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Occurrence and antibiogram profile of *Staphylococcus aureus* isolated from some hospital environment in Zaria, Nigeria

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Staphylococcus aureus **is an opportunistic pathogen and is the most frequent cause of hospital acquired infection. A total sample of 310 was collected for this work; the pathogen was isolated from hands of some healthcare workers/nurses, operation tables, door knobs/door handles, nurses' table tops, bedrails, stretchers, floors, toilets seats, cupboards, and sinks. The total percentage prevalence of the pathogen in Major Ibrahim B. Abdullahi memorial hospital was 16.8% and 20.7% from St. Luke's Anglican hospital. Kirby-Bauer-NCCLS modified single disc diffusion technique was used to determine the antibiogram profile of the pathogen at 0.5 scale MacFarland's standard (1.5 × 10^8 cells/ml). The isolates from the two hospitals were 100% susceptible to vancomycin and 95.7% and 92.6% from Major Ibrahim B. Abdullahi memorial hospital and St Luke's Anlgican hospital respectively were resistant to Ampicillin.**

Key words: *Staphylococcus aureus*, antibiogram profile, pathogen, sensitive, resistant.

INTRODUCTION

Staphylococcus aureus is a Gram positive coccus that occurs in grape-like clusters. It is a eubacterium that is found on the surface of the human skin and mucous membranes (Prescott et al., 2005). They form part of the normal microbial flora of the skin, upper respiratory tract and intestinal tract (Cheesborough, 2005). The pathogen is an opportunistic organism in man and animals and is the most frequent cause of hospital and community infections (Prescott *et al*., 2005). *S. aureus* can cause a range of illness from minor skin infections such as boils, abscesses to life threatening diseases such as pneumonia, meningitis, toxic shock syndrome and sepsis (Lakshmi and Harasreeramulu, 2011).

Drug resistance by the organism is also a major concern (Weinstein, 1998). Both methicillin (oxacillin or cefoxitin) and glycopeptide (vancomycin and teicoplanin) resistance may occur in *S. aureus*. It is found throughout the hospital environment, particularly around patients known to be colonised or infected with the bacterium (Dancer, 2009).

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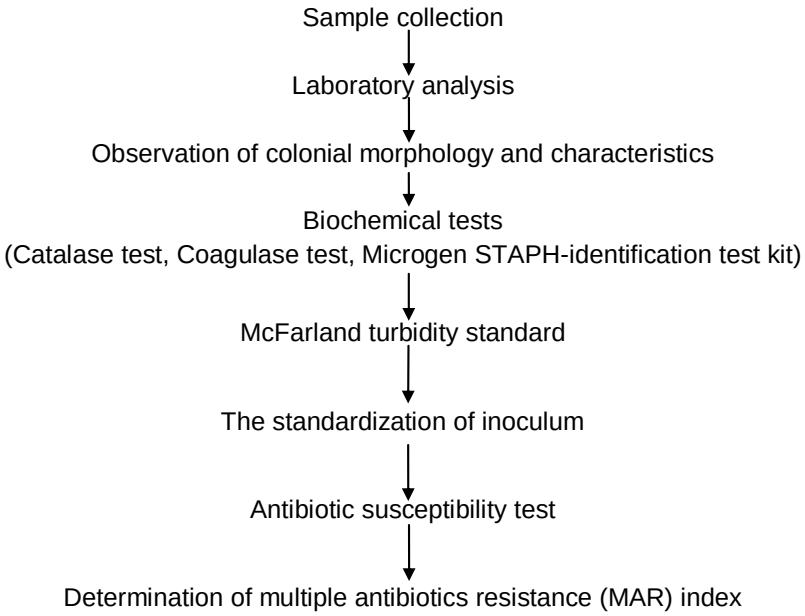

Figure 1. The work flow chart.

Increasingly, nosocomial isolates are resistant to multiple drugs. In the community, *S. aureus* remains an important cause of skin and soft tissue infections, respiratory infections, and (among injection drug users) infective endocarditis (Horst et al., 2011). Scientific evidence suggests that environmental contamination plays an important role in the spread of methicillin-resistant *S. aureus* (MRSA).

The transfer of microorganisms from environmental surfaces to patients is largely through hand contact with the contaminated surfaces (Samuel et al., 2010). It has been estimated that 20 to 40% of nosocomial infections have been attributed to cross infection via the hands of health care personnel (Weinstein, 1991). Contamination of the hands of health care workers (HCWs) could in turn result from either direct patient contact or indirectly from touching contaminated environmental surfaces or patients' skin during routine care activities, sometimes even despite glove use (Kramer et al., 2006, Allegranzi and Pittet, 2009). Many nosocomial infections are caused by pathogens transmitted from one patient to another, by way of healthcare workers who have not washed their hands, or who do not observe simple hospital hygiene measures, and also between patients (Olalekan et al., 2011).

MATERIALS AND METHODS

Study area

The study area of this work encompass some hospitals in Zaria including St Luke Anglican hospital, Wusasa located at 11° 04' N and 007°40' E, then Major Ibrahim B. Abdullahi memorial hospital which is situated at 11° 06' N and 007° 41' E all at Greenwich meridian. These areas were located using Taiwan made Etrex® high-sensitive geographic positioning system (GPS) receiver. A total sample of 310 was collected for this work.

Ethical approval

The ethical approval was obtained from ethical committee of Kaduna state Ministry of Health and was used for sampling. Approval was also obtained from Medical Director, St. Luke's Hospital, Wusasa.

Sampling designing and techniques

Sample collection

The total number of samples collected for this study was 310 and all samples were collected in the morning before commencement of work in each hospital and hand swab of the staff were collected during working hours. Samples for the studies were collected from hands of some of the hospital staff and nurses, floors, toilets seats, operation tables, door knobs/door handles, nurses' table tops, bedrails, stretchers, cupboards, sinks, using sterile swab sticks using sterile cotton swabs wetted with sterile peptone water (Figure 1).

Laboratory analysis

Each sample swab was inoculated into prepared sterile bacteriological peptone water and incubated at 37°C for 24 h for enrichment after which the turbid broth was subcultured unto solid differential media such as Manitol salt agar, Eosin methylene blue agar (EMB), *Pseudomonas centrimide* selective agar and MacConkey agar plates and incubated again at 37°C for 24 h.

Table 1. The prevalence of the pathogens in relation to the two hospital environment.

Hospital	No. of sample screened	*S. aureus*	% prevalence of *S. aureus*
MIBAMH	155	25	16.1
SLAH	155	32	20.6
Total	310	57	18.4

MIBAMH, Major Ibrahim B. Abdullahi Memorial Hospital; SLAH, St. Luke Anglican Hospital.

Discrete colonies were further subcultured onto fresh prepared plates of the selective media and nutrient agar plates to obtain pure cultures. The purified cultures were gram stained and stored on nutrient agar slants for biochemical tests and identification.

Observation of colonial morphology and characteristics

Presumptive morphological identification of the colonies was done by observing their individual appearance on the selective media that were used for the isolation.

Biochemical tests

Catalase test

Three (3) ml of hydrogen peroxide solution was poured into a test tube. With the aid of sterile glass rod, several colonies of the test organism were carefully removed and immersed into 3 ml solution of hydrogen peroxide. Immediate bubbling within few seconds was recorded to be positive test of *Staphylococcus* species.

Coagulase test

A drop of distilled water was added on each end of a slide. A colony of a suspected organism of 24 h culture from blood agar (previously checked by gram staining) was emulsified in each of the drops of the distilled water and made two different suspensions. A loop of the plasma was then added to one of the suspensions and mixed gently. Clumping or agglutinations of the organisms with the plasma within ten (10) seconds indicated a positive result of *S. aureus*; negative result indicates other *Staphylococcus* species.

Microgen STAPH-identification test kit

Other biochemical tests carried out using microgen STAPH-identification kit include sucrose, nitrate, sucrose, trehalose, mannitol, n-acetyl glucoseamine, manose, turanose, alkaline phosphatase, glucosidase, glucuronidase, urease, argenine and l-pyrrolidonyl-α-naphthylamide (www.microgenbioproducts.com, uk).

McFarland turbidity standard

The turbidity standard of the organisms used was 0.5. One percent (1%) v/v solution of sulphuric acid was prepared by adding 1 ml of concentrated sulphuric acid to 99 ml of distilled water and mixed well. 1% w/v solution of barium chloride was also prepared by dissolving 1 g of the dehydrated salt ($BaCl_2.2H_2O$) in 100 ml of distilled water. Then 0.6 ml of the barium chloride was added to 99.4 ml of the sulphuric acid solution and was mixed well. The small portion of the turbid solution was transferred into a test tube which was used to compare with the inoculated organisms in Mueller-Hinton broth (Cheesbrough, 2004).

Antibiotic susceptibility test

The antimicrobial susceptibility pattern was determined using Kirby-Bauer-NCCLS modified single disc diffusion technique (Cheesbrough, 2004). Disc diffusion technique was performed according to Kirby-Bauer method, as described in the guidelines of Clinical and Laboratory Standards Institute, CLSI, 2012. Single antibiotic disc such as ampicillin (10 µg), vancomycin (30 µg), tetracycline (30 µg), cefoxitin (30 µg), ceftazidime (30 µg), linezolid (10 µg) and gentamicin (10 µg), all the discs were obtained from Oxoid England.

The standardization of inoculums

The concentration of each of the suspension of the test organisms and the standard isolates were prepared by picking a 24 h colony of the organism using sterile wire loop into sterile test tube containing sterile normal saline to form turbidity that match with 0.5 scale of MacFarland's standard (1.5×10^8 cells/ml) (Coyle, 2005). *S. aureus*, ATCC 25923 was obtained from National Institute of Pharmaceutical Research and Development (NIPRD), Abuja. The standard strain was used as the antibiotics susceptible control. The cell suspensions was inoculated by streaking on prepared Mueller-Hinton agar using sterile swab stick, then the antibiotic disc was placed on the inoculated medium aseptically with the help of sterile forceps and incubated at 37°C for 24 h. The zones of inhibition created by each of the antibiotics against the test organisms and the standard strains as positive control were measured and the result was interpreted using guideline from CLSI, 2012. The results were recorded as sensitive, intermediate and resistance.

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* is 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 shows the prevalence of the pathogen in the two hospitals. The total sample of 155 was collected from each hospital. Table 1 shows that 16.1 and 20.6% of the

Table 2. The occurrence of the isolate in Major Ibrahim B. Abdullahi Memorial Hospital.

Sample source	Sample size	Total positive isolates	Prevalence of *S. aureus* (%)
NHS	11	4	36.4
NTT/ST	9	3	33.3
DK/DH	25	6	24.0
TS	8	-	-
OT	4	-	-
Sink	14	-	-
Stretcher	14	2	14.3
Floor	35	3	8.6
BR	17	5	29.4
CB	15	2	13.3
Total	155	25	16.1

SHS = 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 3. The occurrence of the isolate in St. Luke Anglican Hospital.

Sample source	Sample size	Total positive isolates	Prevalence of *S. aureus* (%)
NHS	15	6	40.0
NTT/ST	16	5	31.3
DK/DH	20	4	20.0
TS	7	-	-
OT	6	-	-
Sink	10	-	-
Stretcher	10	2	20.0
Floor	30	5	16.7
BR	22	6	27.3
CB	19	4	21.1
Total	155	32	20.6

SHS = 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.

pathogens were isolated from Major Ibrahim B. Abdullahi memorial hospital and St. Luke Anglican Hospital respectively. As it is presented in Table 2 the prevalence of the pathogens isolated from surfaces at Major Ibrahim B. Abdullahi memorial hospital showed that 36.4, 33.3, 24.0, 14.3, 8.6, 29.4 and 13.3% of *S. aureus* was isolated from nurses' hand swab, nurses' table top, door knob/handle, stretcher, floor, bedrail and cupboard respectively. Table 3 shows the distribution of the pathogens on surfaces at St. Luke's Anglican hospital. *S. aureus* was isolated from Nurses' hand swab (40.0%), nurses' table tops (31.3%), door knob/handle (20.0%), stretcher (20.0%), bedrail (27.3%), floor (16.7%) and cupboard (21.1%).

The prevalence of *S. aureus* from hands of the nurses from the two hospitals was slightly lower compared to the earlier report of 42.0% of the pathogen from hand swab as reported by Boyce (2007) and 20% as reported by Ekrami et al. (2011). The level of contamination by this pathogen could also be as a result of inadequate decontamination of the microbial load from the surfaces (Addy et al., 2004).

Page et al. (2009) reported that surfaces can act as reservoirs of microbes which could in turn lead to the spread of infection upon being touched, by either healthcare workers, patients or visitors. The presence of the pathogen in the hand swab 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 by Olalekan et al. (2011). A study by Ferreira et al. (2011) revealed that contaminated hands of healthcare workers played important role in transmission of pathogens within the hospital environment and reported that 29% of nurses working in a general hospital had *S. aureus* on their hands and 78% of those working in a hospital for dermatology

Table 4. The antibiotic profile of the isolate from Major Ibrahim B. Abdullahi Memorial Hospital.

Antibiotic	*Staphylococcus aureus* (N = 25)		
	R	I	S
VA (30 µg)	0 (0.0%)	-	25(100%)
AMP (10 µg)	23 (92.0%)	-	2(8.0%)
TE (30 µg)	4(16.0%)	8(32.0%)	13(52.0%)
LZD (10 µg)	0(0.0%)	-	25(100%)
CAZ (30 µg)	2(8.0%)	11(44.0%)	12(48.0%)
FOX (30 µg)	4(16.0%)	-	21(84.0%)
CN (30 µg)	0(0.0%)	11(44.0%)	14(56.0%)

VA = Vancomycin; AMP = Ampicillin; TE = Tetracycline; LZD = Linezolid; CAZ = Ceftazidime; FOX = Cefoxitin; CN = Gentamicin; NT = Not Tested; R = Resistant; I = Intermediate; S = Sensitive.

Table 5. The antibiotic profile of the isolate from St. Luke's Anglican Hospital.

Antibiotic	*Staphylococcus aureus* (N = 32)		
	R	I	S
VA (30 µg)	0(0.0%)	-	32(100%)
AMP (10 µg)	28(87.5%)	-	4(12.5%)
TE (30 µg)	1(3.1%)	15(46.9%)	16(50.0%)
LZD (10 µg)	0(0.0%)	-	32(100%)
CAZ (30 µg)	0(0.0%)	18(56.3%)	14(43.8%)
FOX (30 µg)	5(15.6%)	-	27(84.4%)
CN (30 µg)	0(0.0%)	16(50.0%)	16(50.0%)

VA = Vancomycin; AMP = Ampicillin; TE = Tetracycline; LZD = Linezolid; CAZ = Ceftazidime; FOX = Cefoxitin; CN = Gentamicin; NT = Not Tested; R = Resistant; I = Intermediate; S = Sensitive.

patients had the organism on their hands.

The antibiotic susceptibility profile of isolates from Major Ibrahim B. Abdullahi memorial hospital as in Table 4 shows that *S. aureus* isolates were highly resistant to ampicillin (92.0%), and 16.0 % were resistant to both tetracycline and cefoxitin, very few were resistant to ceftazidime (8.0%). All the isolates of *S. aureus* were susceptible to vancomycin and linezolid; some were susceptible to tetracycline (52.0%), ceftazidime (48.0%), cefoxitin (84.0%) and gentamicin (56.0%). The antibiotic susceptibility profile of pathogens from St. Luke's Anglican hospital as presented in Table 5 shows that all isolates of *S. aureus* were susceptible to vancomycin and linezolid.

This pathogen was also highly resistant to ampicillin (87.5%). Some were resistant to tetracycline (3.1%) and cefoxitin (15.6%). Resistance to cefoxitin by disc diffusion has been used for the detection of MRSA strains in routine testing because cefoxitin is a potential inducer of the system that regulates *mecA* gene (Philip and Shannon, 1993; Madhusudhan et al., 2011). Methicillin-resistant *S. aureus* (MRSA) bacteria are more prevalent in the hospital environment and can be a challenge to infection control practices in most countries. Oie et al. (2002) and Boyce (2007) reported Methicillin-resistant *S. aureus* (MRSA) that frequently contaminated objects including the floor, bed linens, the patient's gown, overbed tables, door knob/door handle and blood pressure cuffs. Of the total number of *S. aureus* isolates from this hospital, 12.5, 50.0, 84.4, 43.8 and 50.0% were susceptible to ampicillin, tetracycline, cefoxitin cetazidime and gentamicin respectively.

High resistance of *S. aureus* to ampicillin; that is 92.0% from Major Ibrahim B. Abdullahi memorial hospital and 92.6% from St. Lukes' hospital is in agreement with 97.0% of *S. aureus* resistance to ampicillin as reported by Terry-Alli et al. (2011) from South Western Nigeria. This research confirms the earlier report of Dudhagara et al. (2011) that a high percentage of *S. aureus* were resistant to ampicillin and other β – lactam drugs, and is also in agreement with research work carried out by Akindele et al. (2010) that of the 100 total number of *S. aureus* isolated

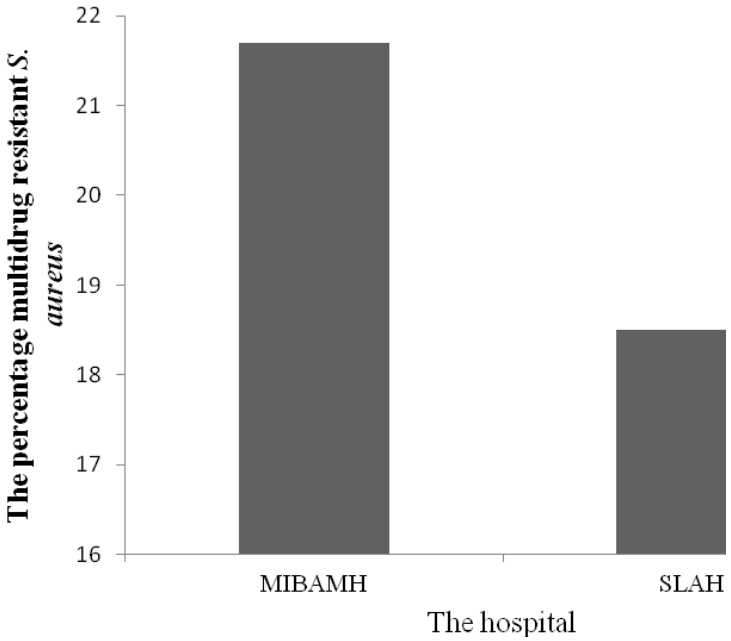

Figure 2. The prevalence of the total multidrug-resistant isolates from the two hospital environment. MIBAMH = Major Ibrahim B. Abdullahi Memorial Hospital; SLAH = St Luke's Anglican Hospital.

Table 6. The Multiple antibiotic resistant (MAR) indexes of the pathogens isolated from the hospitals.

Organism	No. of resistance isolates	MAR Index	Percentage (%) MAR Index
S. aureus	16	0.29	80
	4	0.43	20

from hospital environment 90% of them were resistant to ampicillin. The resistance of *S. aureus* to this antibiotic (AMP) may be as result of the ability of β-lactamase enzyme to break the β-lactam ring of the antibiotic and render it ineffective because *S. aureus* produces β-lactamase in the presence of ampicillin (Oncel et al., 2004). Akindele et al. (2010) 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.

The 100% susceptibility of *S. aureus* to vancomycin from the two hospitals in this finding agreed with the findings of Terry-Alli et al. (2011) and the 100% susceptibility to linezolid is in agreement with 100% susceptibility of *S. aureus* to linezolid as reported by Kaleem et al. (2010) that 100% of the isolates of *S. aureus* were susceptible to linezolid and vancomycin and slightly higher than 93% susceptibility pattern as reported by Seza and Fatma (2012). The 0.0% resistance of *S. aureus* to gentamicin in this finding is not similar with report of Akindele *et al*. (2010) that 39% of this pathogen was resistant to gentamicin.

Figure 2 shows the multidrug resistance pattern of the pathogen against the selected. The multidrug resistant *S. aureus* from Major Ibrahim B. Abdullahi memorial hospital (21.7%) and St. Luke's Anglican hospital (18.5%) was not higher than 87.75% multidrug resistant *S. aureus* as reported by Fagade et al. (2010). This finding has corroborated the report of Seza and Fatma (2012) that among the Gram-positive microorganisms, staphylococci are the most frequently resistant pathogen to antibiotics. The surfaces of the two hospital environment can serve as important secondary reservoir for multi-resistant microorganisms, such as the MRSA as reported by Carvhalo et al. (2007); this has to be emphasized because of the apparent ability of these pathogens to survive on dry surfaces. Therefore, the spread of multidrug resistant *S. aureus* in this research can be a great threat to everyone in the two hospital environments and the public.

Table 6 indicates the multiple antibiotic resistant index of the pathogen against the drugs. The multiple antibiotic resistance (MAR) index gives an indirect suggestion of the probable source(s) of the organism. The MAR indices

in this work were greater than 0.20, this indicates this pathogen might have been originated from an environment where antibiotics are often used as reported by Olayinka et al. (2004).

Conclusion and recommendations

The widespread use of antimicrobials, especially over- or inappropriate use of antibiotics, has contributed to an increased incidence of antimicrobial-resistant organisms. Hospital-acquired infections are often caused by antimicrobial-resistant microorganisms. Resistance to antimicrobial agents is a problem in communities as well as health care facilities, but in hospitals, transmission of bacteria is amplified because of the highly susceptible population. Factors that could be associated with transmission of resistant strains of these microorganisms include poor attention to hygiene, overcrowding, lack of an effective infection control program, and shortage of trained infection control providers.

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 would 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. Cleaning remove pathogens from a surface and can be able to reduce residual organic material to a low level.

Most of these infections can be prevented with readily available, relatively inexpensive strategies by: adhering to recommended infection prevention practices, especially good hand hygiene and wearing gloves; paying attention to well-established processes for decontamination and cleaning of soiled surfaces, followed by use of disinfectants should remain the most effective means to reduce transmission of nosocomial pathogens. There is convincing evidence that improved hand hygiene can reduce infection and cross-transmission rates. Therefore, there are local as well as international guidelines for hand-hygiene practices. Healthcare workers (HCWs) should be encouraged to decontaminate (clean) their hands with an antiseptics before and after all patients' contacts.

Conflict of interests

The authors did not declare any conflict of interest.

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