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The Prevalence and Antibiotic Resistance Pattern of Gram-Negative Pathogens Isolated from Inanimate Hospital Sources in A Maternity Centre in Lagos State, Nigeria

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ABSTRACT

Background: Antibiotic resistance Gram-negative bacteria are becoming responsible for the increased morbidity and mortality, particularly in hospitalized patients. With the increasing use of electronic healthcare, shared use of medical equipment and imperfect hand hygiene, the role of frequently touched environmental surfaces for the potential dissemination of these resistant organisms is now of greater importance, posing a real challenge to conventional infection-control practices. We sought therefore to determine the prevalence and antibiotic resistance pattern of Gram-negative pathogens from inanimate hospital sources. **Methods:** Environment swab samples were collected from Lagos Island maternity hospital for the isolation of Gram-negative pathogens. Antibiotic susceptibility test, multiple antibiotic indexing, phenotypic and PCR genotypic confirmation of ESBL, carbapenemase gene and plasmid profiling were used to detect and determine the resistance pattern of these isolates. **Result:** A total prevalence of 35(40.2%) Gram-negative pathogens were recorded in this study. Among the isolates, 33(94.3%) were resistant to ceftriaxone and 23(65.7%) resistant to cefepime, 19(54.3%) were resistant to ciprofloxacin and 21(60%) were resistant to ofloxacin. Absolute resistance of 100% was observed among gentamicin, tigecycline and ampicillin. The carbapenems showed the least resistance where 15(42.9%) were resistant to imipenem, 11(31.4%) doripenem and 10(28.6%) to meropenem. A similar trend of a very high multiple antibiotics resistance (MAR) index was observed among the isolates from all sampling areas. Out of the 35 isolates, 20 (57.1%) were identified as ESBL producers while 4 (11.4%) phenotypically emerged as carbapenamase producers. Genotypically 4 (20%) carried the blaCTX-M gene and 1 (5%) carried blaTem gene while out of 6 *Pseudomonas aeruginosa*, 2(33.3%) carried Integron 1 gene. All isolates showed no carbapenamase gene while 11(55%) showed the presence of plasmid with a high band size of 23130kbp.**Conclusion:** This study established the detection of the extended-spectrum beta-lactamase (ESBL) gene indicating an increase in the rate of antibiotic resistance in Gram-negative bacteria and their occurrence in inanimate hospital environments.

INTRODUCTION

The history of nosocomial infections can be traced to the origin of hospitals themselves and have been defined by the World Health Organization as infections that develop in a patient during his/her stay in a hospital or other types of clinical facilities which were not present at the time of admission (Mbim *et al.,* 2016). It is a major global safety concern for both patients and healthcare professionals, these infections, often caused by multi-resistant pathogens, take a heavy toll on patients and their families by causing illness, prolonged hospital stay, potential disability, excess costs and sometimes death (Rocha *et al.,* 2015). The widespread use of antibiotics continues to influence this menace giving rise to antibiotic-resistant bacteria in the hospital setting and in the environment. The environment of the hospital is an obviously important focus for the selection and spread of multi-resistant bacteria and a possible direct source of nosocomial infections. Gram-negative bacteria are increasingly becoming responsible for increased morbidity and mortality, particularly in hospitalized patients (Vaibhavi and Sudhir, 2016). Antibiotic-resistant Gram-negative bacteria are a major public health threat, increasing in prevalence globally in both community and institutional settings, the perceived hazards of gram-negative antimicrobial resistance stem from two interlinked concern which includes diminishing therapeutic options for the treatment of infections caused by these bacteria and the potentially greater negative impact on both clinical outcomes and healthcare costs. Gram-negative pathogens of particular importance include extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae, *Acinetobacter baumannii* and *Pseudomonas aeruginosa*, these organisms are also major pathogens in hospitals, where the prevalence of bed rails, the bed surface, and the supply cart, on the basis of their observed frequency of contact (Huslage *et al.,* 2010). Developing an understanding of which sites are more likely to be contaminated with pathogens can guide infection control practices and direct new innovations. Environmental contamination with resistant organisms assumes greater importance when patients are managed in wards with shared facilities, as bacterial contamination of near-patient surfaces, computers and medical equipment has been demonstrated in healthcare environments (Brady *et al.,* 2009; Po *et al.,* 2009). With the increasing use of electronic healthcare, shared use of medical equipment, and imperfect hand hygiene, the role of frequently touched environmental surfaces for the potential dissemination of multidrug resistance organisms is now of greater importance (Thean *et al.,* 2013). As the struggle in the management of nosocomial infections deepens worldwide due to the varying but increasing resistance pattern of the organisms, the regional and geographical variations in their prevalence and antimicrobial susceptibility patterns, constant screening of resistant genes in nosocomial gram-negative organisms in hospital equipment and fomites will be of greater importance in tackling or improving our approach to management or treatment of nosocomial infection. The study was therefore set out to determine the prevalence of ESBL and carbapenemase-producing Gram-negative pathogens from inanimate hospital sources, establish the antibiotic profile and the MAR index as well as determine the plasmid profile of the isolates.

MATERIALS AND METHODS

Study Centre:

Lagos Island Maternity Hospital. The choice of sampling site was based on the fact that it is a specialist hospital owned by Lagos State Government, which caters to all aspects of Obstetric and Gynaecological problems. It is also a referral secondary centre for many private Hospitals, other Lagos State Government Hospitals and also tertiary Institutions from Lagos environs and Other States. It has the highest number of obstetric and gynaecological patients in Lagos state, due to the high number of specialist consultants and because they offer quality services at a very subsidized and affordable

rate. It is a very busy hospital that at each point in time has more than 100 patients on admission, therefore standardization and hygiene control in this setting can be compromised thereby contributing to the transmission of nosocomial pathogens which can escalate the already worrisome maternal and infant mortality in Nigeria.

Sample Collection:

A total number of 112 samples were collected from the hospital in February 2017 based on a non-probability sampling method known as the convenience sampling method. Samples were collected from Intensive Care Units, emergency rooms, theatres, labour rooms and four different wards: the surgical ward, and the paediatric ward. Environmental swabs samples were collected from an inanimate area of approximately 10 cm^2 (patients' beds, door handles, patients' tables, treatment trolley, tap and sink, infusion stand, humidifier, stretcher, kidney dish, galliput, bed railings, treatment bench-top, drugs tray, floors, and bed covers) by means of a sterile swab moistened with sterile saline water. The swabs were labelled and then transported immediately to the University of Lagos Microbiology Department laboratory for analyses within one hour of collection.

Preliminary identification was based on colonial morphology, Gram staining and oxidase test. Confirmation of the isolates was done using Microbact 12E identification system.

Antibiotic Susceptibility Test and Multiple Antibiotic Resistances (MAR) indexing:

All the isolates obtained were standardized to 0.5 McFarland turbidity standard. The peptone water was inoculated with the test organism and incubated at 37°C for 18hrs. The antibiotic profile of the isolates was carried out by Kirby Bauer's disk diffusion method (Bauer *et al*., 1967). Antibiotic susceptibility testing was done using the following antibiotics: ceftriazone 30µg, ciprofloxacin 5µg, tigecycline 15µg, imipenem 10µg, ofloxacin 5µg, doripenem 10µg, amoxicillin 30µg, meropenem 10µg, cefepime 30µg and gentamicin 10µg.MAR index for test isolates was calculated according to the formula:

No. of antibiotics to which all isolates were resistant/No. of antibiotics tested x No. of isolates as recommended by Downing *et al*., (2011).

All the Gram-negative bacterial strains were subjected to various phenotypic and genotypic methods for the screening and confirmation of the beta-lactamases present in the isolated Gram-negative strains.

Phenotypic Characterization:

• ESBL- Phenotypic screening of extended-spectrum beta-lactamases by the double-disk synergy test (DDST). The inoculum was swabbed onto the Mueller-Hinton agar plate. An amoxycillin with the clavulanic acid disc was placed in the centre of the plate and one cefotaxime disc and ceftazidime disc were placed at a distance of 20 mm (Centre to centre) from the amoxicillin with clavulanic acid disk.

• Carbapenemase- Phenotypic detection of carbapenemases by imipenem-ethylene diamine tetra acetic acid combined disk test**.** One disc of imipenem (10µg) alone and one with imipenem (10µg) in combination with Ethylene diamine tetra acetate (EDTA) solution were placed at a distance of 20 mm, from centre to centre, on a Mueller Hinton agar plate inoculated with a bacterial suspension of 0.5 McFarland turbidity standards of each Gram-negative bacterial isolate.

Genotypic Confirmation and Characterization of The Beta-Lactamase Genes in The Gram-Negative Strains:

Resistant genes responsible for beta-lactamase production were detected in the bacteria strains genotypically by Multiplex PCR. The prevalence of ESBLs and Carbapenemes among these isolates was studied using specific primers.

RESULTS

A total of 112 swab samples were collected from the hospital environment and analyzed for the prevalence of Gram-negative pathogens, their antibiogram, multidrug resistance indices, and plasmid profiling and were screened for bla_{SHV}, bla_{TEM}, bla_{CTX}, IMP

and INTEGRON 1 resistance genes. Out of 87 bacteria isolated, 35(40.2%) were confirmed as Gram-negative organisms which included 6(17.1%) *Pseudomonas aeruginosa*, 5(14.3%) *Proteus mirabilis, Citrobacter koseri, Salmonella spp* with 4(11.4%) each, *Acinetobacter baumanii, Enterobacter aerogenes and Hafnia alvei* having 3(8.6%) each, *E.coli, Klebsiella ormithinolytica, Serratia liquefaciens and Providencia rettgeri* with the lowest prevalence showing 1 (2.9%) (Table 1).

Table 2, shows the distribution of the pathogens from different sampling areas. The antibiotic sensitivity profile was interpreted according to CLSI (2016). The isolates showed varying resistance, among the cephalosporin 33(94.3%) were resistant to ceftriaxone and 23(65.7%) were resistant to cefepime, among the fluroquinolones 19(54.3%) were resistant to ciprofloxacin and 21(60%) were resistant to ofloxacin. Absolute resistance of 100% was observed among gentamicin, tigecycline and ampicillin. The carbapenems showed the least resistance where 15(42.9%) were resistant to imipenem, 11(31.4%) to doripenem and 10(28.6%) to meropenem. Commonly clinically relevant pathogens like *Acinetobacter baumaniii* showed high resistance to tigecycline (100%), ceftriaxone (65%), augmentin (100%), gentamicin (100%) and 33% resistance to cefepime, ciprofloxacin and ofloxacin while *Pseudomonas aeruginosa* showed resistance to ciprofloxacin (83.3%), imipenem, doripemen and meropenem (50%) each and 100% resistance to tigecycline, ceftriaxone, Augmentin and gentamicin (Table 3).

The multiple antibiotics resistance indexing based on isolates from different sampling points was also calculated and a similar trend of MAR Index was observed among the isolates from all sampling sites, where they all showed a MAR index above 0.2, the high-risk sources showing the highest MAR index were represented in isolates from floor and humidifier 0.9 and 0.8 respectively as described in Table 4.

Table 1: Distributions and frequency of isolates

S/NO	Oxidase	Motility	Nitrate	Lysine	Ornithine	H_2S	Glucose	Mannitol	Xylose	ONPG	INDOLE	Urease	V-P	Citrate	TDA	Gelatin	Malonate	Insositol	Sorbitol	Rhamnose	Sucrose	Lactose	Arabinose	Adonitol	Raffinose	Salicin	Arginine	Organism Isolated	Frequency
1	+	+	+	+	+	-	+	+	+	+	+	+	-	+	-	+	+	-	+	+	+	+	+	-	-	-	+	*Citrobacter koseri*	*4*
2	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	+	+	+	-	+	+	+	+	+	+	+	+	*Klebsiella ornithinolytica*	*1*
3	+	+	+	+	-	+	+	-	+	-	-	+	-	+	-	+	+	-	-	-	-	-	+	-	-	-	+	*Acinetobactacter baumannii*	*3*
4	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	-	-	-	+	+	+	-	-	-	-	*Serratia liquefaciens*	*1*
5	+	+	+	+	+	+	+	-	+	+	-	+	-	+	-	+	-	-	-	-	-	-	-	-	-	-	+	*Proteus mirabilis*	*4*
6	+	+	+	+	-	-	-	-	+	+	-	+	-	+	-	+	+	-	-	-	-	-	-	-	-	-	+	*Pseudomonas aeruginosa*	*6*
7	+	+	+	+	+	+	+	+	+	+	-	+	-	+	+	+	+	+	-	-	-	+	+	-	-	-	+	*Salmonella spp*	*4*
8	+	+	+	+	-	-	+	+	+	+	+	+	+	+	-	+	+	+	+	-	-	-	+	+	-	-	-	*Providencia rettgeri*	*1*
10	+	+	+	+	+	+	-	-	-	+	+	+	+	-	+	+	+	+	-	-	-	+	+	-	-	-	+	*Hafniaalvei*	*3*
11	-	-	-	-	+	-	+	+	+	+	-	-	+	-	-	+	+	+	-	-	-	+	+	-	-	-	+	*Enterobacter americana*	*2*
12	-	+	-	+	+	+	+	+	-	+	-	-	+	-	-	+	+	-	-	-	-	-	-	-	-	-	+	*Klebsiella pneumoniae*	*2*
13	-	+	+	+	+	-	+	-	+	+	-	+	-	+	-	-	-	+	+	-	+	+	+	-	-	-	+	*Escherichia coli*	*1*
14	+	+	+	+	+	-	-	-	+	+	+	+	+	+	+	+	+	-	-	-	-	+	+	-	+	-	+	*Enterobacter aerogenes*	*3*
																												Total	**35**

Table 2: Percentage occurrence of Gram-negative organisms isolated from sampling areas.

Sampling Area	Frequency	Organism Isolated	Percentage(%)
Emergency	7	*Proteus mirabilis* *Pseudomonas aeruginosa* *Citrobacter koseri* *E.coli* *Acinetobacter baumanii*	20
Labour room	2	*Klebsiella pneumonia* *Acinetobacter baumanii*	5.7
Wards	14	*Serratia liquafaciens* *Pseudomonas aeruginosa* *Salmonella spp* *Klebsiella ornithionolytica* *Proteus mirabilis* *Enterobacter americana*	40
Consulting room	5	*Proteus mirabilis* *Klebsiella pneumonia* *Salmonella spp* *Hafnia alvei* *Enterobacter aerogenes*	14.3
ICU	0		
Theatre	7	*Pseudomonas aeruginosa* *Acinetobacter baumanii* *Citrobacter koseri* *Proteus mirabilis* *Providentia rettgeri*	20
Total	**35**		

Table 3: Antibiotic profile of the isolates from Lagos Island maternity.

Antibiotics		***Pseudomonas aeruginosa (n=6)***	***Citrobacterkoseri n=4)***	***Proteus mirabilis (n=5)***	***Acinetobacterbaumannii n=3)***	***Klebsiellapneumoniae n=2)***	***Enterobacteraerogenes n=3)***	***Escherichia coli n=1)***	***Salmonella spp n=4)***	***Providenciarettgeri n=1)***	***Hafniaalvei n=3)***	***Serratialiquafaciens n=1)***	***Klebsiellaornithinolytica n=1)***	***Enterobacterameri cana n=1)***
Ciprofloxazin	R S	5(83.3%)	3(75%) 1(25%)	2(40%) 3(60%)	2(66.7%)	1(50%)	2(66.7%) 1(33.3%)	1(100%)	3(75%)	1(100%)	2(66.7%)		1(100%)	1(100%)
Tigacycline	R S	6(100%)	4(100%)	5(100%)	3(100%)	1(50%)	3(100%)	1(100%)	4(100%)	1(100%)	3(100%)		1(100%)	1(100%)
Imipenem	R S	1(16.7%) 1(16.7%)	2(50%) 1(25%)	2(40%) 2(40%)	2(66.7%)	1(50%)	- -	1(100%)	2(50%)	1(100%)	2(66.7%) 1(33.3%)		1(100%)	1(100%)
Ceftriazone	R S	6(100%)	4(100%)	5(100%)	2(66.7%) 1(33.3%)	2(100%)	3(100%)	1(100%)	4(100%)	1(100%)	39(100%)	1(100%)	1(100%)	1(100%)
Meropenem	R S	1(16.7%) 5(83.3%)	1(25%) 3(75%)	1(20%) 4(80%)	3(100%)	2(100%)	3(100%)	1(100%)	1(25%) 3(75%)	1(100%)	1(33.3%) 2(66.6%)	1(100%)	1(100%)	1(100%)
Augumentin	R S	6(100%)	4(100%)	5(100%)	3(100%)	2(100%)	3(100%)	1(100%)	4(100%)	1(100%)	4(100%)	1(100%)	1(100%)	1(100%)
Gentamicin	R S	6(100%)	4(100%)	5(100%)	3(100%)	2(100%)	3(100%)	1(100%)	4(100%)	1(100%)	3(100%)	1(100%)	1(100%)	1(100%)
Oflozaxin	R S	5(83.35)	2(50%) 2(50%)	3(60%) 2(40%)	3(100%)	2(100%)	2(66.7%) 1(33.3%)	1(100%)	4(100%)	1(100%)	2(66.7%) 1(33.3%)	1(100%)	1(100%)	1(100%)
Doripenem	R S	1(16.7%) 5(83.3%)	1(25%) 3(75%)	3(60%) 2(40%)	2(66.7%)	2(100%)	2(66.7%) 1(33.3%)	1(100%)		1(100%)	1(33.3%) 1(33.3%)	1(100%)	1(100%)	1(100%)
Cefepime	R S	5(83.3%) 1(16.7%)	2(50%) 1(25%)	3(60%)	3(100%)	1(50%) 1(50%)	2(66.7%) 1(33.3%)	1(100%)	3(75%)	1(100%)	3(100%)		1(100%)	1(100%)

Table 4: Multiple antibiotics resistance (MAR) indices for the isolates from different sampling points.

S/N	Sampling points	No of antibiotics	No resistance isolates	MAR Index
1	Bed railings	30	20	0.7
2	Bed covers	60	40	0.7
3	Equipment	140	89	0.6
4	Humidifier	10	8	0.8
5	Door handles	0	0	0
6	Sink	70	38	0.5
7	Floor	40	37	0.9

With susceptibility patterns exhibited by isolates, ESBL and Carbapenemase producers were phenotypically detected. ESBL-producing Gram-negative pathogens showed synergism between clavulanic acid (AUG, 30 µg) and cefotaxime while carbapenemase producers showed a 7mm higher difference in EDTA imipenem to imipenem. Out of the 35 isolates, 20(57.1%) were identified as ESBL producers while 4(11.4%) phenotypically emerged as carbapenamase producers. *Acinetobacter baumanii*, *Citrobacter koseri* and *P. mirabilis* expressed the highest resistance genes (ESBLand carbapenamase) amongst all isolates. Out of the 20 ESBL-producing Gram-negative bacteria detected, 4 (20%) showed co-resistance. Among these, 2 were identified as *Proteus mirabilis* 2(50%) was the most frequently isolated co-resistant pathogen followed by, *Acinetobacter baumanii*1 (25%) and *Citrobacter koseri1* (25%).

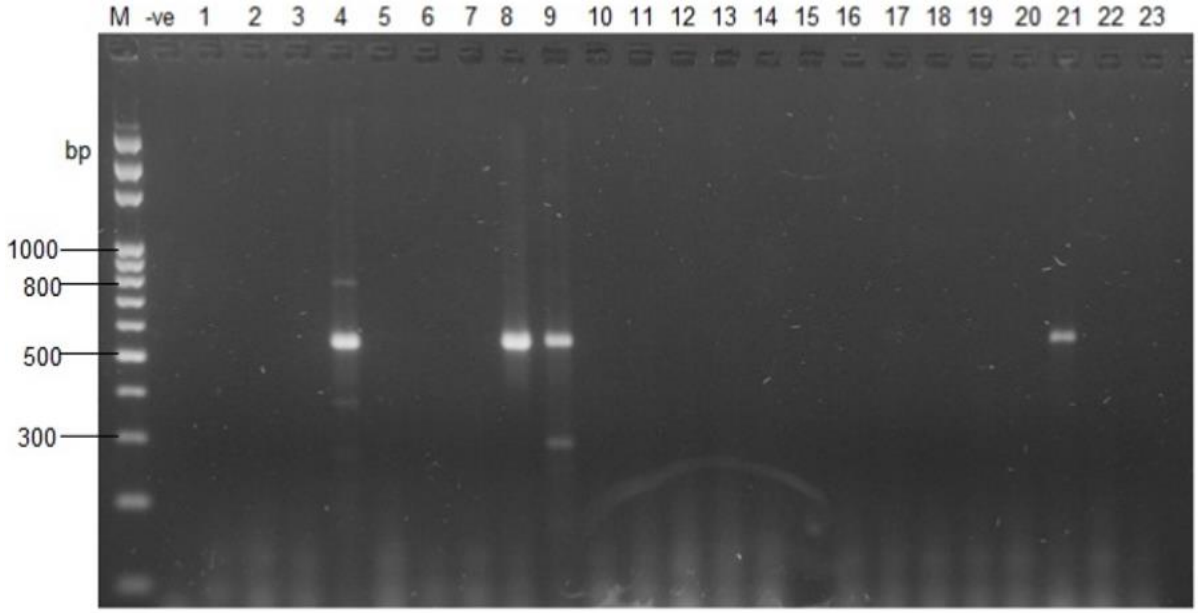

Fig. 1: Gel electrophoresis showing multiplex PCR products after amplification with specific primers for bla_{SHV} (747bp), bla_{Tem} (822bp) and bla_{CTX-M} (543bp), lane 8, 9 and 21; positive for bla_{CTX-M}. Lane M, molecular marker; lane –ve, negative control.

Genotypic detection of ESBL, IPM and INTEGRON 1 gene by PCR amplification was carried out on the twenty isolates that showed positive phenotypically. Out of which 4(20%) carried bla_{CTX-M} gene and 1 carried the bla_{Tem} gene. The highest genotypic detection was shown in *Proteus mirabilis* which carried both bla_{CTX-M} and bla_{Tem} genes. The genes encoding CTX-M were the most common showing on 4 isolates, while out of the 6 *Pseudomonas aeruginosa*2(33.3%) carried integron 1 gene. All twenty isolates showed no carbapenamase gene (Fig1). The plasmid profiling revealed that out of the twenty isolates, 11(55%) showed a single plasmid with the same band size of 23130kp

DISCUSSION

A prevalence of 40.23% nosocomial Gram-negative pathogen was observed in this study. Elsewhere, contamination has been documented to occur on fomites but in varying degrees. The contamination rate from this study was similar to the 38.6% reported by Temitope and colleagues (2014). This could be attributed to similarities of the studied items, while Ckikere and Omoni (2008) reported a much lower prevalence of 19.64%. However, a higher rate of 47.8% was recorded by Maryam and co-researchers (2014). The contamination of fomites in this study could be attributed to some irregular practices observed during sample collection.

In the hospital wards, it was observed that water containing detergent were used irregularly to clean door knobs and tables, while the IV stands were not cleaned at all. The bed covers were only changed frequently by patients that can afford it but the general hospital bed covers are changed rarely, this was also noticed in the ICU. The cloths and mops used in cleaning are however not adequately cleaned and disinfected, as most often, the water containing the disinfectant was not changed until the cleaning was completed. Poor hygiene was also noticed in the labour rooms, which is contrary to the recommended WHO nosocomial infection control guidelines. It has also been shown that if the water-disinfectant mixture used in cleaning is not changed regularly, the mopping procedure actually can spread heavy microbial contamination throughout the healthcare facility (Mangicaro, 2012). Although surface cleaners play a role in reducing the spread of infections, not all disinfectants work equally well at killing all pathogens (Boskey, 2011), some pathogens are more susceptible to specific detergents than others. Therefore, bacterial contamination of fomites in this study reflects a regular daily risk of exposure to several hospital-acquired infections.

The percentage distribution of the Gram-negative bacteria reported in this study correlated with a report by Emmanuel (2012), Temitope and colleagues (2014) that also reported a similar array of Gram-negative bacteria. The isolation of these Gram-negative bacteria especially the clinically relevant ones like *Acinetobacter baumanii* and *Pseudomonas aeruginosa* is quite worrisome. These organisms survive for months on hospital equipment or surfaces thereby acquiring more resistance, their presence on various ward surfaces and instruments could therefore provoke severe infections in the form of wound contamination following surgical procedures, or those associated with catheterization such as urinary tract complications that may well persist as a result of bio-film formations in urogenital organs. The presence of *Proteus, K. pneumoniae and E. coli* which are enteric bacteria, was indicative of fecal pollution and poor personal hygiene especially irregular hand-washing practices among healthcare workers that handle most equipment. Also, the presence of *Acinetobacter baumanii* is worrisome because this organism has been shown to be multidrug-resistant and has the ability to survive on dry surfaces for a long time while maintaining its ability to multiply and infect. While the presence of *P. aeruginosa* is of clinical importance because it has been shown to inhibit non-sterile areas in healthy individuals and can also infect tissues, especially in immune-compromised patients (Cogen *et al.,* 2008; Iwatsuki *et al.,* 2016).

Furthermore, the antibiotic sensitivity result of these organisms indicated a high resistance to major antibiotics, all isolates presented resistance to most readily available and affordable antibiotics like gentamicin, tigecycline and ampicillin with 100% resistance and quinolones with 54.3% resistance to ciprofloxacin and 50% were resistant to ofloxacin. Similar to this Seguija and co-researchers (2016) reported 70% gentamicin and 90% ciprofloxacin resistance against Gram negative isolate. Most resistance to ciprofloxacin and other quinolones may evolve rapidly even in the course of treatment and as a result of its widespread use to treat minor infections readily, this is a cause for concern because many clinicians fall back on the quilonones for the treatment of Gram-negative pathogen in the face of multi-drug resistance (Akujobi and Chika, 2010). The most sensitive antibiotics to these isolates were carbapenem with 68.6% sensitive to meropenem, 22.9% to imipenem and 40% to doripenem, which was similar to a report by Seguija*and* colleagues(2016) that the most effective antibiotics against enterobacteriaceae (*P. mirabilis*, *E. coli* and *K. pneumoniae*) were imipenem and ertapenem with a sensitivity rate of 100%, this also correlated with report Balan and colleagues (2013) stating that *E. coli* was highly sensitive to imipenem (78.3%) and *Klebsiella pneumoniae* was highly

sensitive to imipenem (76%). Although sensitive, carbapenems antibiotics are one of the most expensive antibiotics and are also toxic to the body system, thus not affordable by most patients and if overused can further compromise the health of the patient. The result of the multiple antibiotic resistance index (MAR) of these isolates in relation to their sampling points showed a high MAR index which was above 0.2, thus the organisms were considered to have originated from high-risk sources where antibiotics are often used, this was similar to MAR index recorded in the university of Ilorin teaching hospital (Rasak, 2001). High-risk sources showing the highest MAR index were represented in isolates from floor and humidifiers at 0.9 and 0.8 respectively. Increasing their resistance concern was the genotypic detection of bla_{CTX-M}, bla_{Tem}, and the presence of integron 1 resistance cassette genes, which can further stem the increasing prevalence of nosocomial infection. In this study *Proteus mirabilis* was the highest ESBL producer encoding both bla_{CTX-M} and bla_{Tem} gene, ESBL production of this organism has been reported in several epidemiological settings, with a percentage that exceeds 20% of the same area. This trend is a matter of serious concern since *P. mirabilis* is a common cause of human infections and account for approximately 3% of nosocomial infection while its ESBL-producing strains are usually resistant to several antibiotic agents and can results too difficult to treat infections (Luzzaro, 2000). Also, the emergence and proliferation of multidrug-resistant *P.mirabilis* could pose a threat, especially in catheterized patients with malignancy as a cause of subsequent nosocomial infection (Nagano *et al*., 2003). Of optimum concern also was *Pseudomonas aeruginosa* which was isolated from an emergency room humidifier with a very high MAR index and also a carrier of the integron 1 resistance gene. This is of optimum concern because *Pseudomonas aeruginosa* is a major pathogen of Ventilator-associated pneumonia majorly associated with mechanical ventilation. It occurs in approximately 10 to 20% of patients, who are on ventilators for longer than 48 hours and is associated with significant increases in length of hospital stay, mortality, and costs (Anton *et al.*, 2010). Also, the integron which serves as a mobile genetic element plays an important role in the dissemination of resistance genes among bacteria, genes carried by integrons usually encode multiple resistance mechanisms such as beta-lactams, aminoglycosides and other antimicrobial agents (Elbourne and Hall, 2006).

The increasing prevalence of ESBL in the health care system is probably caused by the high or inappropriate use of antibiotics in combination with the spread of bacteria between patients and of resistance genes between bacteria. Thus, twenty phenotypically positive for ESBL were also checked for the presence of plasmid, out of which 11(55%) of the isolates possessed plasmid which connotes the transfer of genes among these organisms, thereby increasing the resistance. Similarly, Rasak, 2001 reported that all isolates tested for the presence of plasmid (61.2%) were positive. The relationship between multiple antibiotic resistance patterns and plasmid profiles observed generally suggests the significant role of plasmids in the development of multidrug resistance in bacteria species. A comparison of plasmid sizes and numbers showed that all the isolates have the same plasmid sizes which indicated that they are of the same origin that is from the hospital environment. The widespread antibiotic resistance observed in the isolates that possessed plasmids can be associated with the increased and abusive use of antibiotics. Thus, the presence of plasmid in these resistant organisms can serve as a threat to a patients' health and can also increase genetic transfer thereby fueling the prevalence of nosocomial infection. A limitation of this study was that the direct involvement of these inanimate sources in disease transmission was not investigated due to the non-compliance of the healthcare workers. However, numerous authors have reported health-associated infections from various global regions

(Allergranzi *et al.,* 2012; Madani *et al.,* 2009; Rothe *et al*., 2013; Sasahara *et al*., 2011; Greco *et al*., 2011; CDCP, 2016) with some documenting genotypic similarities of clinical isolates to hospital surfaces (De Gialluly *et al*., 2006; Riggs *et al.,* 2007).

Conclusion and Recommendation

This study established the presence of antibiotic-resistant Gram-negative pathogens in an inanimate hospital environment and the detection of the extended beta-lactamase (ESBL) gene, indicating an alarming increase in the rate of antibiotic resistance in Gram-negative bacteria. Therefore, evidence-based on this study thus indicates an urgent need to alert and educate hospital staff about the potential health risks associated with the use of fomites. It is also important that routine detection of resistant patterns in the form of ESBL from fomites samples is done regularly. Healthcare workers should also comply with the recommended WHO nosocomial infection control guidelines, to aid in the improvement of patient management strategies and as a lift towards fighting increasing antibiotic resistance here in Nigeria. Further studies on plasmid-mediated resistance and efflux pump system are recommended. In addition, formulation and appropriate use of antibiotic policy should be observed and epidemiological microbiological surveillance should be routinely done to constantly check the trend of resistance. It will be necessary to establish regular surface cleaning interventions as part of an effective infection control policy.

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