Journal of Advances in Medical and Pharmaceutical Sciences
10(4): 1-15, 2016; Article no.JAMPS.29316
ISSN: 2394-1111

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Prevalence and Antimicrobial Susceptibility Profile of Bacteria Isolated from the Environment of two Tertiary Hospitals in Calabar Metropolis, Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Authors ENM, UOE and CIM designed the study, wrote the protocol and interpreted the data. Authors ENM, UOE and CIM anchored the field study, gathered the initial data and performed preliminary data analysis. While authors CIM and ENM managed the literature searches and produced the initial draft. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JAMPS/2016/29316
Editor(s):
(1) Palmiro Poltronieri, National Research Council of Italy, CNR-ISPA, Italy and Institute of Sciences of Food Productions, Operative Unit in Lecce, Italy.
Reviewers:
(1) Ronald Bartzatt, University of Nebraska, USA.
(2) Eliton da Silva Vasconcelos, Federal University of São Carlos, Brazil.
(3) Anonymous, Nagoya City University, Japan.
Complete Peer review History: http://www.sciencedomain.org/review-history/17038

Original Research Article

Received 2nd September 2016
Accepted 24th October 2016
Published 26th November 2016

ABSTRACT

Objective: The objective of this study was to determine the susceptibility pattern of the bacterial isolates obtained from the environment of two hospitals (General hospital – GH and infectious disease hospital – IDH).
Methods: A total of 240 swabs and air samples were collected from two hospitals with 20 samples each from wards, pharmacies, blood banks, theatres, laboratories and intensive care units. Bacterial isolates were obtained from these samples using standard microbiological techniques. Identified isolates were then subjected to antimicrobial sensitivity, minimum inhibitory and

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bactericidal concentrations determination. The antibiotics used in this study were gentamycin, ofloxacin, norfloxacin, ceporex, ciprofloxacin, reflacine, ampicillin,levofloxacin, ampicillin+cloxacillin, amoxicillin, erythromycin, amoxicillin+clavulanic acid, streptomycin, sulfamethoxazole+ trimethoprim, nalidixic acid, chloramphenicol, cefoxitin and ceftazidime. Mean counts were analysed using student t-test and simple descriptive statistics.

Results: A total mean count of 2940cfu was recorded from both hospitals of which 1949cfu and 991cfu were obtained from GH and IDH, respectively. A comparism of the mean counts for both hospitals unit by unit showed that pharmacy unit alone was significant (p =0.01). A total of 130 bacteria isolates were recovered in this study of which 80(61.5%) and 50(38.5%) were isolated from GH and IDH, respectively. In GH, the lowest MIC and MBC of 470 and 230mg/ml was recorded by *P. aeruginosa, S. aureus and Proteus species* against ciprofloxacin while other isolates exhibited moderate resistance to this antibiotic. In IDH, the lowest MIC and MBC were recorded by *P. aeruginosa*, *C. freundii*, *K. pneumoniae* and *S. marcesens* against ampicillin while lowest MIC and MBC was recorded by *S. aureus* against cefoxitin.

Conclusion: The findings in this study reveal that airborne sources and inanimate surfaces of hospitals are an important reservoir of multidrug resistant nosocomial pathogens.

Keywords: Nosocomial infection; Calabar; resistance; antibiotics and end point.

1. INTRODUCTION

Nosocomial infections has been described by the World health Organization (WHO) as one of the major classes of infectious diseases having a huge economic impact worldwide; with airborne agents of microbial origin in hospital environments being the major culprit of infections [1]. Microbial contaminants have been reported to survive on dry surfaces including those commonly touched by healthcare staff and patients for prolong periods and serve as source of hand transfer of microorganisms known to cause infections [2]. As recommended by Mbim et al. [3], indoor air quality and surfaces of hospital settings should become a critical part of hospital and health care facilities infection control and management protocols in order to reduce the prevalence of nosocomial infections. The significance of environmental modes of transmission in the epidemiology of nosocomial infections has gained popularity in the last two decades and patients susceptible to cross contamination often showed considerable morbidity and mortality [4]. A number of studies have highlighted the dominant prevalence of bacteria in the hospital settings compared to fungi and viruses [2,3]. This is also greatly influenced by the number of occupants and visitors to hospital facilities and units as most of the isolates usually recovered were similar to those that often make up the microbiota of the skin. In addition, the nature and type of infection control protocols as well as hygiene practice has been reported to be greatly influenced by microbial contamination. Among the numerous bacteria that have been associated with nosocomial infections, the commonly reported species include *Staphylococci, Micrococci, Pseudomonas* and *Enterococci* species and in particular members of *Enterobacteriaciae* [2,3]. The burden of nosocomial infections has further been complicated by the insurgence of increased resistance to antibiotics including methicillin-resistant *Staphylococcus aureus* among Gram positive organisms and multi-drug resistant (MDR) *P. aeruginosa* among Gram negative organisms. A recent study has shown that hospital environments harbour microorganisms showing multi resistant to different routinely used antibiotics [5]. Furthermore, these genes are plasmid borne and are of great public health concern [5]. Despite considerable awareness of microbial hazards in hospital settings and their control measures, reports on air and surface bound microbial contamination as well as their associated resistance is still poorly understood. Therefore, this study was aimed at evaluating the prevalence and antimicrobial susceptibility of bacteria isolated from two tertiary hospitals in Calabar metropolis.

2. MATERIALS AND METHODS

2.1 Study Area/Site

This study was undertaken in two tertiary hospitals including General hospital and Infectious Disease Hospital all in Calabar Metropolis. Calabar metropolis comprises of both Calabar municipality and south local government areas [5].

2.2 Source and Size of Sample

A total of 240 swab samples were collected from two hospitals with 20 from each of the following units: wards, pharmacies, blood banks, theatres, laboratories and intensive care units. The swab samples from these units were collected from table tops (10), laboratory coats (5), sinks (3) and door handles (2). Microbiological air qualities of the units were analysed using settle plate technique.

2.3 Antibiotics Used in the Study

The antibiotics used in this study were gentamycin (CN) (10µg), ofloxacin (OFL) (10µg), norfloxacin (NB) (10µg), ceporex (CEP) (10µg), ciprofloxacin (CPX) (10µg), reflacine (PEF) (10µg), ampicillin (AM) (10µg), levofloxacin(LEV) (20µg), ampicillin+cloxacillin (APX) (20µg), amoxicillin (AMX) (25µg), erythromycin (E) (30µg), amoxicillin+clavulanic acid (AU) (30µg), streptomycin (S) (30µg), sulfamethoxazole+ trimethoprim (SXT) (30µg), nalidixic acid (NA) (30µg), chloramphenicol (CH) (30µg) (Optun laboratories, Nigeria), cefoxitin (FOX) (30µg) and ceftazidime (CAZ) (30µg) (Oxoid, UK).

2.4 Treatment of Swab Materials

Briefly, sterile swab sticks soaked in physiological saline were used to swab the aforementioned sample sites and units and processed following standard techniques described previously [6].

2.5 Microbiological Analysis of the Hospital Air

This was carried out using the settle plate technique described by the Center for Disease Control and Prevention (CDC) [7]. Briefly, plates containing nutrient agar, MacConkey agar and blood agar in triplicates exposed to air for 1hr were wrapped in aluminium foil and transported immediately to the laboratory where they were incubated for 24-48hrs at 37℃.

2.6 Determination of mean Loads, Purification and Characterization of Isolates

After incubation, colonies on each plate were counted and recorded following procedures by Benson [8]. Discrete colonies were then identified and characterized following standard microbiological techniques [9].

2.7 Antimicrobial Susceptibility Testing

The antibiotic sensitivity tests of isolates were carried out following procedures of Kirby-Bauer disc diffusion as recommended by the Clinical Laboratory Standard Institute (CLSI) [10]. After incubation, the zones of inhibition were measured and compared with zone diameter interpretative chart to determine susceptibility of the isolates to antibiotics.

2.8 Determination of MIC and MBC

This was carried out as described by CLSI [10]. Briefly, 2-3 colonies of the isolate were inoculated into 5ml of sterile peptone broth and incubated for 30mins. Different concentrations (mg/ml) of 62.50, 31.25, 15.62, 7.81, 3.91, 1.95, 0.98, 0.49 and 0.25 were prepared for amoxicillin+clavulanic acid. For ciprofloxacin, ampicillin+cloxacillin and ampicillin, 50, 25, 12.5, 6.25, 3.13, 1.56, 0.78, 0.39 and 0.20mg/ml were prepared. For cefoxitin, 7.50, 3.75, 1.875, 0.94, 0.47 and 0.23 mg/ml were prepared Tubes containing diluents were kept as controls. Consequently, 0.5ml of the inoculums were introduced into the control and teststubes and incubated at 37℃ for 24hrs and then observed for growth. The MBC was determined by selecting the tubes that showed no growth (turbidity) during the MIC tests. A loopful from each test tube was then sub-cultured into plates of freshly prepared nutrient agar and incubated at 37℃ for 24hours. The least concentration in the MIC tests with no growth in the sub-culture plate was recorded as MBC.

2.9 Data Analysis

The data obtained from the study were managed and analysed using MS Excel 2010 version. Descriptive statistics and student t-test were used to analyse the mean bacteria counts from both hospitals.

3. RESULTS

The results of the present study are presented in the Tables 1-5 and Figures 1-3. Table 1 shows the mean microbial count from sampled sites and units of General hospital (GH) and Infectious Disease Hospital (IDH). A total mean count of 2940cfu was recorded of which 1949cfu and 991cfu were obtained from GH and IDH, respectively. For GH, the counts were 554, 463, 337, 325 and 270cfu for air, door handles, table tops, sinks and laboratory coats, respectively. On

the other hand, the counts for IDH were 328, 230, 180, 163 and 90cfu for sinks, air, table tops, and laboratory coats, respectively. Comparism of the mean counts for both hospitals unit by unit showed that pharmacy unit alone was significant (p =0.01). A total of 130 bacteria isolates were recovered in this study of which 80(61.5%) and 50(38.5%) were isolated from General hospital and IDH, respectively. Figures 1 and 2 show the distribution of the bacteria isolates from both hospitals according to sampled sites while Figures 3 and 4 show the distribution of isolates from both hospitals according to units.

Tables 2 and 3 show the antimicrobial susceptibility of the isolates to the test antibiotics from GH and IDH, respectively. The MIC and MBC of the isolates to the test antibiotics are presented in Tables 4 and 5. In GH, the lowest MIC and MBC of 1560 and 3133µg/ml was recorded by *P. aeruginosa, S. aureus and Proteus species* against ciprofloxacin while other isolates exhibited moderate resistance to this antibiotic. An MIC and MBC of 1953 and 3906µg/ml respectively were recorded by *S. marcesens* against amoxicillin+clavulanic acid.

4. DISCUSSION

A total mean load of 2940cfu from both General Hospital (GH) and Infectious Disease Hospital (IDH) of which 1949 cfu was obtained from GH and 991cfu from IDH. This difference could be due to the difference in the influx of the persons both visitors and patients to both facilities. This was higher than the 461-465cfu reported by Genet et al. [11] but lower than the maximum load of 9734/m^3 reported by Fekaku and Getachewu [12]. The mean loads of 480 (24.6%) and 253 (25.5%) recorded in our study in laboratory and intensive care units of GH and IDH was higher than the 17.43% reported by Garcia-Cruz et al. [13]. The blood bank unit of GH recorded 274(14.1%) and that of IDH 230(23.2%). This is worrisome as studies have shown that it could act as a source of contaminations to blood and its products [14,15]. Furthermore, analysis of the mean loads in the sampled units revealed that only the pharmacy unit of both hospitals was significant (P=0.01).

In both hospitals, more Gram negative isolates than positive were isolated and this is consistent with earlier reports [13,16,17]. However, the prevalence of Gram negative bacteria (30.0 – 33.8%) observed was lower than those previously reported [18,19]. The isolates recovered from sinks of both hospitals were *E. coli*, *P. aeruginosa*, *Salmonella* species, *Klebsiella* species, *Citrobacter freundii*, *S. aureus* and Coagulase-negative *Staphylococci* giving a microbial contamination of 39.3 – 39.5% and was lower than the 79.5% reported by Mashouf et al. [20]. Furthermore, the finding of *P. aeruginosa* on

Table 1. Mean Count of Bacteria according to Hospitals

GH Units	Table tops	Laboratory coat	Door handles	Sinks	Air	Mean load	%
Pharmacy	72	121	31	89	111	424	21.8
Theatre	79	21	51	106	130	387	19.9
Laboratory	101	69	216	30	64	480	24.6
Ward	43	29	68	6	54	200	10.3
Blood bank	20	18	74	52	110	274	14.1
ICU	22	12	23	42	85	184	9.4
Total mean loads	337	270	463	325	554	1949	
%	17.3	13.9	23.8	16.7	28.4		
IDH units							
Pharmacy	42	32	15	45	28	162	16.3
Laboratory	19	8	23	44	28	122	12.3
Ward	28	11	36	67	82	224	22.6
Blood bank	49	13	36	74	58	230	23.2
ICU	42	26	53	98	34	253	25.5
Total mean loads	180	90	163	328	230	991	
%	18.2	9.1	16.4	33.1	23.2		

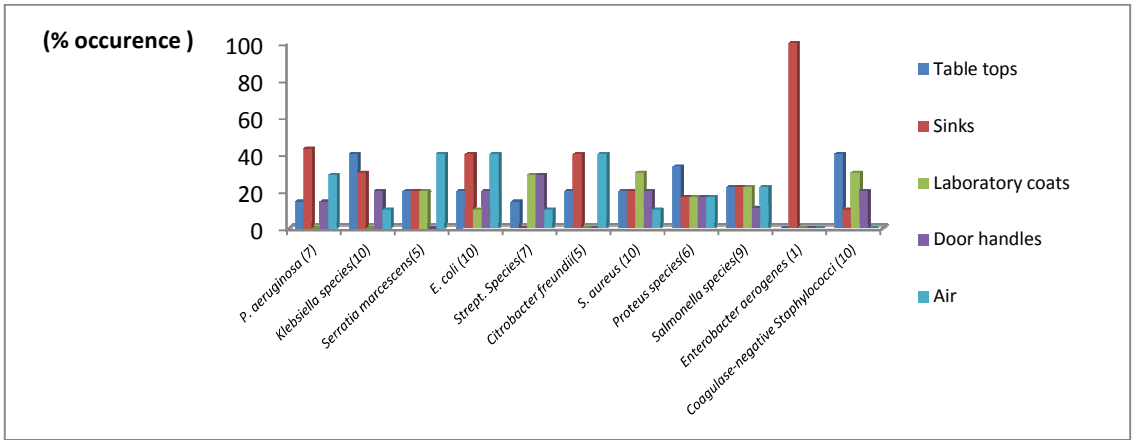

Fig. 1. Percentage occurrence of bacteria recovered according to sampled sites/materials from GH

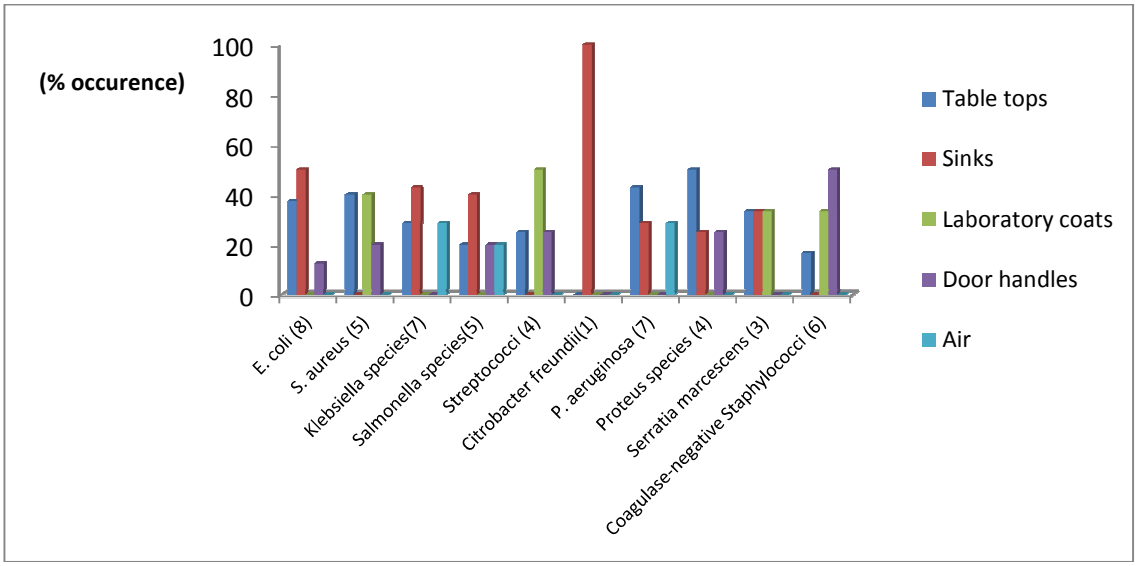

Fig. 2. Percentage occurrence of bacteria recovered according to sampled sites/materials from IDH

sinks confirmed reports of Udeze et al. [21] that sinks are the most common abode for this organism especially in hospital settings. The Gram positive bacteria recovered from table tops of GH and IDH are considerably similar and were lower than 57.1% reported by Ferreira et al. [22] and the 50% reported by Chrinius et al. [23]. The most frequent isolates from table tops of GH included *Klebsiella species, E. coli, Salmonella species, Proteus species and S. aureus* strains while those of IDH were *E. coli, P. aeruginosa, Klebsiella species, Proteus species* and *S. aureus*. The finding of *P. aeruginosa, E. coli* and *K. pneumonia*e on table tops of both hospitals is consistent with an earlier report [13].

The 23.5–25% prevalence of Gram positive bacteria on door handles is consistent with the 19% reported by Oie et al. [24] but lower than the 38% observed by Carvalho et al. [25] and the 53.8% reported by Chrinius et al. [23].This has been attributed to the poor hygienic nature of users of these doors [26]. The most frequently isolated organisms from door handles of GH were Coagulase-negative *Staphylococci*, *S. aureus*, *Streptococci*, *K. pneumonie* and *E. coli* while in IDH, Coagulase-negative *Staphylococci*, *S. aureus, Streptococci, E. coli* and *Salmonella species* were isolated. The finding of *S. aureus, K. pneumoniae* and *E. coli* is consistent with report of Nworie et al. [26].

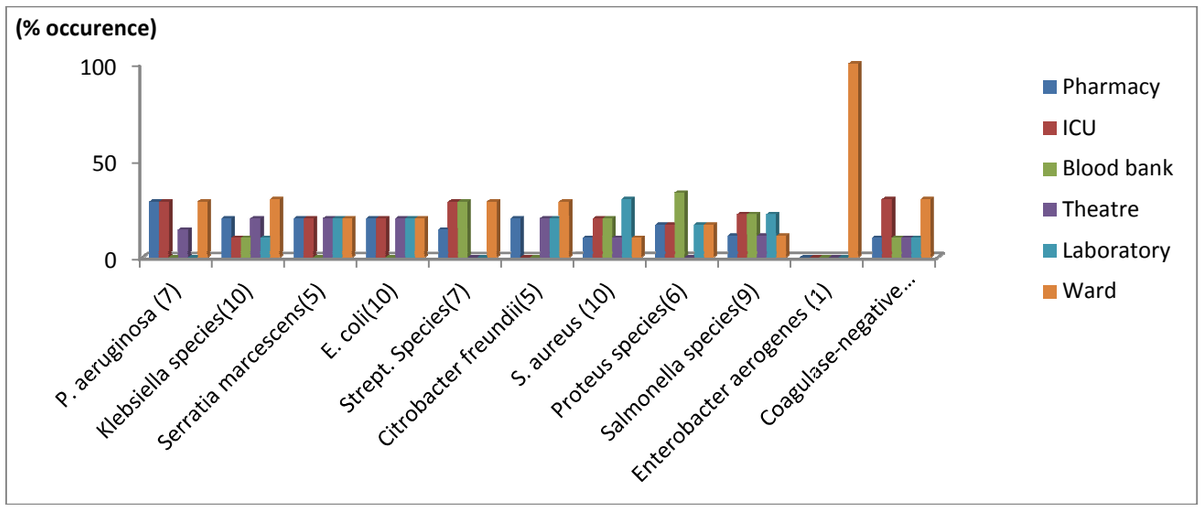

Fig. 3. Percentage occurrence of bacteria recovered according to sampled Units from GH

Table 2. Antimicrobial susceptibility of isolates from GH

Organism	OFX	PEF	CPX	AU	CN	S	CEP	NA	SXT	NB	E	CH	APX	LEV	AM	FOX	AMX	CAZ	MAR
P. Aeruginosa (7)	2(28.6)	4(57.1)	4(57.1)	6(85.6)	3(42.9)	2(28.6)	4(57.1)	3(42.9)	2(28.6)	NA	NA	NA	NA	NA	7(100)	NA	6(85.7)	7(100)	0.60
K. Pneumoniae (10)	4(40.0)	5(50.0)	5(50.0)	2(20.0)	5(50.0)	3(30.0)	5(50.0)	2(20.0)	6(60.0)	NA	NA	NA	NA	NA	10(100.0)	NA	8(80.0)	6(60.0)	0.51
Serratia marcescens (5)	1(20.0)	2(40.0)	3(60.0)	4(80.0)	1(20.0)	2(40.0)	1(20.0)	2(40.0)	1(20.0)	NA	NA	NA	NA	NA	5(100)	NA	4(80.0)	4(80.0)	0.50
E. coli (10)	3(30.0)	4(40.0)	5(50.0)	4(40.0)	4(40.0)	3(30.0)	6(60.0)	5(50.0)	2(20.0)	NA	NA	NA	NA	NA	7(70.0)	NA	8(80.0)	10 (100)	0.51
Strept. Species (7)	NA	NA	5(71.4)	NA	2(28.6)	2(28.6)	NA	NA	NA	2(28.6)	3(42.8)	5(71.4)	0(0.0)	2(28.6)	0(0.0)	NA	0(0.00)	NA	0.30
C. Freundii (5)	0(0.0)	3(60.0)	0(0.0)	1(20.0)	4(80.0)	3(60.0)	2(40.0)	1(20.0)	3(6.00)	NA	NA	NA	NA	NA	3(60.0)	NA	4(80.0)	4(80.0)	0.52
S. Aureus (10)	NA	NA	3(30.0)	NA	6(60.0)	4(40.0)	NA	NA	NA	3(30.0)	4(40.0)	6(60.0)	7(70.0)	4(40.0)	9(90.0)	8(80.0)	7(70.0)	NA	0.55
Proteus species (6)	1(16.7)	2(33.3)	3(50.0	4(66.7)	3(50.0)	3(50.0.0)	4(66.7)	2(33.3)	4(66.7)	NA	NA	NA	NA	NA	6(100)	NA	5(83.3)	5(83.3)	0.58
Salmonella species (9)	2(22.2)	5(55.5)	4(44.4)	4(44.4)	3(33.3)	2(22.2)	4(44.4)	3(33.3)	6(66.7)	NA	NA	NA	NA	NA	9(100)	NA	8(88.9)	7(77.8)	0.53
Enterobacter Aerogenes(1)	0(0.0)	1(100.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(100.0)	0(0.0)	0(0.0)	NA	NA	NA	NA	NA	1(100)	NA	1(100)	1(100)	0.42
CONs (10)	NA	NA	2(20.0)	NA	1(10.0)	0(0.0)	NA	NA	NA	1(10.0)	3(30.0)	3(30.0)	1(10.0)	1(10.0)	3(30.0)	3(30.0)	3(30.0)	NA	0.19

KEY: OFX-Ofloxacin, PEF-Reflacine, CPX-Ciprofloxacin, AU- amoxicillin+clavulanic acid, CN-Gentamycin, S-Streptomycin, CEP-Ceporex, NA-Nalidixic acid, SXT- sulfamethoxazole+ trimethoprim, NB-Norfloxacin, E-Erythromycin ,CH-Chloramphenicol, APX- ampicillin+ cloxacillin, LEV-Levofloxacin, AM-Ampicillin, FOX-Cefoxitin, AMX, AML -Amoxicillin, CAZ-Ceftazidime. CONs= Coagulase negative Staphylococci.

Table 3. Antimicrobial susceptibility of isolates from IDH

Organism	OFX	PEF	CPX	AU	CN	S	CEP	NA	SXT	NB	E	CH	APX	LEV	AM	FOX	AMX	CAZ	MAR
E. coli (8)	2(25)	5(62.5)	2(25)	2(25)	2(25)	1(12.5)	6(75)	2(25)	4(50)	NA		NA	NA		7(87.5)	NA	6(75)	5(62.5)	0.46
S. aureus (5)	NA	NA	1(20)	NA	3(60)	2(40)	NA	NA	NA	2(40)	2(40)	3(60)	2(40)	1(20)	5(100)	5(100)	4(80.0)	NA	0.55
Klebsiella species(7)	3(42.9)	4(57.1)	3(42.9)	3(42.9)	2(28.5)	5(71.4)	1(14.3)	2(28.5)	3(42.9)	NA	NA	NA	NA	NA	6(85.7)	NA	5(71.4)	6(85.7)	0.51
Salmonella species(5)	2(40)	4(80)	3(60)	1(20)	2(40)	1(20)	4(80)	1(20)	4(80)	NA	NA	NA	NA	NA	5(100)	NA	4(80)	4(80)	0.58
Strept. Species(4)	NA	NA	3(75)	NA	1(25)	0(0.00)	NA	NA	NA	2(50)	2(50)	3(75)	0(0.00)	1(20)	0(0.00)	NA	0(0.0)	NA	0.30
C. freundii (1)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	1(100)	0(0.00)	1(100)	NA	NA	NA	NA	NA	1(100)	NA	1(100)	1(100)	0.33
P. aeruginosa (7)	1(14.3)	2(28.6)	3(42.9)	4(57.1)	2(28.6)	1(14.3)	5(71.4)	1(14.3)	1(14.3)	NA	NA	NA	NA	NA	6(85.7)	NA	7(100)	6(85.7)	0.46
Proteus species (4)	1(25)	2(50)	1(25)	0(0.00)	1(25)	0(0.00)	3(75)	1(25)	2(50)	NA	NA	NA	NA	NA	4(100)	NA	4(100)	3(75)	0.45
S. marcescens (3)	1(33.3)	1(33.3)	0(0.00)	1(33.3)	1(33.3)	0(0.00)	3(100)	0(0.00)	2(66.7)	NA	NA	NA	NA	NA	3(100)	NA	2(66.7)	2(66.7)	0.44
Coagulase-negative Staphylococci(6)	NA	NA	2(33.3)	NA	1(16.7)	1(16.7)	NA	NA	NA	2(33.3)	2(33.3)	1(16.7)	1(16.7)	2(33.3)	3(50)	2(33.3)	3(50.0)	NA	0.30

Keys:OFX-Ofloxacin, PEF-Reflacine, CPX-Ciprofloxacin, AU- amoxicillin+clavulanic acid, CN-Gentamycin, S-Streptomycin, CEP-Ceporex, NA-Nalidixic acid, SXT- sulfamethoxazole+ trimethoprim, NB-Norfloxacin, E-Erythromycin, CH-Chloramphenicol, APX- ampicillin+ cloxacillin, LEV-Levofloxacin, AM-Ampicillin, FOX-Cefoxitin, AMX, AML- Amoxicillin, CAZ-Ceftazidime. CONs= Coagulase negative Staphylococci.

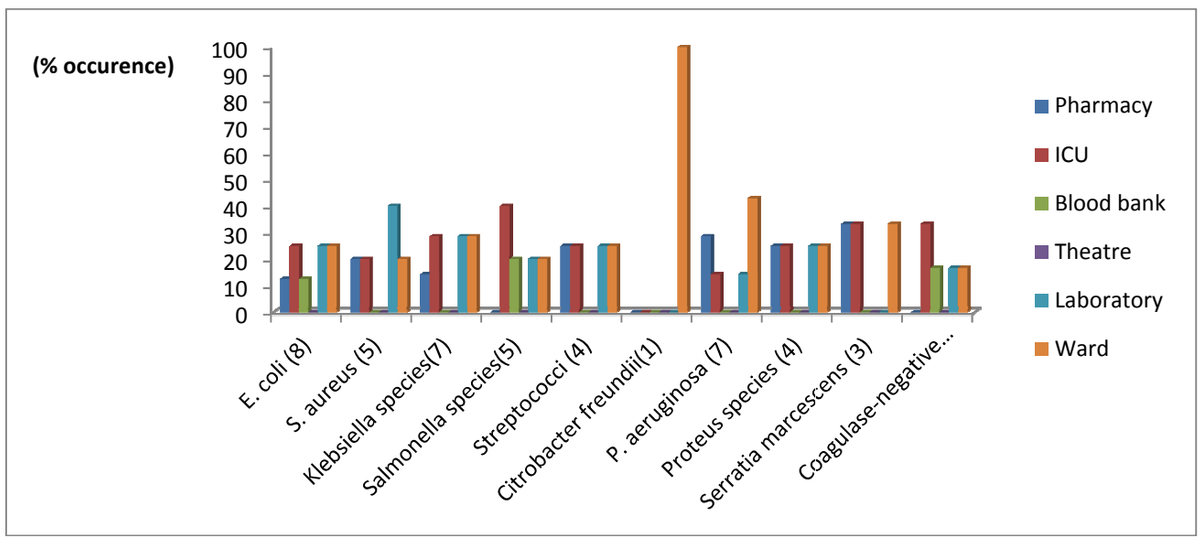

Fig. 4. Percentage frequency of bacteria recovered according to sampled units from IDH

From the laboratory coats of staff, the prevalence rate of 2.6-7.9% and 29.4-33.3% for Gram negative and positive bacteria, respectively were observed and was consistent with the report of Pydi et al. [27]. The presence of microbial contaminants in laboratory coats of staff from both hospitals points to the poor hygiene protocols of both hospitals. The most frequently isolated organisms from laboratory coats of healthcare staff of GH were Coagulase-negative *Staphylococci*, *S. aureus, Streptococci* and *E. Coli* while *S. aureus, Streptococci* and Coagulase-negative *Staphylococci* were from IDH. One of the most frequent isolates was *S. aureus* and this was consistent with previous reports [27,28].

Microbial quality of the air could be considered as a significant reflection of the hygienic condition of the hospitals. The prevalence rates of 5.3-15.9% and 16.7-17.6% for Gram negative and positive bacteria respectively were observed. The most frequently isolated organisms in GH was *Streptococci, S. marcescens, C. freundii* and *P. aeruginosa* while in IDH, *P. aeruginosa* and *K. pneumoniae* were more frequently isolated. The male wards of GH and IDH studied recorded the highest level of contamination of 23.7-28.0% with the most frequent isolates being *Klebsiella species*, Coagulase-negative *Staphylococci*, *Streptococci*, *Citrobacter freundii*, *E. coli and P. aeruginosa.* These were similar to the isolates reported by Luksamijarulkul [30].

The bacteria isolates in this study exhibited varied pattern of resistance to antibiotics. Generally, isolates showed extreme resistance to β-lactam antibiotics compared to other antibiotics classes including the quinolones, aminoglycosides, fluoroquinolones, macrolides, chloramphenicol and sulfamethoxazole+ trimethoprim. *Pseudomonas aeruginosa* isolated from GH exhibited resistance to ceftazidime, amoxicillin + clavulanic acid, amoxicillin and ampicillin, reflacine, ciprofloxacin and ceprorex while those from IDH showed resistance to amoxicillin, ceftazidime, ampicillin, ceporex and amoxicillin + clavulanic acid. The 85.7% - 100% resistance to ceftazidime observed in this study is higher than those earlier reported [31-34].

The 28.6 – 42.9% resistance of *P. aeruginosa* to gentamycin recorded in this study is moderately lower than the 47% and 69.4% reported earlier [33,34]. Similarly, 42.9–57.1% resistance to ciprofloxacin observed in this study is in accordance with the 61.2% reported by Du et al. [33] and 30% reported by Pitt et al. [33]). According to Chrinius et al. [23] multiple antibiotics resistance (MAR) indices give an indirect clue of the probable source (s) of the organisms. The multiple antibiotic resistance index range of 0.49-0.61 exhibited by *P. aeruginosa* employed in this study further confirms the fact that these isolates originated from the environment where antibiotics are often used since they possess an index higher than 0.20.

Strains of *K. pneumoniae* have caused dramatic therapeutic challenges globally as they are resistant to various antibiotics [35]. *K. pneumoniae* isolates from GH exhibited extreme resistance to ampicillin, amoxicillin, ceftazidime, sulfamethoxazole+trimethoprim and moderate resistance to reflacine, ciprofloxacin, gentamycin and ceprorex while those from IDH exhibited high resistance to ampicillin, ceftazidime, streptomycin and moderate resistance to amoxicillin, reflacine, ofloxacin, ciprofloxacin and amoxicillin + clavulanic acid. The resistance of *K. pneumoniae* to ceftazidime and ampicillin is consistent with an earlier report [36]. *K. pneumoniae* isolates from IDH exhibited 42.9% resistance to amoxicillin+ clavulanic acid and is consistent with 48.6% resistance previously reported [37-39]. The 42.9-50% resistance to ciprofloxacin observed in this study is slightly higher than 37% and 35% reported by Feizabadi et al. [35], Koksal et al. [37], Tlamcani et al. [38] and El-Bouamri et al. [39]. This confirms the assertion of Maina et al. [36] who observed that *K. pneumoniae* strains exhibit resistance to other unrelated antimicrobial agents in addition to hydrolysing β-lactam drugs. The 28.5 - 50% resistance of *K. pneumoniae* to gentamycin observed in this study is consistent with 33% and 40.5% reported by Feizabadi et al. [35] and Koksal et al. [37], respectively. However, it was lower than the 63.6% resistance reported by Maina et al. [36]. Consistently, the assertion that *K. pneumoniae employed* in this study were isolated from environments where antibiotics were often used was further confirmed by MAR index 0.51. *E. coli* strains recovered from GH exhibited extreme to moderate resistance to ceftazidime, amoxicillin, ampicillin, ceporex, ciprofloxacin, nalidixic acid, reflacine, amoxicillin+clavulanic acid and gentamycin, respectively. However, similar isolates recovered from IDH also showed extreme to moderate resistance to ampicillin, ceporex, amoxicillin, ceftazidime and sulfamethoxazole+trimethoprim. The 62.5% - 100% resistance to ceftazidime

observed in this study is higher than 19%, 28.8% and 54.60% recorded by Amaya et al. [40], Oteo et al. [41] and Akter et al. [42], respectively.

The 85.7% resistance of *E. coli* strains recovered from IDH to ampicillin observed in this study is also higher than the 19% observed by Amaya et al. [40] but consistent with the 86.5% resistance reported by Akter et al. [39]. The 40% resistance to amoxicillin+clavulanic acid observed in this study is moderately lower than the 59.9% resistance observed by Oteo et al. [41]. Furthermore, the 50% resistance of *E. coli* strains to ciprofloxacin observed in this study is somewhat higher than the 19% reported by Amaya et al. [40] but lower than the 60%, 69.3% and 89.3% resistance reported by Wang et al. [43], Akter et al. [42] and Oteo et al. [41], respectively. Consistently, as noted by Akter et al. [42], *E. coli* is one of the commonly isolated organisms from urinary tract infections, its resistance to commonly used antibiotics calls for concern. Meanwhile, the 40% resistance to gentamycin by *E. coli* strains is moderately higher than the 19% and 35.58% reported by Amaya et al. [39] and Akter et al. [42] but lower than the 70% reported by Oteo et al. [41]. The high resistance exhibited by this species was further confirmed by the high 0.46 – 0.60 MAR index range and low MIC and MBC observed in these isolates. The 60% susceptibility to gentamycin observed in this study is consistent with the 65.48% reported by Akter et al. [42]. Resistance in *E. coli* according to Kaye et al. [44] could be via plasmid mediated genes encoding hyper production of enzymes found either singly or in combination; conferring resistance to antimicrobial agents.

Proteus species recovered from GH recorded extreme to moderate resistance to ampicillin, ceftazidime, amoxicillin, sulfamethoxazole+ trimethoprim, ceporex, amoxicillin+clavulanic acid, streptomycin, gentamycin, ciprofloxacin, nalidixic acid and reflacine. Those from IDH also exhibited similar resistance to amoxicillin, ceftazidime, ampicillin, ceporex, sulfamethoxazole+trimethoprim, reflacine, nalidixic acid, gentamycin, ciprofloxacin and ofloxacin. The 75-83.3% resistance of *Proteus species* to ceftazidime observed in this study is slightly lower than the 90-100% reported by Pal et al. [45] but higher than the 64% reported by Kwiecinska-Pirog et al. [46] and the 41% reported by Al-Bassam and Al-Kazaz [47]. However, the resistance to ceftazidime observed in this study is contrary to reports of Cao et al. [48]. Harada et al. [49] and Nijssen et al. [50] who reported 70.2%, 95.3% and 100% susceptibility of *Proteus* isolates to ceftazidime. Furthermore, *Proteus* isolates in this study exhibited 100% resistance to ampicillin and this was higher than the 20% observed by Harada et al. [48] and the 45% reported by Aragon et al. [51]. The *Proteus species* employed in this study exhibited multiple antibiotic resistances cutting across β-lactam drugs, fluoroquinolones and aminoglycosides as is observable in the multiple antibiotic resistance indices of 0.60 and 0.48 recorded from GH and IDH, respectively. The 0.45 – 0.58 MAR index range exhibited by *Proteus* isolates observed in this study is further confirmed by the low MICs and MBCs. *Proteus species* were however, susceptible to amoxicillin+clavulanic acid, streptomycin, and ofloxacin.

C. freundii recovered from GH exhibited 100% resistance to ampicillin, 80% to ceftazidime, amoxicillin and gentamycin, 60% to ampicillin, sulfamethoxazole+trimethoprim, streptomycin and reflacine, 40% to ceporex and 20% to amoxicillin+clavulanic acid. Similar isolate from IDH exhibited 100% resistance to ceftazidime, ampicillin, amoxicillin, ceporex and sulfamethoxazole+trimethoprim. The resistance of *C. freundii* to ceftazidime observed in this study is consistent with report of Metri et al.[52]who recorded 76.4% but moderately higher than 50% reported by Pepperell et al. [53]. The resistance to ampicillin observed in this study is consistent with 75% reported by Tula and Iyoha [54] and the 96.4% reported by Metri et al. [52] The 100% resistance of *Salmonella species* observed in this study to ampicillin is consistent with the 96% and 94.78% reported by Agada et al. [55] and Elumalai et al. [56], respectively. Furthermore, the 80-88.9% resistance by *Salmonella species* to amoxicillin observed in this study is consistent with the 97.2% reported by Muhammad et al.[57] but higher than the 18.7% recorded by Rayamajhi et al. [58]. *Salmonella species* from GH recorded an MAR index of 0.53 while those from IDH recorded 0.58. The 0.53 – 0.58 multiple antibiotic resistance index exhibited by *Salmonella species* employed in this study further confirms the acquisition of plasmid mediated resistance to antimicrobial agents.

The *S. marcescens* isolated from GH showed extreme to moderate resistance to ampicillin, amoxycillin, ceftazidime, amoxicillin+clavulanic acid, ciprofloxacin, reflacine, streptomycin,

Table 4. MICs and MBCs of antimicrobial agents against bacteria recovered from GH (mg/ml)

Organisms	CPX		AU		APX		AMX		AM		FOX	
	A	B	A	B	A	B	A	B	A	B	A	B
P. aeruginosa (7)	1.56	3.13	0.98	1.95	NA	NA	6.25	12.50	25.00	50.00	NA	NA
K. pneumoniae (10)	0.78	1.56	0.49	0.98	NA	NA	1.56	3.13	6.25	12.50	NA	NA
Serratia marcescens(5)	0.78	1.56	1.95	3.91	NA	NA	3.13	6.25	6.25	12.50	NA	NA
E. coli (10)	0.78	1.56	0.49	0.98	NA	NA	1.56	3.13	3.13	6.25	NA	NA
Strept. Species (7)	0.39	0.78	NA	NA	0.20	0.39	0.20	0.78	0.20	0.39	NA	NA
C. freundii (5)	0.78	1.56	0.98	1.95	NA	NA	3.13	6.25	12.50	25.00	NA	NA
S. aureus (10)	1.56	3.13	0.98	1.95	1.56	3.13	1.56	3.13	3.13	6.25	1:32	1:16
Proteus species (6)	1.56	3.13	1:64	3.91	NA	NA	3.13	6.25	3.13	6.25	NA	NA
Salmonella species (9)	0.39	0.79	1:512	0.49	NA	NA	1.56	3.13	3.13	6.25	NA	NA
Enterobacter aerogenes (1)	0.78	1.56	1:256	0.98	NA	NA	1.56	3.13	1.56	3.13	NA	NA
Coagulase-negative Staphylococci (10)	0.39	0.78	NA	NA	0.78	1.56	1.56	3.13	1.56	3.13	230.00	470.00

KEY: A -Minimum inhibitory concentration, B-Minimum bactericidal concentration, NA-Not Applicable. CPX-Ciprofloxacin, AU-amoxicillin+clavulanic acid, APX- ampicillin+cloxacillin, AM-Ampicillin, FOX-Cefoxitin, AMX - Amoxicillin.

Table 5. MICs and MBCs of antimicrobial agents against bacteria recovered from IDH (mg/ml)

Organisms	AU		AU		APX		AMX		AM		FOX	
	A	B	A	B	A	B	A	B	A	B	A	B
E. coli (8)	1.56	3.13	0.98	1.95	NA	NA	3.13	6.25	6.25	12.50	NA	NA
S. aureus (5)	1.56	3.13	0.98	1.95	1.56	3.13	3.13	6.25	3.13	6.25	470.00	938.00
Klebsiella species (7)	0.78	1.56	1.95	3.91	NA	NA	3.13	6.25	6.25	12.50	NA	NA
Salmonella species (5)	0.78	1.56	0.49	0.98	NA	NA	1.56	3.13	3.13	6.25	NA	NA
Strept. Species(4)	0.39	0.78	NA	NA	0.20	0.39	0.39	0.78	0.39	0.78	NA	NA
C. freundii (1)	1.56	3.13	0.98	1.95	NA	NA	3.13	6.25	12.50	25.00	NA	NA
P. aeruginosa (7)	1.56	3.13	0.98	1.95	NA	NA	6.25	12.50	6.25	12.50	NA	NA
Proteus species (4)	1.56	3.13	3.91	7.81	NA	NA	3.13	6.25	3.13	6.25	NA	NA
S. marcescens (3)	0.78	1.56	1.95	3.91	NA	NA	3.13	6.25	6.25	12.50	NA	NA
Coagulase-negative Staphylococci(6)	0.78	1.56	NA	NA	1.56	3.13	1.56	3.13	1.56	3.13	470.00	230.00

KEY: A --Minimum inhibitory concentration, B--Minimum bactericidal concentration, NA--Not Applicable. CPX- Ciprofloxacin, AU- amoxicillin+clavulanic acid, APX- ampicillin+ cloxacillin, AM-Ampicillin, FOX-Cefoxitin, AMX - Amoxicillin.

nalidixic acid, ampicillin, ofloxacin, gentamycin, ceporex and sulfamethoxazole+trimethoprim. Meanwhile, isolates from IDH recorded similar resistance to ampicillin, ceporex, ceftazidime, amoxicillin, sulfamethoxazole+trimethoprim, reflacine, amoxicillin+clavulanic acid, gentamycin and ofloxacin. The 66.7 – 80% resistance to ceftazidime observed in this study is consistent with the 67.2% reported by Sethuraman et al. [59]. However, it is contrary to reports of Shih et al. [60] and Liou et al. [61] who reported 99% and 93.8% susceptibility of *S. marcesens* to ceftazidime. The 20-40% resistance to aminoglycosides (gentamycin and streptomycin) by *S. marcescens* observed in this study is moderately lower than the 74.1% reported by Sethuraman et al. [59] and consistent with the 40.4% resistance observed by Liou et al. [61].

The high resistance of *E. aerogenes* to ceftazidime observed in this study is consistent with reports of Mordi and Hugbo [62]. *Streptococci* exert varying degrees of resistance to antimicrobial agents. *Streptococci* isolated from GH exhibited 71.4% resistance to chloramphenicol, 42.9% to erythromycin and 28.6% resistance to levofloxacin, norfloxacin, streptomycin and gentamycin. Similar isolates from IDH exhibited 75% to chloramphenicol, 50% to erythromycin and norfloxacin. In addition, *Streptococcal* isolates employed in this study were susceptible to all β-lactam drugs including ampicillin, ampicillin+cloxacillin and amoxicillin.

The sensitivity of *Streptococci* employed in study to β-lactam drugs is consistent with reports of Cifti et al. [63] and Khan et al. [64] who observed in their studies that *Streptococci* isolates were susceptible to penicillin and cephalosporins. Furthermore, isolates in this study, exhibited 71.4 – 75% resistance to chloramphenicol. In addition, the 71.4%-75% resistance of *Streptococci* to chloramphenicol observed in this study is extremely higher than the 30% reported by Boswihi et al. [65]. Furthermore, the 42.50% resistance of *Streptococci* to erythromycin

observed in this study is somewhat higher than the 12.6% reported by Boswihi et al. [65] and extremely higher than the 3.8% reported by Cifti et al. [62]. Widdowson and Klugman [66] have suggested that resistance of *Streptococcus species* to macrolides could be due to modification of the ribosome via methylation of an adenine residue in domain V of the 23S rRNA coupled with the efflux mechanism of antibiotics from the cell usually encoded by *mefC*.

S. aureus species recovered from GH exhibited 90% resistance to ampicillin, 80% to cefoxitin, 70% to ampicillin+cloxacillin and amoxicillin, 60% to chloramphenicol and gentamycin, 40% to levofloxacin, erythromycin and streptomycin and 30% to norfloxacin and ciprofloxacin. Similar isolates from IDH exhibited 100% resistance to ampicillin and cefoxitin, 80% to amoxicillin, 60% to chloramphenicol, gentamycin and 20% to levofloxacin and ciprofloxacin. The 90-100% and 70-80% resistances to ampicillin and amoxicillin, respectively observed in this study are consistent with the 89.6% reported by Umeuzoke and Aritiatu [67]. Consistently, resistance of *S. aureus* as noted by Pantosti et al. [68] occurs via a variety of mechanisms; one of which is the production of β-lactamases which inactivate the β-lactam drugs, rendering them ineffective. Resistance of *S. aureus* strains to penicillins heralded the use of the synthetic antibiotic such as methicillin developed to treat penicillin-resistant *S. aureus* infections. Sadly, resistance was developed to this antibiotic [69]. In this study, *S. aureus* strains exhibited 80-100% resistance to cefoxitin. Cefoxitin from reports has the potential to replace oxacillin for phenotypic detection of *mecA* mediated resistance because it is a more powerful inducer of the system that regulates *mecA* [70,71]. Currently, the increase in resistance of *S. aureus* to methicillin has become an epidemiological and clinical challenge most especially because resistance to this antibiotic implies resistance to all β-lactam antibiotics [72]. The resistance observed in this study against fluoroquinolones could be due majorly to spontaneous mutation and positive selection among these isolates. Pantosti et al. [68] observed that efflux pumps in *S. aureus* evade most fluoroquinolones and tetracyclines. Resistance of 30-60% was exhibited by *S. aureus* against aminoglycosides. In addition, the 40% resistance of *S. aureus* to erythromycin observed in this study is consistent with reports of Umeuzoke and Aririatu [67] who reported 66.7% susceptibility of these isolates to erythromycin. The findings in this study confirmed the significant contamination of air and surfaces with bacteria including multidrug resistant strains and the need for the adoption of objective monitoring systems and procedures as stipulated in the 2010 CDC monitoring tool kit reported by Russotto et al. [73].

5. CONCLUSION AND RECOMMENDATIONS

This study revealed the significant contribution of airborne sources and inanimate surfaces of hospitals as active agents of nosocomial infections. Furthermore, some of these microorganisms showed multidrug resistance. Bacterial contamination of hospital air and surfaces could influence hospital-associated infections especially where cross contamination is effective. However, further research to evaluate this relationship is advocated. Alert on the possible cross contamination from air and surfaces to patients could reduce considerably, the rates of nosocomial infections reported globally.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Ethical approval for this study was gotten from the Cross River State Health Research Ethics Committee (CRS-HREC) with approval number (RP/REC/2015/338).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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