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Prevalence and Antibiogram Pattern of Some Nosocomial Pathogens Isolated from Hospital Environment in Zaria, Nigeria

[*1]Chrinius Hammuel, [2]Edward D. Jatau and [2]Clement M.Z. Whong
[1]Department of Basic Research, National Research Institute for Chemical Technology, Zaria, Nigeria; [2]Department of Microbiology, Ahmadu Bello University Samaru Campus, Zaria, Nigeria.
Corresponding e-mail address: chrizoy2k@yahoo.com

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Abstract - Many ordinary surfaces and hands of healthcare givers in hospitals are sometimes inadequately decontaminated with routine disinfection techniques. It is necessary to determine the distribution of these pathogens in the hospitals. In this study 160 swab samples were collected from ten different surfaces including nurses' hand swab, Nurses' table top, door knob/handle, toilet seat, operation table, sink, stretcher, floor, bedrail, and cupboard. Biochemical tests were used to identify the bacteria. Kirby-Bauer-Clinical and Laboratory Standards Institute (CLSI) modified single disc diffusion technique was used to determine the antibiogram profile of the pathogens at 0.5 scale McFarland's standard (1.5×10^8 cells/ml). The total percentage prevalence of *Staphylococcus aureus* was 50.80%, *Pseudomonas aeruginosa* 28.60% and *Escherichia coli* 20.60%. Out of 20.60% of *E. coli* isolates7.7% were found to be *E. coli* O157:H7. *S. aureus* isolates were highly resistant to ampcillin and cefoxitin *P. aeruginosa* and *E. coli* were resistant to tetracycline. The multiple antibiotic resistance indexes of the pathogens were more than 0.2. Among the isolates, *S. aureus* showed more multidrug resistance (31.30%) and *E. coli* had the least multidrug. Frequently touched surfaces within the hospital environment are contaminated by *Pseudomonas aeruginosa*, *Escherichia coli* and *Staphylococcus aureus*. These pathogens can be transfer from surfaces to patients and to surfaces again through healthcare workers. The widespread use of antimicrobials, especially over- or inappropriate use of antibiotics, has contributed to an increased incidence of antimicrobial-resistant organisms.

Keywords: Nosocomial bacteria; Prevalence; Multidrug resistance; *Staphylococcus aureus*; *Pseudomonas aeruginosa; Escherichia coli*

Introduction

Nosocomial pathogens are organisms causing diseases that are acquired from the hospital and healthcare environment within few days of admission and are responsible for nosocomial infections (Medubi *et al.*, 2006). The hospital exists as a closed community, it is therefore not surprising that certain microorganisms become predominant and cause diseases. The pathogens can be expelled from an infected or colonized patient either through direct contact, aerosol droplets or faeces to the environmental surfaces. These pathogens can be contracted by the contaminated environmental surfaces, and equipment of the hospital through the healthcare workers and even by the patients. Therefore, environmental surfaces in Healthcare centres act as reservoir for bacteria and can as well serve as vectors of the bacteria pathogens (Boyce *et al.*, 1997). Depending on the environmental conditions, these pathogens may remain infectious on the surfaces for weeks after the contamination event (Carvahlo *et al.*, 2007).

The transmission of microorganisms from environmental surfaces to patients is largely via hand contact with the surfaces (Kampf and Kramer, 2004). Otter *et al.* (2011) reported that surfaces can play important role in the epidemic and endemic transmission of the major pathogens linked to healthcare associated infections. Nosocomial infection caused by the nosocomial pathogens has pose a problem of enormous magnitude globally, hospital localities have proven favourable in transmission of infection due to existing suitable pathogens-host-environment relationship (Samuel *et al.*, 2010). Many ordinary surfaces such as upholstery, side

table/bench, floors, carpets and many other areas in the hospital environment may not adequately be decontaminated and can become reservoirs of pathogens (Byers *et al.*, 1998). Commonly used disinfection techniques are sometimes incapable of eradicating fomite reservoir of nosocomial pathogens such as methicillin resistant S*taphylococcus aureus* (MRSA).

The emergence of antibiotic resistant micro-organisms (e.g., *Staphylococcus aureus* and *Pseudomonas aeruginosa*) is increasing rapidly around the globe creating a serious threat; many of the pathogens that cause nosocomial infection have a high level of resistance to antibiotic treatment (Jones and Pfaller, 1998). Infections from drug resistant *Escherichia coli, Staphylococcus aureus,* and *Pseudomonas aeruginosa* are becoming common (Oli *et al.*, 2013).

This research was designed to investigate the prevalence and to determine the antibiogram pattern of the most common potential nosocomial bacteria particularly *Staphylococcus aureus, Pseudomonas aeruginosa, and Escherichia coli,* in hospital environment.

Materials and Methods

Study Area and Sample Collection

The study area of this work is Gambo Sawaba, general hospital, Kofan Gaya, Zaira. A total number of 160 samples were collected from different surfaces of Gambo Sawaba General Hospital, Zaria: hands of some of the hospital nurses, floors, toilets seats, operation tables, door knobs/door handles, Nurses' table tops, bedrails, stretchers, cupboards, sinks, using sterile swab sticks using wetted sterile cotton swabs.

Laboratory Analysis

Each sample swabbed was pre-enriched in prepared sterile bacteriological peptone water and incubated at 37°C for 24 hours. After which the turbid broth was subcultured on media such as Mannitol salt agar, Eosin methylene blue agar (EMB), *Pseudomonas* cetrimide agar and MacConkey agar plates. Discrete colonies were further subcultured onto fresh prepared plates of the selective media and onto nutrient agar plates to obtain pure cultures. Presumptive morphological identification of the colonies was done by observing their individual appearance on the selective media that were used for the isolation. The colonies were gram stained and stored on nutrient agar slants for biochemical tests and identification.

Biochemical tests

The following biochemical tests were employed for this study: Catalase and coagulase tests for identification of *Staphylococcus aureus*, citrate and oxidase tests for identification of *Pseudomonas aeruginosa*, and indole, methyl red (MR), Voges Prokauer (VP) and citrate tests for identification of *Escherichia coli* (Cheesborough, 2005).The isolates were confirmed using Microgen® kits i.e. Gram negative (GNA or GNA + GNB) for *P. aeruginosa* and *Escherichia coli* and STAPH identification kits from Microgen Bioproducts Ltd, U.K. (www.microgenbioproducts.com). The identification of each pathogen was carried out according to the manufacturer's instructions.

The serology test for identification of *E. coli* O157:H7

The isolates of *Escherichia coli* were cultured for 24 hours at 37°C on Sorbitol MacConkey agar plate (the morphology of *E. coli* on this medium is colourless) and were used to carry out serology test for the identification of *E. coli* O157:H7. The identification of these pathogens was carried out according to manufacturer's instruction.

Antibiotic susceptibility test

The antimicrobial susceptibility pattern was determined using Kirby-Bauer-CLSI modified single disc diffusion technique (Cheesbrough, 2004). Single antibiotic disc of Ampicillin (10µg), Vancomycin (30µg), Tetracycline (30µg), Cefoxitin (30µg), Chloramphenicol (30µg), Imepenem (10µg), Ceftazidime (30µg), Linezolid (10µg) and Gentamicin (10µg), all the discs were obtained from Oxoid England and all the results of the antimicrobial susceptibility were interpreted using CLSI (2008).

The standardization of inoculums

Twenty four (24) hours cultured organism was suspended into test tube of sterile normal saline using sterile wire loop to form turbidity that match with 0.5 scale of McFarland's standard (1.5×10^8 cells/ml) (Coyle, 2005). The standard strains used as the antibiotics susceptible control were *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 9027. The cell suspensions was inoculated by streaking on prepared Mueller-Hinton agar using sterile swab sticks, then the antibiotic disc was placed on the inoculated medium aseptically with help of sterile forceps and incubate at 37°C for 24 hours. The zones of inhibition created by each of the antibiotics against the test organisms and the standard strains were measured and the result was interpreted using guideline by CLSI (2008).

Determination of multiple antibiotics resistance (MAR) index

The multiple antibiotics resistance index was determined for each of the selected bacterial isolate using a formula MAR = x/y, where x was the number of antibiotics to which test isolate displayed resistance and y is the total number of antibiotics to which the test organism has been evaluated for sensitivity (Olayinka *et al.*, 2004; Tula *et al.*, 2013).

Results and Discussion

Table 1 indicates the occurrence of the pathogens isolated from the surfaces of this general hospital. The total prevalence rate of the three pathogens from this hospital was 39.40%. Of the pathogens, 32 (50.80%) were tested positive for *S. aureus,* 18 (28.60%) for *P. aeruginosa* and 13 (20.60%) were tested positive for *E. coli*. The 100% prevalence rate of *S. aureus* from hand swab of the nurses from this hospital was higher compare to the earlier report of Boyce (2007) and Ekrami *et al.* (2011). The high prevalence of the *S. aureus* from hand swab 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 as reported earlier by Olalekan *et al.* (2011).

The higher prevalence rate of *S. aureus* on bedrail in this hospital is in agreement with 100% prevalence on bedrail as reported by Boyce (2007). Also, 38% of *S. aureus* reported on door handle by Carvalho *et al.* (2007) is lower to the prevalence rate of the pathogen on door knob/door handle of 53.8% from this hospital. The prevalence rate of 53.8% and 42.2 of *S. aureus* and *E. coli* on door knobs/door handles in confirms the early report of Nworie *et al.* (2012) from some parts of Abuja metropolis that the contamination of door knob/door handle can be as a result of poor hand hygiene after using toilet. And if hand hygiene practices are suboptimal, microbial colonisation is more easily established and/or direct transmission to patients or a fomite in direct contact with the patient may occur (Allegranzi and Pittet, 2009). It has been reported that organisms are capable of surviving on hands of health care workers for at least several minutes following contamination (Allegranzi and Pittet, 2009).

The isolation of *Pseudomonas aeruginosa* from the sinks confirms the report of Udeze *et al.* (2012) that sinks are the most common place in hospital environment were *P. aeruginosa* are predominantly found. Also, the prevalence rate of *P. aeruginosa* on operation table of the hospital was still higher than a work reported by Pal *et al.* (2010) that 9.6% of the pathogen was isolated from operation table in a hospital in India. The presence of this pathogen on operation table can contaminate open wounds of the patients in course of the operation.

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Table 1. The occurrence of the bacterial isolates in the hospital

Sample source	Sample size	Total positive isolates	Total % of isolates	*S. aureus*	Percentage (%)	*P. aeruginosa*	Percentage (%)	*E. coli*	Percentage (%)
NHS	20	5	25.0	5	100	-	-	-	-
NTT/ST	11	6	63.6	5	83.3	-	-	1	16.7
DK/DH	23	13	56.5	7	53.8	-	-	6	46.2
TS	13	9	69.0	-	-	4	44.4	5	55.6
OT	5	3	60	2	66.7	-	-	1	33.3
Sink	12	5	41.7	-	-	5	100	-	-
Stretcher	11	4	36.3	3	75.0	1	25.0	-	-
Floor	31	5	16.1	-	-	5	100	-	-
BR	17	6	35.2	6	100	-		-	-
CB	17	7	41.1	4	57.1	3	42.9	-	-
Total	160	63	39.4	32	50.8	18	28.6	13	20.6

NHS = Nurses' hand swab, NTT/ST = Nurses table top/staff table, DK/DH = Door knob/Door handle, TS= Toilet seat, OT= Operation table, BR = Bedrail, CB = Cup board.

Table 2. The serotype of *Escherichia coli* for *E. coli* O157:H7

Number of isolates	Test latex	Control text	Interpretation	Total number of *E. coli* O157:H7 identified
13	+	-	*E. coli* O157 present	1(7.7%)

One of the most frequently used surfaces in the hospital environment was found contaminated by *P. aeruginosa* (44.4%). This could be as a result of inadequate decontamination of the surfaces. This corroborates the report of Sabra (2013) that *P. aeruginosa* can be isolated from toilet seat and other moist environment in hospitals. This pathogen may be seeded (forming biofilm) into toilets remain on the toilet seat for a long time after multiple flushing and cleaning with antimicrobial fluids. The prevalence of *E. coli* O157:H7 (7.7%) from one of the toilet seats in this work as seen in Table 2 is above the prevalence of 13.7% as reported by Wagner *et al.* (2004). Enterohaemorrhagic strains of *Escherichia coli*, especially *E. coli* O157:H7, have been emerged as important enteric pathogens in recent years. Various serotypes have been implicated in human disease, but *E. coli* O157:H7 is the most common (Wagner *et al.*, 2004).

The high percentage of ampicillin resistant *S. aureus* in this research as shown in Table 3 confirms the earlier report of Dudhagara *et al.* (2011). The resistance of the *S. aureus* to this antibiotic (AMP), may be as result of the ability of β-lactamase enzyme to break the β-lactam ring in the antibiotic and rendered it ineffective because *S. aureus* produces β-lactamase in the presence of ampicillin (Oncel *et al.*, 2004). The 100% susceptibility of *S. aureus* to vancomycin in this finding agreed with the findings of Terry-Alli *et al.* (2011) and the 100% susceptibility to linezolid. Linezolid has an advantage over vancomycin for treating MRSA because it has an intravenous preparation and an oral tablet that has excellent bioavailability. The 0.0% resistance of *S. aureus* to gentamicin in this finding is not similar to the report of Akindele *et al.* (2010) that 39% of this pathogen was resistant to gentamicin. The antimicrobial profile of *S. aureus* showed that 25.0%, of the isolates were resistant to cefoxitin. Resistance to cefoxitin by disc diffusion can be used for the detection of MRSA strains in routine testing because cefoxitin is a potential inducer of the system that regulates *mec*A gene (Madhusudhan *et al.*, 2011). For this reason, the resistant of *S. aureus* to cefoxitin in this finding is considered resistant to methicillin.

The 0.0% of the antimicrobial resistance profile of *E. coli* to gentamicin, cefoxitin, ceftazidime and chloramphenicol as seen in Table 3 in this finding is in agreement with research findings of Mukhtar and Saeed (2011) in Sudan, who also found that *E. coli* was 0.0% resistance to gentamicin, cefoxitin, ceftazidime and chloramphenicol. This pathogen was resistant to tetracycline (46.2%) and ampicillin (7.7%) from the hospital. The resistance of *E. coli* to ampicillin could be as a result of production of β-lactamse enzyme which has the ability to deactivate the efficacy of this β-lactam drug as reported by Hassan *et al.* (2011). In this research, gentamicin, cefoxitin, ceftazidime and chloramphenicol were the most active antibiotics against *E. coli.*

The multiple antibiotic resistance (MAR) indices give an indirect suggestion of the probable source(s) of the organism. The MAR indices in this work were greater than 0.20, as seen in Table 4, this confirms the report of Olayinka *et al.* (2004) that the MAR index greater than 0.20 indicates that the organisms must have been originated from an environment where antibiotics are often used (Olayinka *et al.*, 2004). Thus, the result of the multiple antibiotic index in this work can be reported that these pathogens might have been originated were these antibiotics are used. As it is shown in Figure 1, the multidrug resistance of *S. aureus* from this hospital was 31.30%, and was not higher than 87.75% multidrug resistance of *S. aureus* as reported by Fagade *et al.* (2010). This finding corroborates the report of Seza and Fatma (2012) that among the Gram-positive microorganisms, staphylococci are the most frequently resistance pathogen to antibiotics.

Table 3. The antibiotic susceptibility profile of the isolates

	Pseudomonas aeruginosa (N = 20)			*Staphylococcus aureus* (N = 32)			*Escherichia coli* (N = 13)		
Antibiotic	R	I	S	R	I	S	R	I	S
VA		NT		0 (0%)	-	32 (100%)		NT	
AMP		NT		32 (100%)	-	0 (0.0%)	1 (7.7%)	4 (30.8%)	8 (61.5%)
TE	5 (25.0%)	0 (0%)	15 (75.0%)	2 (6.3%)	8 (25.0%)	22 (68.7%)	6 (46.2%)	4 (30.8%)	3 (23.0%)
LZD		NT		0(0.0%)	-	32 (100%)		NT	
CAZ	0 (0.0%)	0 (0.0%)	20 (100%)	2(6.3%)	12 (37.5%)	4 (56.2%)	0 (0.0%)	6 (41.2%)	7 (53.8%)
C	2 (10.0%)	0 (0.0%)	18 (90.0%)		NT		0 (0.0%)	5 (38.5%)	8 (61.5%)
IMP	0 (0.0%)	0 (0.0%)	20 (100%)		NT			NT	
FOX		NT		8 (25.0%)	-	24 (75.0%)	0 (0.0%)	0 (0.0%)	13 (100%)
CN	1 (5.0%)	0 (0.0%)	19 (95.0%)	0 (0.0%)	15 (46.9%)	17 (53.1%)	0 (0.0%)	6 (41.2%)	7 (53.8%)

Table 4. The Multiple antibiotic index (MAR) of the isolates

P. aeruginosa	Combination of antibiotics	MAR Index	*E. coli*	Combination of antibiotics	MAR Index	*S. aureus*	Combination of antibiotics	MAR Index
1	CN, TE, C	0.60	1	AMP, TE	0.30	6	AMP, FOX	0.28
1	TE, C	0.40				1	AMP, CAZ	0.28
						1	AMP,CAZ, FOX	0.43
						1	AMP, TE, FOX	0.43
						1	AMP, TE	0.38

Notation for Table 3 and Table 4:
VA = Vancomycin FOX = Cefoxitin AMP = Ampicillin CN = Gentamicin TE = Tetracycline OX = Oxacillin
LZD = Linezolid NT = Not Tested CAZ = Ceftazidime R = Resistant C = Chlorampehnicol I = Intermediate
IMP = Imipenem S = Sensitive

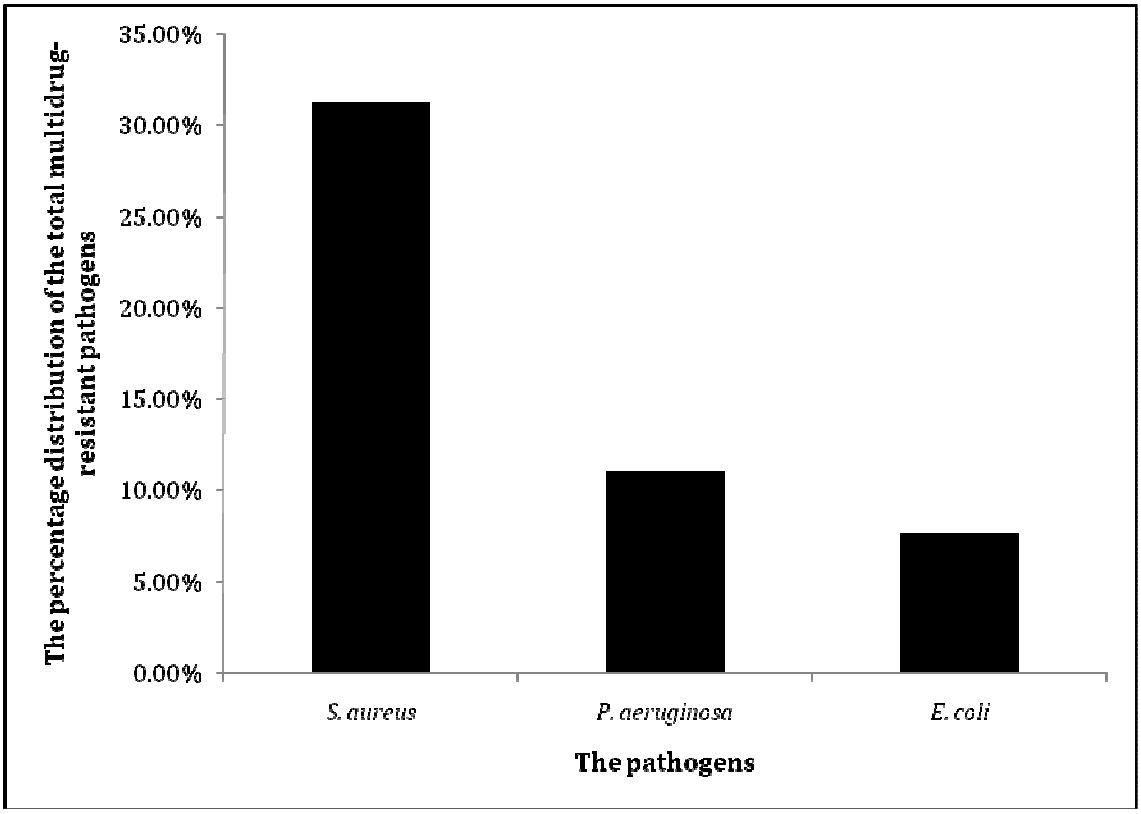

Figure 1. The multidrug-resistant isolates from the hospital environment

The multidrug resistance of *P. aeruginosa* from the hospital was 11.10% which confirms the report of Hota *et al.* (2009) that outbreaks of multidrug-resistant *P. aeruginosa* colonization or infection can occurred in urology wards, a burn unit, haematology/oncology units, and adult and neonatal critical care units and that various medical devices and environmental reservoirs can be implicated in the outbreaks of the pathogen. The multidrug resistant isolates of *E. coli* (7.70%) from this hospital is similar to report of Ibrahim *et al.* (2012) that 7.0% of *E. coli* isolated from hospital in Sudan were multidrug resistant. The occurrence of MDR is very common and mainly in Gram negative bacteria. Multidrug resistance *E. coli* are widely distributed in hospitals and are increasingly being isolated from community. Thus, there is urgent need to find out new antimicrobial agents (Ibrahim *et al.*, 2012).

Conclusions

We therefore, conclude that inanimate surfaces near infected patients and those frequently touched surfaces within the hospital environment are reservoirs of *Pseudomonas aeruginosa*, *Escherichia coli* and *Staphylococcus aureus*. The hands of healthcare workers can readily acquire pathogens after contact with contaminated hospital surfaces or patients and can transfer these pathogens to subsequently touched patients and inanimate surfaces. This can lower the quality of healthcare services being provided in these hospitals. The widespread use of antimicrobials, especially over- or inappropriate use of antibiotics, has contributed to an increased incidence of antimicrobial-resistant organisms or multidrug resistant microorganisms in the hospital environment.

In view of multiple studies indicating the environment to be an important source of bacterial transmission, more stringent routine environmental decontamination practices in healthcare facilities with regular monitoring is necessary in the MDRO containment bundle.

Thorough cleaning and disinfection of the environment should remain one of the topmost effective preventive measures intended to provide reassurance that patients as well as staff are not put at unnecessary risks during their stay in the hospital setting. The hospital authorities should take the initiative by forming infection control team which continuously monitors the prevalence and incidence of such pathogens.

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