacConkey agar), sterile swab stick, glass wares (Petri dishes, round bottom flask, graduated cylinder, conical flask glass, slides) and antibiotic sensitivity disc. Other apparatus, reagents and equipment include incubator, autoclave, forceps, inoculating loop, Bunsen burner, aluminum foil, cotton wool, weighing balance, work bench, peptone water, distil water, safranin, alcohol and Lugol's iodine (Cheesbrough, 2006).

Procedure for Sterilization

All glass wares were sterilized as described by Cheesbrough (2006).

Sampling Design and Techniques

Air samples were taken by exposure of prepared media in plates (set plates) into air for 20 minutes while surface swabs were collected using sterile swab sticks. The selected surfaces analyzed include hospital floors, door knobs, nurse table tops, bedrails, stretchers, operation tables, toilet seats and sinks for the purposes of isolating microorganisms from these sources

and transported with sealed sterile polythene bag to the laboratory for analysis within thirty minutes. These samples were inoculated into nutrient agar plates and blood agar under aseptic condition as described by Cheesbrough (2006).

Method of Isolation

Cultural method

The swabs collected from the selected surfaces by the researchers and other trained medical laboratory scientist were streaked on the bacteriological media with the use sterile swab sticks to make an inoculum on the plates. These swabs samples were taken before cleaning of the hospitals while, the bacteria from air samples were isolated by plate exposure method before incubation and later streaked with the use of a sterile wire loop to make axenic culture (Prescott, Harley, and Klein, 2008).

Isolation and characterization of isolates

The organisms cultured from the air and the swabs were directly inoculated on nutrient agar by the use of sterile inoculating loop and later sub-cultured on blood agar, chocolate and MacConkey agar near bunsen burner. The inoculated media were incubated at 35°C for 24 hours and then examined for bacterial growth. The organisms isolated were identified by cultural characteristics, microscopy (Gram stain) and biochemical tests which include catalase, coagulase test, indole test, citrate test, oxidase test and carbohydrates fermentation (Prescott, Harley, and Klein, 2008).

In-vitro Determination of Antibiotic Susceptibility

Antibiotic susceptibility of the bacterial isolates was performed by disk diffusion method according to Clinical Laboratory Standards Institute guidelines. The bacteria were sub-cultured to prepare the inoculum with the sterile inoculating loop. Then, one isolated colony was transferred into 10ml of sterile distilled water prepared in the test tube and mixed properly. The liquid now serve as source of inoculum containing 10^6 CFU/ml of bacterial suspension. A sterile swab stick was properly dipped into mixed inoculum and excess fluid was removed before rotating the swab on the surface of the prepared Mueller-Hinton agar plates. This spread was done four different planes by rotating the planes 180° each time. This was to ensure that every part of the plate was inoculated. Forceps was flamed until red hot and cooled and then used to pick the antibiotics disc and it was also used to place the discs on the inoculated plates. The discs were pressed down on the agar so as to be in complete contact with agar. The plates were incubated at 37°C for 18 hours. The antibiotics multidisc employed contained Ofloxacin (OFL) 5µg, Ceftriazone (CTR) 30µg, Gentamicin (GEN) 10µg, Cefuroxime (CRX) 10µg, Cloxacillin (CXC) 10µg, Augmentin (AUG) 30µg, Ceftazidime (CAZ) 30µg, and Erythromycin (ERY) 30µg. Zones of inhibition were used to determine the level of susceptibility of the isolates to these test antibiotics (CLSI, 2012).

Statistical Analysis

Statistical Package for the Social Sciences (SPSS) software version 23 was used for data analysis through the use of descriptive statistics which include frequency tables,

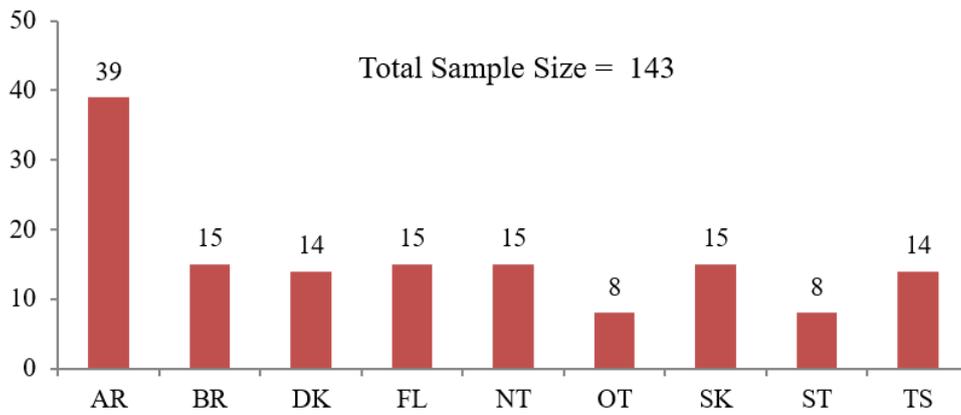


Figure 1. Distribution of Bacteriological Samples

Key: AR- Air Sample, BR- Bed Rails, DK- Door knobs, ST- Stretchers, SK-Sink, TS - Toilet seat, OT - Operation table, FL - Floor, TS - Toilet Seat, NT - Nurse Table Top

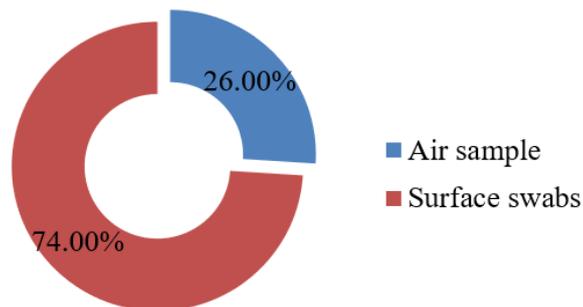


Figure 2. Occurrence of the Bacterial Isolates in Relation to Air Sample and Surface Swab

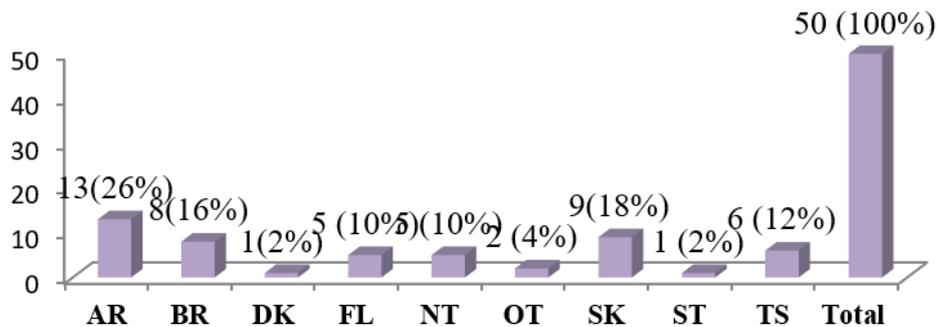


Figure 3. Incidence of Bacterial Isolates from Hospital Air Sample and Selected Surfaces

Key: AR- Air Sample, BR- Bed Rails, DK- Door knobs, ST- Stretchers, SK-Sink, TS - Toilet seat, OT - Operation table, FL - Floor, TS - Toilet Seat, NT - Nurse Table Top

percentage, pie chart and bar chart as described by Pallant (2007).

RESULTS AND DISCUSSION

The bacteriological assessment of the three referral hospitals is presented below. **Figure 1** depicts the distribution of bacteriological samples while **Figure 2** represents the occurrence of the bacterial isolates in relation to air sample and surface swab. **Figure 3** reveals the incidence of bacteria isolated from the air sample and selected surfaces of the referral hospitals. One hundred and forty-three (143) bacteriological samples were collected for analysis and fifty bacterial isolates were obtained which consist of 13 (26%) and 37 (74%) bacterial isolates from air sample and surface swabs respectively (**Figure 2**). The selected surfaces comprise bed

rails, doorknobs, floor, nurse table top, operation tables, sinks, stretchers and toilet seats. The bacteriological assessment of the indoor air in different wards and the selected surfaces is shown in **Figure 3**. Thus, bacteria isolated from hospital air include 13 (26%), bed rails 8 (16%), door knobs 1 (2%), floor 2 (4%), nurse table top 5 (10%), operation table 2 (4%), sink 9 (18%), stretchers 1 (2%) and toilet seat 6 (12%). The cultural, morphological and biochemical characteristics of the bacterial isolates are shown in **Table 1** as described in Bergey's Manual 9th edition (Bergey, 2000).

A total of fifty bacterial were isolated from the three-referral hospital viz: City Hospital 13 (26%), Federal Medical Centre Makurdi 20 (40%) and Saint Theresa Hospital 17 (34%) which comprises of 17 (34%) *S. aureus*, 9 (18%) *S. spp*, 9 (18%) *P. aeruginosa*, 4 (8%) *E. coli* and 5 (10%) *Klebsiella spp* (**Table 2** and **3**). The bacteria isolates involved in this work are potential

Table 1. Morphological and Biochemical Characteristics of Bacterial Isolates

Isolate code	Cultural morphology on nutrient agar	Shape	Gram reaction	MacConkey agar	Mannitol salt agar	Blood/haemolysis	Catalase	Coagulase	Indole	Citrate	Oxidase	Motility	Glucose	lactose	Sucrose	Starch hydrolysis	Identification
SBR ₂	Opaque, milky and golden yellowish colour	Coccci in clusters	+	No growth	Growth, mannitol fermented	β-haemolysis	+	+	-	+	-	-	+	+	+	+	<i>Staphylococcus aureus</i>
ST ₁	Flat growth with greenish colour and offensive odour	Rods	-	Flat growth with an offensive odour	ND	β-haemolysis	+	-	-	-	+	+	-	-	-	-	<i>Pseudomonas aeruginosa</i>
SNT ₂	Flat growth with greenish colour and offensive odour	Rods	-	Flat growth with an offensive odour	ND	β-haemolysis	+	-	-	-	+	+	-	-	-	-	<i>Pseudomonas aeruginosa</i>
ST ₂	Opaque, milky and golden yellowish colour	Coccci in clusters	+	No growth	Growth, mannitol fermented	β-haemolysis	+	+	-	+	-	-	+	+	+	+	<i>Staphylococcus aureus</i>
SNT ₄	Opaque and milky growth	Coccci in clusters	+	No growth	Growth, mannitol not fermented	α-haemolysis	+	-	-	-	-	-	+	+	+	-	<i>Staphylococcus spp</i>
SSK ₄	Opaque and milky growth	Coccci in clusters	+	No growth	Growth, mannitol not fermented	α-haemolysis	+	-	-	-	-	-	+	+	+	-	<i>Staphylococcus spp</i>
SSK ₁	Opaque and milky growth	Coccci in clusters	+	No growth	Growth, mannitol not fermented	β-haemolysis	+	-	-	-	-	-	+	+	+	-	<i>Staphylococcus spp</i>
SBR ₃	Opaque and milky growth	Coccci in clusters	+	No growth	Growth, mannitol not fermented	α-haemolysis	+	-	-	-	-	-	+	+	+	-	<i>Staphylococcus spp</i>
SBR ₅	Opaque, milky and golden yellowish colour	Coccci in clusters	+	No growth	Growth, mannitol fermented	β-haemolysis	+	+	-	+	-	-	+	+	+	+	<i>Staphylococcus aureus</i>
SFL ₃	Convex and entire growth	Short rods	-	Growth	ND	ND	+	ND	+	-	-	+	+	+	-	-	<i>Escherichia coli</i>
SFL ₂	Convex and entire growth	Short rods	-	Growth	ND	ND	+	ND	+	-	-	+	+	+	-	-	<i>Escherichia coli</i>
SFL ₁	Opaque, milky and golden yellowish colour	Coccci in clusters	+	No growth	Growth, mannitol fermented	β-haemolysis	+	+	-	+	-	-	+	+	+	+	<i>Staphylococcus aureus</i>
SPD AR ₂	Flat growth with greenish colour and offensive odour	Rods	-	Flat growth with an offensive odour	ND	β-haemolysis	+	-	-	-	+	+	-	-	-	-	<i>Pseudomonas aeruginosa</i>
SPD AR ₃	Opaque, milky and golden yellowish colour	Coccci in clusters	+	No growth	Growth, mannitol fermented	β-haemolysis	+	+	-	+	-	-	+	+	+	+	<i>Staphylococcus aureus</i>
SMW AR ₂	Convex and entire growth	Short rods	-	Growth	ND	ND	+	ND	+	-	-	+	+	+	-	-	<i>Escherichia coli</i>
SMW AR ₃	Convex and entire growth	Short rods	-	Growth	ND	ND	+	ND	+	-	-	+	+	+	-	-	<i>Escherichia coli</i>
STH DK ₂	Opaque, milky and golden yellowish colour	Coccci in clusters	+	No growth	Growth, mannitol fermented	β-haemolysis	+	+	-	+	-	-	+	+	+	+	<i>Staphylococcus aureus</i>
CMwAR ₃	Opaque, milky and golden yellowish colour	Coccci in clusters	+	No growth	Growth, mannitol fermented	β-haemolysis	+	+	-	+	-	-	+	+	+	+	<i>Staphylococcus aureus</i>
CST ₁	Opaque, milky and golden yellowish colour	Coccci in clusters	+	No growth	Growth, mannitol fermented	β-haemolysis	+	+	-	+	-	-	+	+	+	+	<i>Staphylococcus aureus</i>
CBR ₂	Flat growth with greenish colour and offensive odour	Rods	-	Flat growth with an offensive odour	ND	β-haemolysis	+	-	-	-	+	+	-	-	-	-	<i>Pseudomonas aeruginosa</i>
CFL ₂	Opaque, milky and golden yellowish colour	Coccci in clusters	+	No growth	Growth, mannitol fermented	β-haemolysis	+	+	-	+	-	-	+	+	+	+	<i>Staphylococcus aureus</i>
CNT ₃	Convex and entire growth	Short rods	-	Growth	ND	ND	+	ND	+	-	-	+	+	+	-	-	<i>Escherichia coli</i>
CTS ₂	Convex and entire growth	Coccci in clusters	+	No growth	Growth, mannitol not fermented	α-haemolysis	+	-	-	-	-	-	+	+	+	-	<i>Staphylococcus spp</i>
COT ₁	Opaque, milky and golden yellowish colour	Coccci in clusters	+	No growth	Growth, mannitol fermented	β-haemolysis	+	+	-	+	-	-	+	+	+	+	<i>Staphylococcus aureus</i>
COT ₄	Opaque, milky and golden yellowish colour	Coccci in clusters	+	No growth	Growth, mannitol fermented	β-haemolysis	+	+	-	+	-	-	+	+	+	+	<i>Staphylococcus aureus</i>

Key: +: Positive -: Negative ND: Not determined

Table 1 (continued). Morphological and Biochemical Characteristics of Bacterial Isolates

Isolate code	Cultural morphology on nutrient agar	Shape	Gram reaction	MacConkey agar	Mannitol salt agar	Blood agar	Catalase	Coagulase	Indole	Citrate	Oxidase	Motility	Glucose	lactose	Sucrose	Starch hydrolysis	Identification
CSK ₄	Convex and entire growth	Short rods	-	Growth	ND	ND	+	ND	+	-	-	+	+	+	-	-	<i>Escherichia coli</i>
CBR ₃	Convex and entire growth	Short rods	-	Growth	ND	ND	+	ND	+	-	-	+	+	+	-	-	<i>Escherichia coli</i>
CNT ₄	Opaque, milky and golden yellowish colour	Cocci in clusters	+	No growth	Growth, mannitol fermented	β-haemolysis	+	+	-	+	-	-	+	+	+	+	<i>Staphylococcus aureus</i>
CSK ₃	Opaque, milky and golden yellowish colour	Cocci in clusters	+	No growth	Growth, mannitol fermented	β-haemolysis	+	+	-	+	-	-	+	+	+	+	<i>Staphylococcus aureus</i>
CTS ₁	Opaque, milky and golden yellowish colour	Cocci in clusters	+	No growth	Growth, mannitol fermented	β-haemolysis	+	+	-	+	-	-	+	+	+	+	<i>Staphylococcus aureus</i>
FFw AR ₂	Mucoid, convex and entire growth	Short rods	-	Large light pink mucoid colonies	ND	ND	+	ND	-	+	-	-	+	+	+	+	<i>Klebsiella</i> spp
FMT AR ₁	Mucoid, convex and entire growth	Short rods	-	Large light pink mucoid colonies	ND	ND	+	ND	-	+	-	-	+	+	+	+	<i>Klebsiella</i> spp
FPdAR _{1A}	Mucoid, convex and entire growth	Short rods	-	Large light pink mucoid colonies	ND	ND	+	ND	-	+	-	-	+	+	+	+	<i>Klebsiella</i> spp
FPd AR _{1B}	Convex and entire growth	Short rods	-	Growth	ND	ND	+	ND	+	-	-	+	+	+	-	-	<i>Escherichia coli</i>
FAR	Mucoid, convex and entire growth	Short rods	-	Large light pink mucoid colonies	ND	ND	+	ND	-	+	-	-	+	+	+	+	<i>Klebsiella</i> spp
FAR Pd AR _{2A}	Flat growth with greenish colour and offensive odour	Rods	-	Flat growth with an offensive odour	ND	β-haemolysis	+	-	-	-	+	+	-	-	-	-	<i>Pseudomonas aeruginosa</i>
FAR Pd AR _{2B}	Convex and entire growth	Short rods	-	Growth	ND	ND	+	ND	+	-	-	+	+	+	-	-	<i>Escherichia coli</i>
FBR Mw ₁	Opaque, milky and golden yellowish colour	Cocci in clusters	+	No growth	Growth, mannitol fermented	β-haemolysis	+	+	-	+	-	-	+	+	+	+	<i>Staphylococcus aureus</i>
FBR Mw ₃	Opaque, milky and golden yellowish colour	Cocci in clusters	+	No growth	Growth, mannitol fermented	β-haemolysis	+	+	-	+	-	-	+	+	+	+	<i>Staphylococcus aureus</i>
FNT ₄	Flat growth with greenish colour and offensive odour	Rods	-	Flat growth with an offensive odour	ND	β-haemolysis	+	-	-	-	+	+	-	-	-	-	<i>Pseudomonas aeruginosa</i>
FBR MW ₁	Opaque, milky and golden yellowish colour	Cocci in clusters	+	No growth	Growth, mannitol fermented	β-haemolysis	+	+	-	+	-	-	+	+	+	+	<i>Staphylococcus aureus</i>
FMW SK ₁	Opaque, milky and golden yellowish colour	Cocci in clusters	+	No growth	Growth, mannitol fermented	β-haemolysis	+	+	-	+	-	-	+	+	+	+	<i>Staphylococcus aureus</i>
FSK ₁	Flat growth with greenish colour and offensive odour	Rods	-	Flat growth with an offensive odour	ND	β-haemolysis	+	-	-	-	+	+	-	-	-	-	<i>Pseudomonas aeruginosa</i>
FPD TS ₄	Opaque, milky and golden yellowish colour	Cocci in clusters	+	No growth	Growth, mannitol fermented	β-haemolysis	+	+	-	+	-	-	+	+	+	+	<i>Staphylococcus aureus</i>
FPD SK ₃	Flat growth with greenish colour and offensive odour	Rods	-	Flat growth with an offensive odour	ND	β-haemolysis	+	-	-	-	+	+	-	-	-	-	<i>Pseudomonas aeruginosa</i>
FPD SK _{2A}	Convex and entire growth	Short rods	-	Growth	ND	ND	+	ND	+	-	-	+	+	+	-	-	<i>Escherichia coli</i>
FPD SK _{2B}	Flat growth with greenish colour and offensive odour	Rods	-	Flat growth with an offensive odour	ND	β-haemolysis	+	-	-	-	+	+	-	-	-	-	<i>Pseudomonas aeruginosa</i>
FPD SK ₃	Flat growth with greenish colour and offensive odour	Rods	-	Flat growth with an offensive odour	ND	β-haemolysis	+	-	-	-	+	+	-	-	-	-	<i>Pseudomonas aeruginosa</i>
FPD TS ₅	Opaque, milky and golden yellowish colour	Cocci in clusters	+	No growth	Growth, mannitol fermented	β-haemolysis	+	+	-	+	-	-	+	+	+	+	<i>Staphylococcus aureus</i>
FMw FL ₂	Flat growth with greenish colour and offensive odour	Rods	-	Flat growth with an offensive odour	ND	β-haemolysis	+	-	-	-	+	+	-	-	-	-	<i>Pseudomonas aeruginosa</i>

Key: +: Positive -: Negative ND: Not determined

Table 2. Incidence of Bacterial Isolates in Relation to Sample Source

Sample Source	Total positive Isolates (%)	<i>Pseudomonas aeruginosa</i>	<i>Klebsiella</i> spp	<i>Escherichia coli</i>	<i>Staphylococcus Aureus</i>	<i>Staphylococcus</i> spp
AR	13 (18.3)	1(18.2)	5(100)	4(36.4)	3(5.1)	0 (0)
BR	8 (11.3)	1 (12.5)	0 (0)	1(9.1)	4(12.8)	2(16.7)
DK	1 (1.4)	0 (0)	0 (0)	0 (0)	1(2.6)	0 (0)
FL	5 (7.0)	1(9.1)	0 (0)	2(18.2)	2(5.1)	0 (0)
NT	5 (7.0)	2(18.2)	0 (0)	1(9.1)	1(2.6)	1(16.7)
SK	9 (12.7)	3(27.3)	0 (0)	2(18.2)	2(7.7)	2(16.7)
ST	1 (1.4)	0 (0)	0 (0)	0 (0)	1(2.6)	0 (0)
OT	2 (2.8)	0 (0)	0 (0)	0 (0)	2(5.1)	0 (0)
TS	6(8.5)	1(9.1)	0 (0)	0 (0)	4(10.3)	1(16.7)
Total	50 (100)	9(100)	5(100)	11(100)	36(100)	10(100)

AR- Air Sample, BR- Bed Rails, DK- Door knobs, ST- Stretchers, SK-Sink, TS - Toilet seat, OT - Operation table, FL - Floor, TS - Toilet Seat, NT - Nurse Table Top

Table 3. Incidence of Bacterial Isolates in Hospitals from the Three Referral Hospitals

Hospitals	<i>Staphylococcus aureus</i>	<i>Staphylococcus</i> spp	<i>Pseudomonas aeruginosa</i>	<i>Escherichia coli</i>	<i>Klebsiella</i> spp
CHM	8(16%)	1(2%)	1(2%)	3 (6%)	0 (0)
FMC	2 (4%)	4 (8%)	6 (12%)	3 (6%)	5(100)
STH	7 (14%)	4 (8%)	2 (4%)	4 (8%)	0 (0)
Total	17 (34%)	9 (18%)	9(18%)	10 (20%)	5(10%)

Key: CH- City Hospital, FMCW- Federal Medical Centre Wadata, STH – Saint Theresa Hospital Figures in brackets are the percentage rates of occurrence of the bacterial isolates

cause of nosocomial infections. This is in agreement with the findings of Bereket *et al.* (2012) which revealed genus *Staphylococcus*, *Escherichia coli* and *Pseudomonas aeruginosa* as commonly isolated nosocomial pathogens. There is lower incidence of Gram-negative bacterial isolates 15 (30%) than Gram positive bacterial isolates 35 (70%), which suggests that most of the bacterial are from human source (Prescott, Harley and Klein, 2008).

The Gram-negative bacteria are *Klebsiella* spp (10%), *Pseudomonas aeruginosa* (18%) and *Escherichia coli* (20%). This is also in agreement with findings by Hassan, Aftab and Riffat, (2015) that, 3-7% of nosocomial bacterial infections are related to *Klebsiella pneumoniae*. *Klebsiella* is a Gram-negative, non-motile, encapsulated, rod-shaped bacillus present within the nasopharynx and gastrointestinal tract of humans and nonhuman primates. *E. coli* is one of the common Gram-negative bacteria often associated with wound sepsis and endotoxin-induced shock (Hassan, Aftab and Riffat, 2015). It often causes urinary tract and wound infections in patient with compromised immunity. Drach (2005) revealed *Klebsiella* spp and *E. coli* among others as the microorganisms related to urinary catheterization. Bereket *et al.* (2012) reported that *Pseudomonas aeruginosa* can transiently colonize the respiratory and gastrointestinal tracts of hospitalized individuals. Furthermore, giving the preponderance of these organisms in the present study, there is a high possibility of patients contracting such infections. According to Brooks *et al.* (2010), *Klebsiella* can cause respiratory tract infection, urinary tract infections as well as wounds infections in a poor ventilated environment.

The percentage of occurrence of *Staphylococcus aureus* were (34%) and other *Staphylococcus* spp were (18%). Out of 26 (Twenty-six) Gram positive cocci isolated, *Staphylococcus aureus* were 17 (65.4%) while *Staphylococcus* spp were 9 (34.6%). This report agrees with the findings by Hassan, Aftab

and Riffat (2015) that *Staphylococcus* spp had the least occurrence among the Gram-positive bacteria associated with hospital acquired infections. *Staphylococcus aureus* being among normal microbiota of human may be responsible for higher number of incidence (65.4%).

Therefore, the nine sources in this study which include air and other eight selected surfaces shown in **Figure 3** and **Table 3** are multi-reservoirs being responsible for hospital contamination. This was also the same with the findings of Wilks, Michels and Keevil (2005) and Michels (2006) that inanimate objects can allow survival of microorganisms for a particular period of time. However, this work is in contrast to a report by Derese *et al.* (2016) where the main isolates in a similar finding executed in selected wards of a referral hospital in Ethiopia were coagulase negative *Staphylococci* (CoNs) 44%, followed by *S. aureus*, 37.4%, and *Klebsiella* spp at 11.6%.

Tables 4-6 shows antibiotics susceptibility pattern of the bacteria isolates from the three hospitals. 28 (56%) bacterial isolates were resistant to almost all the antibiotics employed in the study. *P. aeruginosa* showed highest resistant to the antibiotics considered in the three referral hospitals. The least incidence of *Klebsiella* spp in this finding from air sample of one of the three locations affirms the report of Sani *et al.* (2012) which stated that *Klebsiella* spp was the least commonly isolated bacteria among the Gram-negative facultative *Bacillus* despite the sample source differs. This shows consistency to previous findings on microbiological assessment of indoor and outdoor air quality in a general hospital in north-east Nigeria. The factors which encourage the growth and multiplication of airborne microbes need to be controlled in the Hospital environment to reduce the rate of nosocomial infections (Agwaranze *et al.*, 2020).

Table 4. Zones of inhibition for Bacterial isolates from City Hospital

Isolate code	Antibiotics measured in millimeter								Bacteria
	AUG	CAZ	CRX	CTR	CXC	ERY	GEN	OFL	
CMwAR ₅	35(s)	0(r)	22(s)	27(s)	26(s)	0(r)	0(r)	20(s)	<i>Staphylococcus aureus</i>
CST ₁	0(r)	0(r)	0(r)	0(r)	0(r)	0(r)	0(r)	25(s)	<i>Staphylococcus aureus</i>
CBR ₂	0(r)	0(r)	0(r)	0(r)	0(r)	0(r)	0(r)	0(r)	<i>Pseudomonas aeruginosa</i>
CFL ₂	32(s)	0(r)	30(s)	30(s)	0(r)	0(r)	30(s)	23(s)	<i>Staphylococcus aureus</i>
CNT ₃	33(s)	0(r)	10(r)	25(s)	0(r)	0(r)	0(r)	0(r)	<i>Escherichia coli</i>
CTS ₂	30(s)	0(r)	26(s)	26(s)	0(r)	34(s)	0(r)	0(r)	<i>Staphylococcus spp</i>
COT ₁	0(r)	0(r)	22(s)	22(s)	0(r)	0(r)	22(s)	0(r)	<i>Staphylococcus aureus</i>
COT ₄	0(r)	0(r)	0(r)	0(r)	0(r)	0(r)	0(r)	24(s)	<i>Staphylococcus aureus</i>
CSK ₄	0(r)	0(r)	0(r)	0(r)	0(r)	0(r)	25(s)	28(s)	<i>Escherichia coli</i>
CBR ₃	24(s)	0(r)	24(s)	0(r)	27(s)	33(s)	0(r)	25(s)	<i>Escherichia coli</i>
CNT ₄	35(s)	0(r)	22(s)	27(s)	26(s)	0(r)	0(r)	20(s)	<i>Staphylococcus aureus</i>
CSK ₃	24(s)	24(s)	26(s)	26(s)	20(s)	26(s)	25(s)	22(s)	<i>Staphylococcus aureus</i>
CTS ₁	0(r)	0(r)	0(r)	0(r)	0(r)	0(r)	0(r)	25(s)	<i>Staphylococcus aureus</i>

Key: C- City Hospital, NT- Nurse Table Top, BR- Bed Rails, DK- Door knobs, ST-Stretchers, SK- Sink, TS- Toilet seat, OT- Operation table, FL- Floor, TS - Toilet Seat, AR- Hospital air, r = resistant, s = sensitive

Table 5. Zones of inhibition for Bacterial isolates from Federal Medical Centre, Wadata

Isolate code	Antibiotics measured in millimeter								Bacteria
	AUG	CAZ	CRX	CTR	CXC	ERY	GEN	OFL	
FFw AR ₂	35(s)	30(s)	30(s)	28(s)	8(r)	8(r)	28(s)	30(s)	<i>Klebsiella spp</i>
FMT AR ₁	30(s)	30(s)	34(s)	30(s)	6(r)	8(r)	20(s)	30(s)	<i>Klebsiella spp</i>
FPd AR _{1A}	26(s)	26(s)	36(s)	27(s)	12(r)	8(r)	34(s)	36(s)	<i>Klebsiella spp</i>
FPd AR _{1B}	28(s)	36(s)	36(s)	34(s)	12(r)	10(r)	26(s)	38(s)	<i>Escherichia coli</i>
FAR	8(r)	8(r)	8(r)	8(r)	8(r)	8(r)	8(r)	8(r)	<i>Klebsiella spp</i>
FAR Pd AR _{2A}	0(r)	0(r)	0(r)	0(r)	0(r)	0(r)	0(r)	0(r)	<i>Pseudomonas aeruginosa</i>
FAR Pd AR _{2B}	32(s)	6(r)	36(s)	6(r)	6(r)	6(r)	18(s)	32(s)	<i>Escherichia coli</i>
FBR Mw ₁	28(s)	26(s)	32(s)	6(r)	6(r)	6(r)	24(s)	30(s)	<i>Staphylococcus aureus</i>
FBR Mw ₃	4(r)	4(r)	4(r)	4(r)	4(r)	4(r)	4(r)	4(r)	<i>Staphylococcus aureus</i>
FNT ₄	4(r)	4(r)	4(r)	4(r)	4(r)	4(r)	4(r)	4(r)	<i>Pseudomonas aeruginosa</i>
FBR MW ₁	5(r)	5(r)	5(r)	5(r)	5(r)	5(r)	5(r)	5(r)	<i>Staphylococcus aureus</i>
FMW SK ₁	6(r)	6(r)	6(r)	34(s)	6(r)	6(r)	6(r)	34(s)	<i>Staphylococcus aureus</i>
FPD SK ₁	6(r)	6(r)	6(r)	18(s)	6(r)	6(r)	6(r)	24(s)	<i>Pseudomonas aeruginosa</i>
FPD TS ₄	28(s)	24(s)	28(s)	6(r)	8(r)	12(r)	22(s)	28(s)	<i>Staphylococcus aureus</i>
FPD SK ₃	18(s)	5(r)	26(s)	28(s)	5(r)	5(r)	5(r)	34(s)	<i>Pseudomonas aeruginosa</i>
FPD SK _{2A}	16(i)	6(r)	6(r)	32(s)	6(r)	6(r)	6(r)	30(s)	<i>Escherichia coli</i>
FPD SK _{2B}	4(r)	4(r)	4(r)	4(r)	4(r)	4(r)	4(r)	4(r)	<i>Pseudomonas aeruginosa</i>
FPD SK ₅	6(r)	6(r)	6(r)	6(r)	6(r)	6(r)	6(r)	18(s)	<i>Pseudomonas aeruginosa</i>
FPD TS ₅	22(s)	0(r)	0(r)	18(s)	6(r)	6(r)	6(r)	20(s)	<i>Staphylococcus aureus</i>
FMw FL ₂	8(r)	8(r)	28(s)	22(s)	8(r)	8(r)	8(r)	28(s)	<i>Pseudomonas aeruginosa</i>

Key: F- Federal Medical Centre Wadata, NT- Nurse Table Top, BR- Bed Rails, DK- Door knobs, ST-Stretchers, SK- Sink, TS- Toilet seat, OT- Operation table, FL- Floor, TS - Toilet Seat, AR- Hospital air, r = resistant, s = sensitive

Table 6. Zones of Inhibition for Bacterial Isolates from Saint Theresa Hospital

Isolate code	Antibiotics measured in millimeter								Bacteria
	AUG	CAZ	CRX	CTR	CXC	ERY	GEN	OFL	
SBR ₂	31(s)	0(r)	20(s)	12(r)	12(r)	12(r)	26(s)	27(s)	<i>Staphylococcus aureus</i>
STs ₁	0(r)	0(r)	0(r)	0(r)	0(r)	0(r)	0(r)	0(r)	<i>Pseudomonas aeruginosa</i>
SNT ₂	0(r)	8(r)	0(r)	0(r)	0(r)	9(r)	18(s)	20(s)	<i>Pseudomonas aeruginosa</i>
STS ₂	27(s)	0(r)	0(r)	20(s)	15(i)	0(r)	14(i)	0(r)	<i>Staphylococcus aureus</i>
SNT ₄	28(s)	0(r)	0(r)	0(r)	0(r)	24(s)	26(s)	24(s)	<i>Staphylococcus spp</i>
SSK ₄	28(s)	0(r)	27(s)	18(s)	17(s)	0(r)	27(s)	20(s)	<i>Staphylococcus spp</i>
SSK ₁	0(r)	0(r)	0(r)	0(r)	0(r)	25(s)	0(r)	29(s)	<i>Staphylococcus spp</i>
SBR ₃	15(i)	0(r)	28(s)	23(s)	0(r)	22(s)	30(r)	0(r)	<i>Staphylococcus spp</i>
SBR ₅	0(r)	0(r)	18(s)	25(r)	0(r)	0(r)	0(r)	25(s)	<i>Staphylococcus aureus</i>
SFL ₅	25(s)	0(r)	0(r)	25(s)	0(r)	30(s)	20(s)	16(i)	<i>Escherichia coli</i>
SFL ₂	0(r)	16(i)	0(r)	12(r)	0(r)	0(r)	22(s)	29(s)	<i>Escherichia coli</i>
SFL ₁	22(s)	20(s)	22(s)	0(r)	0(r)	0(r)	0(r)	0(r)	<i>Staphylococcus aureus</i>
SAR ₂	31(s)	0(r)	20(s)	12(r)	12(r)	12(r)	26(s)	27(s)	<i>Pseudomonas aeruginosa</i>
SAR ₃	0(r)	0(r)	18(s)	25(r)	0(r)	0(r)	0(r)	25(s)	<i>Staphylococcus aureus</i>
SAR ₂	0(r)	16(i)	0(r)	12(r)	0(r)	0(r)	22(s)	29(s)	<i>Escherichia coli</i>
SAR ₃	25(s)	0(r)	0(r)	25(s)	0(r)	30(s)	20(s)	16(i)	<i>Escherichia coli</i>
SDK ₂	22(s)	20(s)	22(s)	0(r)	0(r)	0(r)	0(r)	0(r)	<i>Staphylococcus aureus</i>

Key: S- Saint Theresa Hospital, AR- Hospital air, BR- Bed Rails, DK- Door knobs, NT- Nurse Table Top, ST-Stretchers, SK-Sink, TS - Toilet seat, OT- Operation table, FL- Floor, TS - Toilet Seat, r = resistant, s = sensitive

Klebsiella spp were susceptible to ofloxacin, ceftriazone, gentamicin, cefuroxime, augmentin and ceftazidime but resistant to cloxacillin and erythromycin (Table 5). This result corroborates with findings of other scholars that hospitals provide a reservoir of microorganisms, many of which are multi-resistant to antibiotics (Fithamlak *et al.*, 2017).

Meanwhile, *E. coli* isolated from Federal Medical Centre was sensitive to considerable number of the antibiotics with sensitivity to ofloxacin and augmentin streaking (Table 5). Whereas this was in contrast to the strain of *E. coli* isolates from Saint Theresa Hospital (Table 6). This corroborates with the reports of Gelaw *et al.* (2013) and Dessie *et al.* (2016) that stated *E. coli* among gram-negative isolates found resistant to antimicrobial tested.

The highest incidence of *Staphylococcus aureus* in the work give an ample opportunity of looking into antibiogram of the isolates. It was observed that *Staphylococcus aureus* isolated from City Hospital were highly sensitive to ofloxacin (inhibitors of nucleic acid synthesis) and bacteriological assessment of *Staphylococcus aureus* isolated from Saint Theresa Hospital reveals that the bacteria is sensitive to cloxacillin (cell wall inhibitor antimicrobial agent). *Staphylococcus aureus* isolated from Federal Medical Centre Wadata was also sensitive to ofloxacin (a member of fluoroquinolones) which is capable of inhibiting nucleic acid synthesis. This is in agreement with the report of Pollack (2010), that the effectiveness of antimicrobial drugs in chemotherapy is based on the susceptibility of the target microbes.

In general, predisposing factors that make patients susceptible to nosocomial infections had been reported by some researchers, the length of hospital stay remains the most important factors of nosocomial infections (Kleven *et al.*, 2007; Lahsaezadeh *et al.*, 2009). Assessment of environmental quality of hospital wards should be based on comprehensive analysis with multiple indicators, because there may be imbalances in the microbial diversity in the hospital wards, therefore, monitoring of the environmental quality of hospitals is important in the prevention of nosocomial infections (Hart *et al.*, 2015; Ling and Hui, 2019; Loupa, *et al.*, 2016). This research is in line with the study on nosocomial bacterial infections and their antimicrobial susceptibility patterns among patients in Ugandan intensive care units (Peter *et al.*, 2017). It is also consistent with the study on evaluation of the complex indoor environment of hospital wards through the composition of bacteria and microbial ecology of the hospital wards (Ling and Hui, 2019).

CONCLUSION

This investigation has unveiled the hide out of pathogenic bacteria in hospital premises from hospital floor even to the air. Incidence of Gram-negative bacteria is less than incidence of Gram positive bacteria which mean, patients are prone to contract bacterial infection associated with Gram positive bacteria than Gram negative. This study reveals *Klebsiella* spp as the predominant bacteria in hospital air. It further reveals *E. coli*, *Pseudomonas aeruginosa*, *S. aureus* and other *Staphylococcus* spp as bacteria commonly associated with hospital

environment and these bacteria are capable of causing nosocomial infections on patients with compromised immunity. Antibiotics susceptibility pattern of the bacterial isolates reveals the presence of highly resistant species of *P. aeruginosa* and *Klebsiella* in the hospitals which has high public health implication. This report will create awareness and be a good guide to health care workers, patients and the public about the likeliness of contracting nosocomial infection and how to treat such infection.

RECOMMENDATIONS

Based on the findings and conclusion reached above, the following recommendations are hereby made:

1. Healthcare workers should be more careful in carrying out their duty to avoid been infected in the course of work.
2. The *in-vitro* antibiotics susceptibility testing on the bacterial pathogens in this study shows that erythromycin, Cefazidime, Cefuroxime and Cloxacillin may not be effective in the treatment of the hospital acquired infections caused by bacterial pathogens. Therefore, information on resistance patterns of isolates encountered in this study will assist the clinicians in making improvement in management of nosocomial infections.
3. Health care workers, patients and any other individual who visit hospitals should keep proper hygiene through hand wash and the use of hand sanitizer.
4. Use of antiseptics to clean inside hospital environments surfaces more than once in a day and use of recommended air fresheners in hospital wards will ameliorate the high loads of the pathogens and reduces the chances of infection by immuno-compromised individuals.

FUTURE WORK

There should be sound epidemiology and health information system, properly documented for surveillance in hospitals to be able to monitor, control in order to tackle the rate of hospital acquired infections.

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