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Distribution, phenotypic characterisation and antibiogram of bacterial species from hospital environment in Nigeria: Public health implications.

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Abstract
Background: This study was carried out to determine the distribution of antibiotic resistant bacterial pathogens on surface samples from Federal Teaching Hospital, Abakaliki (FETHA), Ebonyi State, Nigeria.
Materials and Methods: A total of 100 surface samples were collected from five (5) different wards including laboratory unit, pediatric ward, post natal ward, GOPD ward and children emergency ward. Isolation, phenotypic characterization and antibiogram study were carried out. Multiple Antibiotic Resistance Index (MARI) was determined for all isolates.
Results: A total of 156 organisms comprising of 40 (25.64%) *Staphylococcus aureus*, 40 (25.64%) *Escherichia coli*, 38 (24.36%) *Vibrio cholera*, 21 (13.46%) *Shigella spp.* and 17 (10.90%) *Salmonella spp.* were isolated and characterized. The result of the antibiotic susceptibility of isolates indicated that all strains were resistant to penicillin, nalidixic acid, cefotaxime, tetracycline, cefpirome, sulphamethoxazole, oxytetracycline and cephalothin. In contrast, the strains were susceptible to gentamicin, imipenem, streptomycin, and azithromycin. The five isolates had an average MARI between the range of 0.81- 0.88.
Conclusions: This investigation has revealed that all the different areas of the hospital harbor appreciable numbers of pathogenic bacteria.

Key words: *Antibiotic Resistance, Hospital environment, nosocomial infection, Nigeria*

Introduction
Various types of surfaces such as stainless steel, plastic, wood and glass are used today in many hospitals. These surfaces are subject to contamination by bacteria, some of which are able to form biofilms. It is wellknown that contamination of surfaces depends on their features, such as smooth, rough, porous, or irregular, and their state, for example before or after the cleaning process, new or old, dry or wet (1). Hospital acquired infections are serious complications in patients care and

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badly affect the mortality and morbidity despite antimicrobial treatment and advances in supportive care (2). It is a major public health concern these days and a cause of significant mortality and morbidity for hospitalized patients (2). Nosocomial infection caused by the nosocomial pathogens has pose a problem of huge degree worldwide, hospital localities have proven favorable in spread of infection due to prevailing suitable pathogens-host-environment relationship (3). Bacterial pollution of hospital equipment is one of the most probable cause of nosocomial infections. These contaminations are developed within a hospital or other type of clinical care facility and are acquired by patients while they are in the facility (4). Besides harming patients, nosocomial infections can affect nurses, doctors, aides, friends, delivery person, guardians and anybody who has contact with the hospital. The Center for Disease Control (CDC) estimates that about 10% of all hospital patients acquire some type of nosocomial infection as a consequence of interaction with some polluted hospital equipment. Roughly 40 million people are admitted to hospitals annually, 2 to 4 million people may develop an infection they did not have upon entering the hospital. Thus, nosocomial infections represent a significant proportion of all infectious diseases acquired by humans (5).

Multi reservoirs have been reported as being responsible for hospital contamination particularly due to stethoscope, in the delivery theater and intensive care units (ICU) (6). Several researches have been carried out on contamination of hospital equipment. Bernard et al. (6) reported 85% contamination of physician's stethoscope with both Gram-negative and Gram-positive bacterial pathogens. Gram-positive pathogens isolated were *Staphylococcus aureus, Staphylococcus epidermidis* and *Enterococcus faecalis*, while Gram-negative pathogens were *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. Bdareen (7) also reported 38.2% coagulase negative Staphylococcus and 23.9% *E. coli* isolated from 50 swab samples collected from instruments, equipment, devices and patients contact equipment from different hospital departments in educational hospital in Maber. Clinical thermometers, stethoscopes, sphygmomanometers, x-ray machines cassette, table coach and stationary grid do come in contact with patients skin during usage thereby putting the patient at risk of developing skin infections, if these equipments are contaminated with these organisms and are not disinfected (5). The occurrence of multi-drug resistance in hospital associated pathogens has resulted in the emergence and reemergence of difficult-to-treat nosocomial infections in patients depicting the pre-antibiotic era. These infections are difficult to eradicate due to resistance to many antibiotics, thus major cause of morbidity and mortality, leading directly and indirectly to an enormous increase in cost of hospital stay for the patients and also emergence of new health hazards for the community (8).

The emergence of antibiotic resistance causes increased mortalities and substantial costs; expenses have recently been estimated to be over 1.5 billion euros every year in Europe alone (8). Resistance genes are commonly encountered on, or associated with, mobile genetic elements (9) such as plasmids (10), integrons (11), and transposons (12). This enables their transfer within and between bacterial cells and species, and their genetic context risk of transfer from a source environment, and onwards into clinically relevant bacteria (13). It is reasonable to assume that such

transfer of genes from the environment will occur in the future, and that we can expect pathogens to pick up additional resistance determinants from the environmental resistome (14).

In Nigeria there are several hospitals that lack appropriate sanitary infrastructure, poor hygienic conditions, proper clinical waste management system and proper water supply, Ebonyi State inclusive. There is scarcity of data concerning resistance profiles of microorganisms isolated from hospital surfaces. The studies about distribution of antibiotic resistance genes and emerging pathogenic diarrheal bacterial species in hospital surfaces is important because the study will attempt to generate original local data and examine the magnitude of drug resistance pathogens in hospital environments in Ebonyi State which will be of immense benefit to the Public health sector in Nigeria as a whole. The aim is to study the distribution of antibiotic resistant bacterial pathogens on formites from Federal Teaching Hospital, Abakaliki (FETHA) in Ebonyi State, Nigeria.

Material and methods

Description of Study area

The study area of this research is Federal Teaching Hospital, Abakaliki (FETHA 1), Ebonyi State. Abakaliki is the capital of Ebonyi State, Nigeria. Ebonyi State is located in the south-eastern part of Nigeria which lies approximately within longitude 70301 and 70E and, latitude 50401 and 60451 N. It has a population of 149,683, and a land mass of about 5,935 square kilometers. Ebonyi State is bounded to the North by Benue State, to the South by Afikpo and Ohaozara local government and to East and West by Enugu State and Cross River State respectively. Federal Teaching Hospital Abakaliki (FETHA 1) is one of the Federal Hospitals in the eastern part of Nigeria. It receives referrals from the four corners of its geopolitical area and beyond.

Ethical Clearance

Ethical clearance was sought and obtained from the Ethical and Research Committee of Federal Teaching Hospital, Abakaliki (FETHA I), Ebonyi State.

Sample collection

A total of 100 surface samples were collected from five (5) different wards including laboratory unit, pediatric ward, post natal ward, GOPD ward and children emergency ward. A breakdown of the total number of samples collected show that twenty (20) samples were collected from five (5) different surfaces namely: floors (F), bedrails (BR), table tops (TT), door handles (DH), and drip stand (DS). Sterile cotton-wool-tipped swab sticks were moistened by dipping in physiological saline and were used to swab the surfaces of interest. The collection was made in early hours of the morning and transported to the Applied Microbiology Laboratory Complex, Ebonyi State University (EBSU) within two (2) hours of collection for analysis.

Sample processing and Isolation of bacteria

Each sample swabbed was pre-enriched in prepared sterile bacteriological peptone water and incubated at 37°C for 24 hours. After this the turbid broth was sub-cultured on nutrient agar and incubated at 37°C for 24 hours. Discrete colonies

obtained from the nutrient agar plates were further sub-cultured onto freshly prepared plates of selective and differential media such as MacConkey agar, Mannitol salt agar base, Salmonella-Shigella agar, Thiosulphate citrate bile salts sucrose agar plates. The petri dishes were placed in inverted position in the incubator for 24 hours at 37°C to obtain pure cultures. Presumptive morphological identification of the colonies was done by observing their individual appearance on the selective media used for the isolation. The colonies were stored in test tubes containing Peptone water for cultural, bacteriological identification and further characterizations following standard methods (15).

Identification of Bacteria

The bacterial isolates were primarily characterized and identified by microscopic examination and standard conventional biochemical and physiological tests. The cultures were examined for colony morphology, cell morphology, motility, Gram stain and sugar fermentation tests according to Bergeys Manual of systematic Bacteriology (16).

Antimicrobial Susceptibility Testing

Bacteria isolates were subjected to in-vitro susceptibility test against commonly used antimicrobial agents using disk diffusion method following guidelines established by the Clinical and Laboratory Standards Institute (17). In brief, by taking pure isolated colony, bacterial suspension was adjusted to 0.5McFarland turbidity standards. The diluted bacterial suspension was then transferred to Mueller-Hinton agar plate using a sterile cotton swab and the plate was seeded uniformly by rubbing the swab against the entire agar surface followed by 24 h incubation. After the inoculums were dried, antibiotic impregnated disks were applied to the surface of the inoculated plates using sterile forceps. The plates were then incubated aerobically at 37°C for 24 h. Finally, the zone of inhibition was measured including the disk diameter. The susceptible and resistant categories were assigned on the basis of the critical points recommended by the CLSI and according to the manufacturer's leaflet attached to them. The standard antibiotic discs (Oxoid, England) and their concentrations used against the isolates were, erythromycin (E 10 µg), azithromycin (AZM 15 µg), tetracycline (TE 10 µg), oxytetracycline (OT 30 µg), sulphamethoxazole (RL 25 µg), streptomycin (S 10 µg), gentamicin (CN 30 µg), penicillin G (P 10 µg), cefuroxime (CXM 30 µg), cephalothin (KF 30 µg), cefotaxime (CTX 30 µg), cefpirome (CPO 30 µg), imipenem (IPM 10 µg), nalidixic acid (NA 30 µg), vancomycin, (VA 10 µg), Oxacillin (OX 10 µg) and norfloxacin (NOR 10 µg). These antibiotics were chosen because they are either used in both human medicine and animal veterinary practice or because previous studies have reported microbial resistance to them.

Multiple Antibiotic Resistance Index (MARI)

MARI values of isolated bacteria against the antibiotics used were computed. MARI is a tool that helps in analyzing health risks and checking antibiotic resistance in a given area. The value of MARI is 0.20 and it differentiates the low risk (<0.20) from the high risk (>0.20). It is calculated by dividing the aggregate resistance of total isolates of an organism to all antibiotics by the product of the total number of antibiotics used and the number of isolates of an organism from the sample site. i.e. x/(y.z), where x represents the aggregate resistance of total isolates

of an organism to all antibiotics, y represents the total number of antibiotics used and z represents the number of isolates of an organism from the sample site. This formula was used since the MARI was being calculated from a sample site (environmental sampling) where many isolates were obtained according to the method of Riaz et al., (18)

Results

Table 1 presents the distribution pattern of five bacterial isolates from the different hospital wards. A total of 27 bacteria comprising of 7 (25.9 %) *Staphylococcus aureus*, 6 (22.2 %) *Escherichia coli*, 7 (25.9 %) *Vibrio cholera*, 0 (0 %) *Salmonella spp.* and 7(25.9 %) *Shigella spp.* was isolated from Laboratory Unit, while 33 bacterial isolates comprising of 8(24.2 %) *Staphylococcus aureus*, 6(18.1 %) *Escherichia coli*,7(21.2 %) *Vibrio cholera*, 6(18.1 %) *Salmonella spp.* and 6(18.1 %) *Shigella spp.* was isolated from Pediatric ward. Twenty eight (28) bacteria isolates comprising (25.0 %) *S. aureus*, 9 (32.1 %) *E. coli*, 6(21.4 %) *V. cholera*, 4(14.2 %) *Salmonella spp*. and 2(7.1 %) *Shigella spp*. was isolated from Post natal ward. while Thirty (35) bacteria comprising of 11(31.4%) *S. aureus*, 11(31.4%) *E. coli*, 7 (20.0 %) *V. cholera*, 3(8.5 %) *Salmonella spp.* and 3(8.5 %) *Shigella spp.* was isolated from GOPD. Thirty three (33) organisms comprising of 7 (21.2 %) *S. aureus*, 8(24.6%) *E. coli*, 11(33.3 %) *V.cholera*, 4 (12.1 %) *Salmonella spp*. and 3 (9.0 %) *Shigella spp*. was isolated from Children emergency ward.

Table 1. Distribution of bacterial isolates from different hospital wards.

Hospital Wards	**Bacterial Isolates**				
	S. aureus	***E. coli***	***V. cholerae***	***Salm. spp***	***Shigella***
Laboratory Unit	7 (25.9)	6 (22.2)	7 (25.9)	0 (0)	7 (25.9)
Pediatric	8 (24.2)	6 (18.1)	7 (21.2)	6 (18.1)	6 (18.1)
Post natal	7 (25.0)	9 (32.1)	6 (21.4)	4 (14.2)	2 (7.1)
GOPD	11 (31.4)	11 (31.4)	7 (20.0)	3 (8.5)	3 (8.5)
Children emergency	7 (21.2)	8 (24.6)	11 (33.3)	4 (12.1)	3 (9.0)

Table 2 shows the total number of bacteria isolated from the different wards/units. From the table it showed that 27 (17.31 %) of the bacteria were isolated from Laboratory Unit, 33 (21.15 %) from Pediatric ward, 28 (17.95 %) from Post natal ward, 35 (22.44 %) were isolated from GOPD, while 33 (21.15 %) bacterial isolates were from Children emergency ward.

Table 2. Total Number of Isolates from hospital wards.

Hospital Ward	Total	Prevalence (%)
Laboratory Unit	27	17.31
Pediatric	33	21.15
Post natal	28	17.95
GOPD	35	22.44
Children emergency	33	21.15
Total	**156**	**100**

Table 3 presents the prevalence of each of the isolated organisms. This comprises of 40 (25.64%) *S. aureus*, 40 (25.64%) *E. coli*, 38 (24.36%) *V. cholera*, 21 (13.46%) *Shigella spp.* and 17 (10.90%) *Salmonella spp.*

Table 3. Total prevalence of isolated organisms.

Bacterial Isolates	Number Isolated	Prevalence (%)
Staphylococcus aureus	40	25.64
Escherichia coli	40	25.64
Vibrio cholera	38	24.36
Salmonella spp.	17	10.90
Shigella spp.	21	13.46
Total	**156**	**100**

The result of the antibiotic susceptibility test for the bacteria isolates using disc diffusion method are shown in Table 4 to 8. It was indicated that strains of *S. aureus* shows susceptibility to gentamicin (100 %), imipenem (92.5 %) and azithromycin (82.5 %). The isolate showed 100 % resistance to cefpirome, nalidixic acid, tetracycline and oxacillin; 97.5, 95.0, 92.5, 90, 87.5, 82.5 and 72.5 % resistance to sulphamethoxazole, cefotaxime, erythromycin, norfloxacin, vancomycin, oxytetracycline and streptomycin, respectively (Table 4).

E. coli was susceptibleto gentamicin (90 %), imipenem (67.5 %), streptomycin (55 %), and azithromycin (35 %). The isolate showed 100 % resistance to penicillin, 97.5 % resistance to nalidixic acid, 95 % resistance to cefotaxime, tetracycline and cefpirome, 92.5 % resistance to sulphamethoxazole, oxytetracycline and cephalothin (Table 5).

Table 4. Inhibition zone diameter for *Staphylococcus aureus.*

Antibiotics	Disc potency (µg)	Number tested	Number sensitive (%)	Number resistant (%)	Number intermediate (%)
CPO	30	40	0 (0)	40 (100)	0 (0)
NA	30	40	0 (0)	40 (100)	0 (0)
IMP	10	40	37 (92.5)	0 (0)	3 (7.5)
TE	10	40	0 (0)	40 (100)	0 (0)
CXM	30	40	2 (5)	33 (82.5)	5 (12.5)
CTX	30	40	1 (2.5)	38 (95)	1 (2.5)
E	10	40	2 (5)	37 (92.5)	1 (2.5)
OT	30	40	1 (2.5)	33 (82.5)	6 (15)
NOR	10	40	1 (2.5)	36 (90)	3 (7.5)
CN	30	40	40 (100)	0 (0)	0 (0)
RL	25	40	1 (2.5)	39 (97.5)	1 (2.5)
AZM	15	40	33 (82.5)	2 (5)	5 (12.5)
S	10	40	4 (10)	29 (72.5)	7 (17.5)
OX	10	40	0 (0)	40 (100)	0 (0)
VA	10	40	2 (5)	35 (87.5)	3 (7.5)

(OX = oxacillin, CPO = cefpirome, E = erythromycin, AZM = azithromycin, TE = tetracycline, OT = oxytetracycline, RL = sulphamethoxazole, S = streptomycin, CN = gentamicin, CXM = cefuroxime, CTX = cefotaxime, IPM = imipenem, NA = nalidixic acid, VA = vancomycin and NOR = norfloxacin.)

Table 5. Inhibition zone diameter for *Escherichia coli.*

Antibiotics	Disc potency (µg)	Number tested	Number sensitive (%)	Number resistant (%)	Number intermediate (%)
P	10	40	0 (0)	40 (100)	0 (0)
CPO	30	40	1 (2.5)	38 (95)	1 (2.5)
NA	30	40	0 (0)	39 (97.5)	1 (2.5)
IMP	10	40	27 (67.5)	2 (5)	1 (2.5)
TE	10	40	0 (0)	38 (95)	2 (5)
KF	30	40	1 (2.5)	37 (92.2)	2 (5)
CXM	30	40	0 (0)	39 (97.5)	1 (2.5)
CTX	30	40	1 (2.5)	38 (95)	1 (2.5)
E	10	40	2 (5)	36 (90)	2 (5)
OT	30	40	1 (2.5)	37 (92.5)	2 (5)
NOR	10	40	3 (7.5)	33 (82.5)	4 (10)
CN	30	40	36 (90)	2 (5)	2 (5)
RL	25	40	1 (2.5)	37 (92.5)	2 (5)
AZM	15	40	14 (35)	19 (47.5)	7 (17.5)
S	10	40	22 (55)	13 (32.5)	5 (12.5)

(E = erythromycin, AZM = azithromycin, TE = tetracycline, OT = oxytetracycline, RL = sulphamethoxazole, S = streptomycin, CN = gentamicin, P = penicillin, CXM = cefuroxime, KF = cephalothin, CTX = cefotaxime, CPO = cefpirome, IPM = imipenem, NA = nalidixic acid and NOR = norfloxacin.)

Vibrio cholera was susceptible to gentamicin (97.4 %), azithromycin (81.6 %) and imipenem (52.6 %). The isolate showed 100 % resistance to penicillin, 97.4 %

resistance to cefpirome and nalidixic acid, 94.7 % resistance to cephalothin, 92.1 % resistance to tetracycline, cefuroxime, oxytetracycline, norfloxacin and sulphamethoxazole (Table 6). *Salmonella spp.* was susceptible to gentamicin (94.1 %), azithromycin (82.4 %), imipenem (76.5 %) and streptomycin (35.3 %). The isolate showed 100 % resistance to penicillin and sulphamethoxazole, 94.1 % resistance to nalidixic acid and cefuroxime, 88.2 % resistance to oxytetracycline, cefotaxime, cephalothin and cefpirome (Table7).

Shigella spp. was 95.2 % susceptible to gentamicin and imipenem. The isolate showed 100 % resistance to penicillin, cefuroxime and cefotaxime, 90.5 % resistance to erythromycin and sulphamethoxazole, 85.7 % resistance to norfloxacin, tetracycline and nalidixic acid (Table 8).

The result presented in table 9 shows the total and average MARI for the bacteria isolates. It was revealed that the five isolates had a total MARI of 32.69, 32.83, 31.97, 14.85 and 18.57 for *S. aureus*, *E. coli*, *V. cholerae*, *Salmonella spp*. and *Shigella spp*. respectively. It was revealed also that the five isolates had an average MARI of 0.81, 0.82, 0.84, 0.87 and 0.88 for *S. aureus*, *E. coli*, *V. cholerae*, *Salmonella spp*. and *Shigella spp*. respectively.

Table 6. Inhibition zone diameter for *Vibrio cholera.*

Antibiotics	**Disc potency (µg)**	**Number tested**	**Number sensitive (%)**	**Number resistant (%)**	**Number intermediate (%)**
P	10	38	0 (0)	38 (100)	0 (0)
CPO	30	38	0 (0)	37 (97.4)	1 (2.6)
NA	30	38	0 (0)	37 (97.4)	1 (2.6)
IMP	10	38	20 (52.6)	11 (28.9)	7 (18.4)
TE	10	38	1 (2.6)	35 (92.1)	2 (5.3)
KF	30	38	1 (2.6)	36 (94.7)	1 (2.6)
CXM	30	38	0 (0)	35 (92.1)	3 (7.9)
CTX	30	38	2 (5.3)	33 (86.8)	3 (7.9)
E	10	38	2 (5.3)	32 (84.2)	4 (10.5)
OT	30	38	2 (5.3)	35 (92.1)	1 (2.6)
NOR	10	38	1 (2.6)	35 (92.1)	2 (5.3)
CN	30	38	37 (97.4)	0 (0)	1 (2.6)
RL	25	38	0 (0)	35 (92.1)	3 (7.9)
AZM	15	38	31 (81.6)	0 (0)	7 (18.4)
S	10	38	2 (5.3)	34 (89.4)	2 (5.3)

(E= erythromycin, AZM = azithromycin, T = tetracycline, OT = oxytetracycline, RL= sulphamethoxazole, S = streptomycin, CN = gentamicin, P = penicillin, CXM = cefuroxime, KF = cephalothin, CTX = cefotaxime, CPO = cefpirome, IPM = imipenem, NA = nalidixic acid, NOR = norfloxacin.**)**

Table 7. Inhibition zone diameter for *Salmonella spp.*

Antibiotics	Disc potency (µg)	No Tested	No sensitive (%)	No resistant (%)	No intermediate (%)
P	10	17	0 (0)	17 (100)	0 (0)
CPO	30	17	0 (0)	15 (88.2)	2 (11.8)
NA	30	17	0 (0)	16 (94.1)	1 (5.9)
IMP	10	17	13 (76.5)	1 (5.9)	3 (17.6)
TE	10	17	0 (0)	14 (82.4)	3 (17.6)
KF	30	17	0 (0)	15 (88.2)	2 (11.8)
CXM	30	17	1 (5.9)	16 (94.1)	1 (5.9)
CTX	30	17	1 (5.9)	15 (88.2)	1 (5.9)
E	10	17	2 (11.8)	13 (76.5)	2 (11.8)
OT	30	17	0 (0)	15 (88.2)	2 (11.8)
NOR	10	17	3 (17.6)	12 (70.6)	2 (11.8)
CN	30	17	16 (94.1)	0 (0)	1 (5.9)
RL	25	17	0 (0)	17 (100)	0 (0)
AZM	15	17	14 (82.4)	1 (5.9)	2 (11.8)
S	10	17	6 (35.3)	4 (23.5)	7 (41.2)

(E = erythromycin, AZM = azithromycin, TE = tetracycline, OT = oxytetracycline, RL = sulphamethoxazole, S = streptomycin, CN = gentamicin, P = penicillin, CXM = cefuroxime, KF = cephalothin, CTX = cefotaxime, CPO = cefpirome, IPM = imipenem, NA = nalidixic acid and NOR = norfloxacin)

Table 8. Inhibition zone diameter for *Shigella spp.*

Antibiotics	Disc Potency (µg)	Number Tested	Number sensitive (%)	Number resistant (%)	Number intermediate (%)
P	10	21	0 (0)	21 (100)	0 (0)
CPO	30	21	3 (14.3)	15 (71.4)	3 (14.3)
NA	30	21	1 (4.8)	18 (85.7)	2 (9.5)
IMP	10	21	20 (95.2)	0 (0)	1 (4.8)
TE	10	21	0 (0)	18 (85.7)	3 (14.3)
KF	30	21	0 (0)	20 (95.2)	1 (4.8)
CXM	30	21	0 (0)	21 (100)	0 (0)
CTX	30	21	0 (0)	21 (100)	0 (0)
E	10	21	0 (0)	19 (90.5)	2 (9.5)
OT	30	21	0 (0)	20 (95.2)	1 (4.8)
NOR	10	21	0 (0)	18 (85.7)	3 (14.3)
CN	30	21	20 (95.2)	0 (0)	1 (4.8)
RL	25	21	0 (0)	19 (90.5)	2 (9.5)
AZM	15	21	4 (19.0)	13 (61.9)	4 (19.0)
S	10	21	1 (4.8)	19 (90.5)	1 (4.8)

(E = erythromycin, AZM = azithromycin, TE = tetracycline, OT = oxytetracycline, RL = sulphamethoxazole, S = streptomycin, CN = gentamicin, P = penicillin, CXM = cefuroxime, KF = cephalothin, CTX = cefotaxime, CPO = cefpirome, IPM = imipenem, NA = nalidixic acid and NOR = norfloxacin)

Table 9. Total and average Multiple Antibiotics Resistance Index (MARI) for the bacteria isolates.

S/N	Bacterial Isolates	Total MARI	Average MARI
1	*Staphylococcus aureus*	32.69	0.81
2	*Escherichia coli*	32.83	0.82
3	*Vibrio cholera*	31.97	0.84
4	*Salmonella* spp.	14.85	0.87
5	*Shigella* spp.	18.57	0.88

Discussion

The bacteria isolated from the surface samples as presented in table 1 were *S. aureus, E. coli, V. cholera, Salmonella spp.* and *Shigella spp*. All of which belongs to the family Enterobacteriaceae. The presence of these pathogenic organisms could be as a result of inadequate and improper decontamination of the various surfaces evaluated. Mohiudin et al. (19) reported that predominating organisms responsible for nosocomial infection in a Tertiary Hospitals of Dhaka city, Bangladesh were *E. coli, Pseudomonas spp., Proteus spp., S. aureus, Klebsiella spp.* and *Acinetobacter spp*. In a similar study in a specialist hospital in Kano, North-western Nigeria, Emmanuel, (20) reported the occurrence of *Escherichia coli, Staphylococcus aureus, Salmonella choleraesius, Proteus Mirabilis, Streptococcus spp., Proteus vulgaris, Pseudomonas aeruginosa, E. faecalis* and Coagulase negative Staphylococcus on fomites in hospital operating rooms. Cesar et al., (21) reported the incidence of *Klebsiella spp. E coli* spp. *Pseudomonas spp.* and *Enterobacter spp*. in a study conducted on indoor surfaces of a hospital located in Xalapa City in Mexico. The detection of Salmonella and Shigella in this study corroborates with the report of Helen et al., (22) who isolated Salmonella and Shigella species from selected hospitals in Anyigba, Kogi State, Nigeria.

Results presented in table 2 shows the distribution patterns of five pathogens isolated from the surfaces of the different hospital wards evaluated. From this it was observed that there was variation in the occurrence and abundance of the organisms in the different wards. Although, all the organisms targeted were isolated from the different wards, Salmonella spp was not isolated from Laboratory Unit 0 (0%).

Results presented in table 3 show the total number of the five pathogens isolated. The highest number, 35 (22.44 %), were obtained from GOPD. This was followed by 33 (21.15 %) from Children emergency ward, 33 (21.15 %) from Pediatric ward 28 (17.95 %) from Post natal ward and 27 (17.31 %) from Laboratory Unit. The results obtained here are fairly comparable with the result obtained by Chrinius et al., (23). The GOPD having the highest number of organisms is justifiable being that it receives patients who normally lack adequate health information relating to hospital environments as their stay is usually brief. Such patients visiting the hospital from homes, industries, markets, farms and government institutions usually harbor the highest loads of microorganisms transmitted from a wide range of sources before getting to the health facility (23)

Table 4 below presents the prevalence of each of the isolated organisms. This comprises of 40 (25.64%) *S. aureus*, 40 (25.64%) *E. coli*, 38 (24.36%) *V.cholera*, 21 (13.46%) and 17 (10.90%) *Shigella spp*. Isolation of more Gram positive organisms is consistent with previous reports (24). Similar study done in selected hospitals in Akoko, Ondo State Southwest Nigeria, Alabi et al., (25) showed that the frequency of isolation of gram positive bacteria was higher than the gram negative which also corroborate the findings of this study and agree with the statement that Gram-positive bacteria have overtaken the Gram-negative as the predominant bacteria isolated from formites (26). The result of this study is consistent with Jalalpoor et al. (27) who reported that Staphylococcus species (54.7%) was the most frequent bacteria isolated in hospital environment; same with 55.6 % reported by Anyadoh-Nwadike et al. (28) in Owerri, Imo State South Eastern, Nigeria. The high prevalence of the *S. aureus* in this work might be as a result of inadequate hand hygiene and this could be one of the attributing factors of the distribution of the pathogen in the hospital environmental surfaces (29). In a similar study on indoor surfaces of a hospital in Mexico, Cesar et al., (21), found 50.45 % of *Klebsiella spp*. 32 % of *Pseudomonas spp*., 9.17 % of *E. coli* spp. and 8.25 % of *Enterobacter spp*. In a another study conducted on inanimate objects in selected hospitals in Ondo State by Temitope et al., (30), the frequency of *Staphylococcus aureus* was 21.4%, *Escherichia coli* 17.5% and *Streptococcus spp*. 15.7%. In a related study, Aloma et al., (31), identified 138 (81.2%) *Staphylococcus aureus*, and 32 (18.8 %) *Pseudomonas aeruginosa* from different surfaces in specialist hospitals in Kaduna, Nigeria.

The result obtained from the antibiotic susceptibility testing for the bacteria isolates from hospital environment samples by disc diffusion method are shown in tables 4 to 8. It was indicated that strains of *S. aureus* shows marked susceptibility to gentamicin (100 %), imipenem (92.5 %) and azithromycin (82.5 %). The isolate showed 100 % resistance to cefpirome, nalidixic acid, tetracycline and oxacillin; 97.5, 95.0, 92.5, 90, 87.5, 82.5 and 72.5 % resistance to sulphamethoxazole, cefotaxime, erythromycin, norfloxacin, vancomycin, oxytetracycline and streptomycin, respectively. The 0.0 % resistance of *S. aureus* to gentamicin in this finding is not similar to the report of Akindele et al. (32) that 39 % of this pathogen was resistant to gentamicin. Mohiuding et al. (19) also reported that resistant rate of S. aureus was relatively lower than that of Gram negative bacteria and this can be attributed to the production of extended spectrum beta-lactamase by Gram negative organism. However, mechanism of antibiotics resistance in these gram-negative bacteria could be attributed to loss of porin, production of β-lactamases and increase expression of efflux pumps. In this study *S. aureus* showed 100 % resistance to cefpirome, nalidixic acid, tetracycline and oxacillin, sulphamethoxazole, cefotaxime, erythromycin, norfloxacin, vancomycin, oxytetracycline and streptomycin. Akindele et al. (32) also reported in their work that β- lactamase production by staphylococci is the recognized mechanism of resistance to β-lactam antibiotics such as ampicillin and penicillin .

E. coli was susceptible to gentamicin (90 %), imipenem (67.5 %), streptomycin (55 %), and azithromycin (35 %). The isolate showed 100 % resistance to penicillin, 97.5 % resistance to nalidixic acid, 95 % resistance to cefotaxime, tetracycline and

cefpirome, 92.5 % resistance to sulphamethoxazole, oxytetracycline and cephalothin. The high percentage of susceptibility of *E. coli* to gentamicin, imipenem, streptomycin, and azithromycin is in agreement with research findings of Mukhtar and Saeed (33) in Sudan, who found that *E. coli* expressed 0.0 % resistance to gentamicin, cefoxitin, ceftazidime and chloramphenicol. This also agrees with the work of Chrinius et al., (23), who reported that *E. coli* isolated from hospital environments was100 % susceptible to gentamicin. This pathogen showed varying levels of resistance to the rest of the antibiotic used. The resistance of *E. coli* to penicillin, cefpirome, cefotaxime and cephalothin could be as a result of production of β-lactamse enzyme which has the ability to deactivate the efficacy of these β-lactam drugs (34).

Vibrio cholera was susceptible to gentamicin (97.4 %), azithromycin (81.6 %) and imipenem (52.6 %). The isolate showed 100 % resistance to penicillin, 97.4 % resistance to cefpirome and nalidixic acid, 94.7 % resistance to cephalothin, 92.1 % resistance to tetracycline, cefuroxime, oxytetracycline, norfloxacin and sulphamethoxazole. *Salmonella spp*. was susceptible to gentamicin (94.1 %), azithromycin (82.4 %), imipenem (76.5 %) and streptomycin (35.3 %). The isolate showed 100 % resistance to penicillin and sulphamethoxazole, 94.1 % resistance to nalidixic acid and cefuroxime, 88.2 % resistance to oxytetracycline, cefotaxime, cephalothin and cefpirome. *Shigella spp*. was 95.2 % susceptible to gentamicin and imipenem. The isolate showed 100 % resistance to penicillin, cefuroxime and cefotaxime, 90.5 % resistance to erythromycin and sulphamethoxazole, 85.7 % resistance to norfloxacin, tetracycline and nalidixic acid.

The result of multiple antibiotics resistance index (MARI) for the bacteria isolates revealed that the five isolates had an average MARI between the range of 0.81-0.88. The MARI indices give an indirect suggestion of the probable source(s) of the organism. The MARI indices in this study were greater than 0.20, as seen in table 9, this confirms the report of Olayinka et al., (35) that the MARI index greater than 0.20 indicates that the organisms must have been originated from an environment where antibiotics are often used (23, 35 and 36). Thus, from the result of the multiple antibiotic index in this work, it could be asserted that these pathogens might have originated from where these antibiotics are used.

Conclusion

This investigation has revealed that all different areas of the hospital harbor appreciable numbers of pathogenic bacteria. It must be of concern that almost all of the surfaces were contaminated with bacteria and are a potential source of cross-infection from the hands of the health care workers to their patients. These pathogens can easily acquire antibiotic resistance and constitute a threat to the life of patients if they eventually find their way as aetiologic agents of surgical site infection.

The result of the antibiotic susceptibility of isolates from hospital environment samples indicated that all strains were resistant to penicillin, nalidixic acid, cefotaxime, tetracycline and cefpirome, sulphamethoxazole, oxytetracycline and cephalothin. Consequent upon the high resistant profile of penicillin, cephalexin

and vancomycin, it is recommended that these antibiotics should not be considered as drugs of choice for treatment of infection caused by these organisms. However, in contrast, gentamicin, imipenem, streptomycin, and azithromycin were considered the most effective antibiotics and subsequently could be used as drugs of choice or as alternatives for treatment of diseases caused by the organisms in the study area.

Finally, further possible investigations should include examining the effect of hand antisepsis or decontamination of surfaces in order to determine whether cleaning these potential sources of infection are associated with a reduced incidence of infection in a hospital.

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References

1. Rached I, Florence A, Valérie M, Isabelle L, Perrine G, Magdalena K, and Michel F . Methods for Recovering Microorganisms from Solid Surfaces Used in the Food Industry: A Review of the Literature. Int J Environ Res Public Health. 2013; 10(11): 6169–6183.
2. Pereira da Fonseca TA, Pessôa R, Felix AC, Sanabani SS. Diversity of Bacterial Communities on Four Frequently Used Surfaces in a Large Brazilian Teaching Hospital. Mawson A, ed. Int J Environ Res Public Health. 2016;13(2):152.
3. Samuel SO, Kayode OO, Musa OI, Nwigwe GC, Aboderin AO, Salami TAT, Taiwo SS. Nosocomial infections and the challenges of control in developing countries. Afri J Clin Exp Microb. 2010; 11(2): 102-110.
4. Yusha'u A, Bukar BS, Abdulkareem A. Bacterial contamination of some hospital equipment in Kano, Nigeria, Inf Contr Hosp Epidem. 2012; 23: 274-276.
5. Willey JM, Sherwood LM, Woolverton CJ. Prescott, Harley and Klein's Microbiology, 7th Edn., Mc Graw Hill Companies, New York, USA. 2008; (12) 70317, 1473-3099.
6. Bernard L, Kereveur A, Durand D, Gonat T. Bacterial contamination of hospital stethoscope. Infect Control Hosp. Epide. 1999; 20(9): 274-276.
7. Bdareen KZ, Bacterial contamination in the hospital and its aggressive behaviours, Department of Medical Laboratories, College of Medical Science, Thamar University, Maber. 2009.
8. Walsh F, Duff YB. The culturable soil antibiotic resistome: A community of multi-drug resistant bacteria. PLoS ONE, 2013; 8(6): e65567.
9. Heuer H, Smalla K. Plasmids foster diversification and adaptation of bacterial populations in soil. FEMS Microbiol Rev. 2012; 36: 1083-1104.
10. Szczepanowski R, Linke B, Krahn I, Gartemann K, Guetzkow T, Eichler W. Detection of 140 clinically relevant antibiotic-resistance genes in the plasmid metagenome of wastewater treatment plant bacteria showing reduced susceptibility to selected antibiotics. Microbiology. 155: 2306- 2319.
11. Gaze WH, Zhang L, Abdouslam Na, Hawkey PM, Calvo-Bado L, Royle J. Impacts of anthropogenic activity on the ecology of class 1 integrons and integron-associated genes in the environment. ISME J. 2011; 5: 1253-1261.
12. Nigro S. J., Hall R. M. Antibiotic resistance islands in A320 (RUH134), the reference strain for *Acinetobacter baumannii* global clone 2. J Antimicrob Chemother. 2012; 67: 335-338.

13. D'Costa V.M, King C.E, Kalan L, Morar M, Sung WWL, Schwarz C. Antibiotic resistance is ancient. Nature. 2011, 477: 457-461.

14. Toleman MA, Walsh TR. Combinatorial events of insertion sequences and ICE in Gram-negative bacteria. FEMS Microbiol Rev. 2011; 35, 912-935.

15. Li D, Qi R, Yang M, Zhang Y, Yu T. Bacterial community characteristics under long-term antibiotic selection pressures. Water Res. 2011; 45: 6063–6073.

16. Cheesbrough, M. District Laboratory Practice in Tropical Countries, Part 2, Cambridge University Press. 2006; 35-195.

17. Clinical Laboratory Standard Institute (CLSI) . Performance Standards for Antimicrobial Disc Susceptibility Test; 8th ed. Approved Standards, m2A8, Wayne, Pa (USA), 2005; 11:64-68.

18. Riaz S, Faisal M, Hasnain S. Antibiotic susceptibility pattern and multiple antibiotic resistances (MAR) calculation of extended spectrum β-lactamase (ESBL) producing *Escherichia coli* and *Klebsiella spp.* in Pakistan. Afri J Biotechnol. 2011; 10(33): 6325-6331.

19. Mohiuddin MD, Ashraful Haq J, Mozammel Hoq MD, Farida H. Microbiology of nosocomial infection in Tertiary Hospitals of Dhaka city and its impact. Bangladesh J Med Microbiol. 2010; 4(2): 32-38.

20. Emmanuel, N. Isolation of Pathogenic Bacteria from Fomites in the Operating Rooms of a Specialist Hospital in Kano, North-western Nigeria. The Pan African Medical Journal, 2012; 12:90.

21. Cesar P., Garcia-Cruz, L., Maria J, and Omar E A. Fungal and bacterial contamination on indoor surfaces of a hospital in Mexico Jundishapur. J Microb. 2012; 5(3): 460-464.

22. Helen Ojomachenwu Ocean, Emmanuel Aniebonam Eze, Chibuzor Nwadibe Eze and Matthew Chekwube Enebe. Drug resistance pattern of Salmonella and Shigella species isolated from selected hospitals in Anyigba, Kogi State, Nigeria. Afri J Microbi Res. 2015; 9(37): 2023-2036.

23. Chrinius H., Edward D.J and Clement M.Z.. Prevalence and Antibiogram Pattern of Some Nosocomial Pathogens Isolated from Hospital Environment in Zaria, Nigeria. Int. J Sci Tech. 2014; 3(3): 131-139.

24. Neely AN, Maley MP; Maley . Survival of enterococci and staphylococci on hospital fabrics and plastic. J Clin Microbiol. 2000; 38 (2): 724–726.

25. Alabi MA, Olowokere T, Sunday RM, Salami ET, Osanaiye F, Afolabi J F . Antibiotic Sensitivity Pattern of Bacteria from Selected Hospitals in Akungba Akoko, Ondo State, Southwest Nigeria. Bact. Journal, 2014; 4 (1), 21-26.

26. Inweregbu K, Dave J, Pittard. Nosocomial infections. Contin Educ Anaesth Crit Care Pain. 2005; 5(1): 4-17.

27. Jalalpoor S, Kermanshahi RK, Nouhi AS, Esfahani ZH. Isolation of bacteria in hospital environment. Isfahan Med School, 2009; 27(100): 632-645.

28. Anyadoh SO, Akerele J, Udum UI. Prevalence of multi drug resistant *Escherichia coli* among pregnant women in Owerri. IJMST, 2010; 3(3):17-20.

29. Olalekan, AW, Asekun-Olarinmoye, EO, Bamidele JO, Abodunrin OL, Olowu, A. A Comparative study of awareness and attitude to nosocomial infections among levels of health care workers in Southwestern Nigeria. Cont J Trop Med. 2011; 5 (2): 5-10.

30. Temitope O, Mutiu AA, Bamidele SF, Rita MS, Ekundayo TS, Folake O, Johnson F, A, Taiwo O. Antibiotic sensitivity pattern of bacteria from selected hospitals in Akungba Akoko, Ondo State, Southwest Nigeria. IOSR J of Pharm Biol Sci. 2013; (IOSR-JPBS) 8: 01-04.

31. Aloma AA, Olonitola OS, Jatau ED. Isolation, characterization and antibiotic susceptibility patterns of *Pseudomonas aeruginosa* and *Staphylococcus aureus* from

hospital environment in Kaduna Metropolis, Kaduna State. Inter J Scientific Res Publ. 2016; 6:141.

32. Akindele AA, Adewuyi IK, Adefioye OA, Adedokun SA, Olaolu AO. Antibiogram and Beta- Lactamase production of *Staphylococcus aureus* isolates from different human clinical specimens in a tertiary health institute in Ile-Ife Nigeria. American-Eurasian J Sci Res. 2010; 5(4): 230-233.

33. Mukhtar AM, Saeed HA. Profile of antibiotic sensitivity and resistance of some pathogenic bacteria isolated from clinical specimens in Sudan. J Sc Tech. 2011; 12(1): 14-19.

34. Hassan SA, Jamal SA. Kamal M. Occurrence of multidrug resistant and ESBL producing *E. coli* causing urinary tract infections. J Basic Applied Sc. 2011; 7(1): 39–43.

35. Olayinka BO, Olonitola OS, Olayinka AT, Agada EA. Antibiotic susceptibility pattern and multiple antibiotic susceptibility and multiple antibiotic resistance index of *Pseudomonas aeruginosa* urine isolates from a University Teaching Hospital. Afr J Clin Exp Microbiol. 2004; 5(2): 198-202.

36. Hammuel C, Idoko MO, Migap HH, Ambrose N. Occurrence and antibiogram profile of *Staphylococcus aureus* isolated from some hospital environment in Zaria. Nigeria Afr J Microb Res. 2015; 9(19): 1304-1311.