

**OCCURRENCE AND ANTIBIOTIC RESISTANT PHENOTYPES OF *STAPHYLOCOCCUS AUREUS* ISOLATED FROM SOME HOSPITAL ENVIRONMENTS IN NASARAWA TOWN, NASARAWA STATE, NIGERIA**Aliyu Y.*¹, Jibril U. Y.², Jibrin S. M.³ and Salawu E. M.⁴^{1,2,3,4}Department of Science Laboratory Technology, School of Applied Sciences, Federal Polytechnic, P.M.B. 001, Nasarawa, Nasarawa State, Nigeria.***Corresponding Author: Aliyu Y.**

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ABSTRACT

This work was aimed at determining the occurrence and antibiogram of *Staphylococcus aureus* isolated from some hospital environments in Nasarawa town, Nasarawa State, Nigeria. Out of the 160 swab samples collected in this study, 52 were positive for *S. aureus* with an overall prevalence rate of 32.56%. Out of these, 28 positive samples were obtained from Henad Medical Centre, 16 positive samples from Alpha Hospital, and 8 positive samples from the Polytechnic Clinic. The prevalence rate for Henad Medical Centre was 40.0%, it was 26.76% for both Alpha Hospital and the Polytechnic Clinic respectively. The Henad Medical Centre was observed to have had a higher prevalence of the bacterium than Alpha Hospital and the Polytechnic Clinic. This may not be unconnected with the number of samples collected from this healthcare centre. A relatively larger sample size (70) was collected from Henad Medical Centre compared to the 60 and 30 samples collected from Alpha Hospital and the Polytechnic Clinic respectively. This was based on the number of patients that visits the three hospitals to seek medical attention. The antibiotic susceptibility profile of the isolates indicate that, majority of them were highly susceptible to ciprofloxacin, gentamicin, rifampicin, chloramphenicol, and levofloxacin. The isolates were observed to have developed high resistance to amoxil, ampiclox, and erythromycin. Eight (8) antibiotic resistance phenotypes were observed with varying combinations of 2,3, and 4 antibiotics. All the isolates had multiple antibiotics resistance (MAR) index of 0.2 and above.

KEYWORDS: *Staphylococcus aureus*, hospital environments, antibiogram, Nasarawa town, Nigeria.**INTRODUCTION**

Staphylococcus aureus is a Gram positive coccus that occur 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 flora of the skin, upper respiratory tract and intestinal tract. The pathogen is an opportunistic organism in man and animals and is the most frequent cause of hospital and community-associated infections (Prescott *et al.*, 2005). *S. aureus* can cause a range of illnesses 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). *S. aureus* cause disease through the production of toxins or through direct invasion and destruction of tissue(s) in adults and children (Medubi *et al.*, 2006).

S. aureus is the most common nosocomial pathogen. Nosocomial pathogens are organisms causing diseases that are acquired from the hospital environment within few days of admission into the hospital or other health

care facilities and is responsible for nosocomial infections (Medubi *et al.*, 2006). Nosocomial infections (i.e. infection acquired from the hospital environment, secondary to the patients, original conditions) have posed a scourge to patients, staff and visitors to hospital over the years and 1.4 million people across the world suffered from hospital acquired infections at any given time (Adebimpe *et al.*, 2011). The hospital exists as a closed community, it is therefore not surprising that certain microorganisms become dominant and may cause diseases. It seems obvious that the occurrence of nosocomial infection is related to the hospital milieu. These pathogens can be contracted from contaminated environmental surfaces and equipments of the hospital, through health care workers and even by the patients (Ijioma *et al.*, 2010). These pathogens can survive for extended periods and can persist despite attempts in removing them (Wren *et al.*, 2008).

Increasingly, nosocomial isolates are resistant to Multiple antibiotics. 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, 1998). Contamination of the hands of healthcare 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).

Drug resistance by *S. aureus* 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). The emergence of antibiotic resistant *S. aureus* is increasing extremely rapidly around the globe, creating a serious threat to the spread and treatment of infectious diseases (Oli *et al.*, 2013). In view of the public health importance of *S. aureus* in nosocomial infections, this work was designed to: 1) Isolate and identify *Staphylococcus aureus* from some hospital environments in Nasarawa town; 2) determine the antibiotic susceptibility profile of the *Staphylococcus aureus* isolates; 3) determine the antibiotic susceptibility patterns of the bacterial isolates; and 4) determine the multiple antibiotic resistance (MAR) indices of the isolates.

MATERIALS AND METHODS

The Study Area

This study was carried out in Nasarawa town. Nasarawa is a town in Nasarawa State in Northern part of Nigeria (NBS, 2009). It has an area of about 5, 704 km² with a population of 189, 835 as at the 2006 census (NBS, 2009). It is approximately 105km from Abuja, the Federal Capital Territory, 37 km from Keffi and 165 km from Lafia, the state capital. The town is located between latitude 8°21'58"N of the equator and longitude 7°5'58E of the Greenwich meridian (NBS, 2009).

Sampling Design and Techniques

The Sample Size

The sample size was determined using a prevalence of 11.85%, WHO prevalence rate of nosocomial infection as reported by Samuel *et al.* (2010). The sample size was

determined using the equation described by Naing *et al.* (2006).

$$n = \frac{Z^2 P (1-P)}{d^2}$$

Where: n= number of samples

p= prevalence rate of contamination of previous study = 11.85% = 0.1185

z= standard normal distribution at 95% confidence limit = 1.96

d= absolute desired precision of 5% = 0.05

Therefore,

$$n = \frac{1.96^2 \times 0.1185 (1-0.1185)}{0.05^2} = 160 \text{ samples}$$

Sample Collection

Samples for the study was collected from bedside of patients (BSP), door knob/handle (DN), operation Table (OPT), Nurses' hands (NSH), cupboard (CPD) and toilet seat (TST) using sterile swab sticks wetted with peptone water and transported to laboratory for analysis within one hour.

Isolation and Identification of *S. aureus*

The swabbed samples were inoculated into sterile bacteriological peptone water and incubated at 37°C for 24 h for enrichment after which the inoculated broth was sub-cultured onto mannitol salt agar (MSA) (HiMedia®, India) and incubated at 37°C for 24 h. The presumptive colonies of *S. aureus* were further sub-cultured onto mannitol salt agar (MSA) and repeatedly sub-cultured order to get pure culture. These isolates were preserved for further bacterial identification. The isolates were identified as *S. aureus* on the basis of Gram staining, colony morphology on mannitol salt agar (MSA) (HiMedia®, India), beta-hemolytic patterns on blood agar enriched with 5% (v/v) sheep blood, catalase test, DNase test, and coagulase tests (Japoni *et al.*, 2004). To perform agglutination tests, the pure colony of *S. aureus* were placed on the clean glass slide using sterile inoculation loop and a drop of respective reagents were added and mixed with the loop. For catalase and coagulase tests 3% hydrogen peroxide and fresh rabbit plasma were used respectively. For the DNase test, suspected colonies of *Staphylococcus aureus* were inoculated onto prepared DNase agar plates. The plates were incubated overnight at 37°C. Presence of DNase was tested by flooding plates with a weak 1N HCl. Polymerised DNA precipitated in the presence of 1N HCl gives an opaque medium. A positive organism shows clear zones around colonies (Japoni *et al.*, 2004).

Antibiotic Susceptibility Test of the Isolates

All the *S. aureus* isolates were subjected to antibiotic sensitivity testing by standard agar disc diffusion method on Muller-Hinton agar (OXOID, England) according to the Clinical and Laboratory Standards Institute (CLSI) recommendations (CLSI, 2012). Sensitivity pattern of the isolates to: ciprofloxacin (10µg), (gentamicin (10µg), streptomycin (30µg), norfloxacin (10µg),

chloramphenicol (30µg), amoxicillin (20µg), erythromycin 30µg), ampiclox (20µg), and levofloxacin (20µg) (Oxoid) and rifampicin (20µg) was evaluated (Antimicrobial Susceptibility Test System, UK).

After incubation, the test plates were examined for confluent growth and zone of inhibition. The diameter of each zone of inhibition was measured in millimeter (mm) using a transparent ruler on the underside of the plate. The results were interpreted using the Clinical Laboratory Standards Institute (CLSI) criteria (CLSI, 2012). Results obtained for each isolate were interpreted as: 1). Sensitive (S): if the observed zone of the inhibition diameter was equal or greater than CLSI sensitive diameter (mm); 2). Intermediate (I): if the observed zone of inhibition diameter fell within the intermediate range between the CLSI resistant and sensitive limits; 3). Resistant (R): if the observed zone of inhibition diameter was less than or equal to the CLSI resistant diameter (mm) according to the Clinical and Laboratory Standards Institute (CLSI) guideline; Performance Standards for Antimicrobial Susceptibility Testing (CLSI, 2012).

Determination of the Multiple Antibiotics' Resistance (MAR) Indices of the *S. aureus* isolates

The multiple antibiotic resistance (MAR) index was determined for each isolate by dividing the number of antibiotics to which the isolate(s) is resistant, by the total number of antibiotics tested (Olayinka *et al.*, 2004).

MAR Index= $\frac{\text{Number of antibiotics to which the isolate is resistant}}{\text{Total number of antibiotics tested}}$

Total number of antibiotics tested.

RESULTS

Table 1 Shows the prevalence (%) of *Staphylococcus aureus* obtained from the 3 hospitals sampled. On the whole, 160 samples were collected for the study. Of the 70(43.8%) samples collected from Henad Medical centre, 28(40.0%) of the samples yielded positive results for *Staphylococcus aureus*; a prevalence of 26.7% (16) was recorded for the 60 samples collected from Alpha Hospital. In the same vein, of the 30(18.8%) samples collected from the Polytechnic clinic, a prevalence of 26.7% was recorded. That is, only 8 samples were positive for *Staphylococcus aureus*.

Table 1: Prevalence of *Staphylococcus aureus* from Hospital Environments in Nasarawa Town.

Hospital	No.(%) of samples	No.(%) Positive
HMC	70(43.8%)	28(40.0%)
ALPH	60(37.5%)	16(26.7%)
TPCL	30(18.8%)	8(26.7%)
Total	160(100%)	52(32.5%)

Key: HMC = Henad Medical Centre; ALPH = Alpha Hospital; TPCL = The Polytechnic Clinic

Table 2 shows the antibiotic susceptibility profile of *Staphylococcus aureus* isolates obtained from Henad Medical Centre, Nasarawa. The antibiotic susceptibility profile showed that virtually all the isolates of *S. aureus* from hospital environment in Henad Medical Centre, Nasarawa were resistant to one or multiple antibiotics. Twenty eight 28(100%) of the *Staphylococcus aureus*

isolates were resistant to amoxil and ampiclox, 20 (71.4%) were resistant to erythromycin, 5 (17.9%) were resistant to norfloxacin. Least resistant was recorded observed for streptomycin, whereby only 2 (7.1%) of the isolates were resistant to the antibiotic. None of the isolates was resistant to ciprofloxacin, gentamicin, rifampicin, chloramphenicol and levofloxacin.

Table 2: Antibiotic Susceptibility Profile of *Staphylococcus aureus* Isolated from Henad Medical Centre, Nasarawa.

(n=28)

Antibiotic	Disc conc. (µg)	S	I	R
Ciprofloxacin	5	17(60.7%)	11(39.3%)	0(0.0%)
Gentamicin	30	16(57.1%)	12(42.9%)	0(0.0%)
Norfloxacin	10	9(32.1%)	14(50.0%)	5(17.9%)
Amoxil	20	0(0.0%)	0(0.0%)	28(100%)
Streptomycin	30	22(78.6%)	4(14.3%)	2(7.1%)
Rifampin	5	24(85.7%)	4(14.3%)	0(0.0%)
Erythromycin	15	5(17.9%)	3(10.7%)	20(71.4%)
Chloramphenicol	30	18(64.3%)	10(35.7%)	0(0.0%)
Ampiclox	20	0(0.0%)	0(0.0%)	28(100%)
Levofloxacin	5	15(53.6%)	13(46.4%)	0(0.0%)

Key: S= susceptible; I= intermediate; R= resistance

Table 3 shows the antibiotic susceptibility profile of *Staphylococcus aureus* obtained from Alpha Hospital, Nasarawa. The antibiotics susceptibility profile of the 16

isolates of *Staphylococcus aureus* obtained from Alpha Hospital, Nasarawa, showed that, all of the 16(100%) of the isolates were resistant to amoxil, 15(93.8%) were

resistant to ampiclox, 11 (68.8%) were resistant to erythromycin, 3(18.8%) were resistant to norfloxacin. Least resistance was observed for streptomycin, whereby, only 2(12.5%) of the isolates were resistant to

the antibiotic. None of the isolates was resistant to ciprofloxacin, gentamicin, rifampin, erythromycin and levofloxacin.

Table 3: Antibiotic Susceptibility Profile of *Staphylococcus aureus* Isolates from Alpha Hospital, Nasarawa. (n=16)

Antibiotic	Disc conc.(µg)	S	I	R
Ciprofloxacin	5	11(68.8%)	5(31.3%)	0(0.0%)
Gentamicin	30	14(87.5%)	2(12.5%)	0(0.0%)
Norfloxacin	10	10(62.5%)	3(18.8%)	3(18.8%)
Amoxil	20	0(0.0%)	0(0.0%)	10(100%)
Streptomycin	30	13(81.3%)	1(6.3%)	2(12.5%)
Rifampin	5	12(75.0%)	4(25.0%)	0(0.0%)
Erythromycin	15	4(25.0%)	1(6.3%)	11(68.8%)
Chloramphenicol	30	12(75.0%)	4(25.0%)	0(0.0%)
Ampiclox	20	0(0.0%)	1(6.3%)	15(93.8%)
Levofloxacin	5	10(62.5%)	6(37.5%)	0(0.0%)

Key: S= susceptible; I= intermediate; R= resistance

Table 4 shows the antibiotic susceptibility profile of *Staphylococcus aureus* obtained from the Polytechnic Clinic. The antibiotics susceptibility profile of the 8 isolates of *Staphylococcus aureus* obtained from the Polytechnic Clinic, showed that 7(87.5%) of the isolates were resistant to amoxil and ampiclox, 6(75.0%) were

resistant to norfloxacin. Least resistant was observed for streptomycin, whereby only 2(25.0%) of the isolates were resistant to the antibiotic. None of the isolates showed resistance to ciprofloxacin, gentamicin, rifampin, chloramphenicol and levofloxacin.

Table 4: Antibiotic Susceptibility Profile of *Staphylococcus aureus* Isolates from the Polytechnic Clinic. (n=8)

Antibiotic	Disc conc. (µg)	S	I	R
Ciprofloxacin	5	6(75.0%)	2(25.0%)	0(0.0%)
Gentamicin	30	8(100%)	0(0.0%)	0(0.0%)
Norfloxacin	10	4(50.0%)	0(0.0%)	4(50.0%)
Amoxil	20	1(12.5%)	0(0.0%)	7(87.5%)
Streptomycin	30	5(62.5%)	1(12.5%)	2(25.0%)
Rifampin	5	6(75.0%)	2(25.0%)	0(0.0%)
Erythromycin	15	0(0.0%)	2(25.0%)	6(75.0%)
Chloramphenicol	30	6(75.0%)	2(25.0%)	0(0.0%)
Ampiclox	20	1(12.5%)	0(0.0%)	7(87.5%)
Levofloxacin	5	5(62.5%)	3(37.5%)	0(0.0%)

Key: S= susceptible; I= intermediate; R= resistance

Table 5 shows the antimicrobial resistance patterns of *Staphylococcus aureus* isolates from hospital environments in Nasarawa town. Eight (8) resistance phenotypes were obtained, all from multiple resistance types with varying combinations of 2, 3 and 4 antibiotics.

No resistance phenotype was found with single antibiotic. The highest frequency (3) was found in combinations of 2 and 3 antibiotics. Multiple antibiotic resistance (MAR) index of *Staphylococcus aureus* is shown in figure 1.

Table 5: Antimicrobial Resistance Patterns of 52 isolates of *Staphylococcus aureus* from Hospital Environment in Nasarawa Town.

No. of antibiotics	Resistance pattern	No. of isolates
2	Amo, Amp	5(9.6%)
2	Amo, Ery	1(1.9%)
2	Nor, Amo	1(1.9%)
3	Amo, Ery, Amp	27(51.9%)
3	Amo, Str, Amp	5(9.6%)
3	Nor, Amo, Amp	5(9.6%)
4	Nor, Amo, Ery, Amp	6(11.5%)
4	Amo, Str, Ery, Amp	2(3.8%)

Symbols: Amo = Amoxil; Amp = Ampiclox; Ery = Erythromycin; Nor = Norfloxacin; Str = Streptomycin

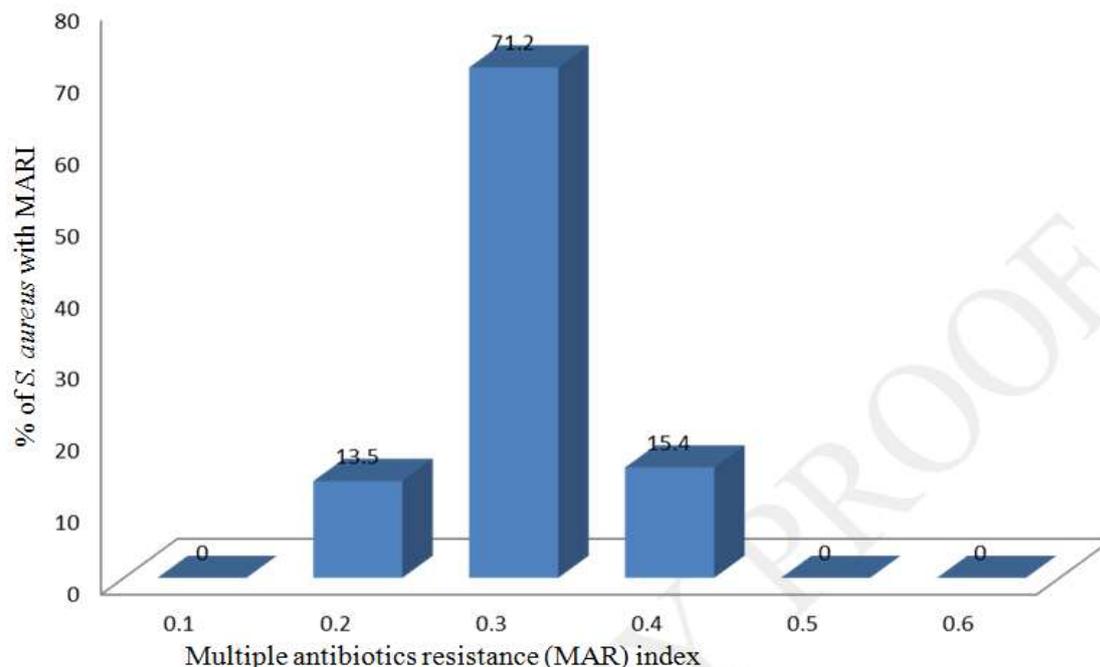


Figure 1: Multiple antibiotics resistance indices of *S. aureus* isolated from some hospital environments in Nasarawa town (100% of the isolated had MARI of 0.2 and above).

DISCUSSION

From the results obtained, out of 160 samples collected for this study, 52 samples were positive for *Staphylococcus aureus*. This shows a total of 32.5% prevalence rate. This is not surprising, however, considering that *Staphylococcus aureus* is a normal flora of the skin and naturally has wide occurrence (Medubi *et al.*, 2006). Out of the 52 positive samples, 28 were obtained from Henad Medical Centre, 16 from Alpha Hospital, and 8 from the Polytechnic Clinic. The prevalence of the organism was higher in Henad Medical Centre, followed by Alpha Hospital, and then the Polytechnic Clinic as shown in table 4. This agrees with the work done by Medubi *et al.* (2006) and is attributed to recontamination by carriers whose habit of putting their fingers into their nostrils and ears which contaminates fingers.

Studies among the three (3) hospitals sampled, revealed that the percentage prevalence rate of Henad Medical Centre was 40.0%. This may be because of the negligence of workers in observing personal hygiene such as hand washing with disinfectants after attending to a patient. The percentage prevalence rate for Alpha hospital and polytechnic clinic was 26.7% for both. The reason for this could not be readily attributed to unhygienic conditions within the hospital because the hospital environment was clean. This is not surprising, however, considering the level of contaminated items they worked with, and the possibility of contaminating their hands with the contaminated items. The lower prevalence rate of the organism observed in Alpha Hospital compared to the prevalence observed in Henad Medical Centre, may be attributed to the number of samples collected for the study. A relatively larger

sample size (70) was collected from Henad Medical Centre compared to the 60 collected from Alpha Hospital. Generally, over 50% of all the hospital personnel had *Staphylococcus aureus* on their hands confirming the wide spread nature of the organism as part of the normal flora.

The result of the antibiotic susceptibility tests showed that, all of the 52 positive isolates obtained from the three hospitals exhibited high susceptibility to ciprofloxacin, gentamicin, rifampicin, chloramphenicol and levofloxacin. However, the isolates were observed to have developed high resistance to amoxicillin, ampiclox and erythromycin. This is in agreement with the findings of Onanuga *et al.* (2005) and Hammuel *et al.* (2015) who observed a very high rate of resistance of *Staphylococcus aureus* to ampicillin, an antibiotic which belong to the same antibiotic class as ampiclox and amoxicillin. These inexpensive antibiotics are widely available to individuals in pharmacies without prescription from authorised health personnel (Newman *et al.*, 2006), and this lends credence to the indiscriminate use of antibiotics which promotes selective pressure favouring the emergence of resistant bacteria (Levy, 2001). Not only are these resistant bacterial strains potential causes of recurrent infections but they are also reservoirs of resistance genes that could be transferred to other pathogens. For this reasons, the antibiotic susceptibility trends seen with the *S. aureus* isolates may also occur with other bacterial pathogens.

Staphylococcus aureus obtained in this study were observed to have exhibited multiple antibiotics resistance to the antibiotics tested. On the whole, eight (8) resistance phenotypes were observed with varying

combinations of 2, 3 and 4 antibiotics. No resistance phenotype was found with single antibiotic. The highest frequency (3) was found in combinations of 2 and 3 antibiotics. This is in consonance with the findings of Onanuga *et al.* (2005) who observed 8 resistance phenotypes among *Staphylococcus aureus* isolated from healthy women in Abuja, Nigeria. Hundred percent (100%) of the isolates in this study were found to have had multiple antibiotics resistance (MAR) index of 0.2 and above. MAR index gives an indirect suggestion of the probable source of an organism. MAR index greater than 0.2 indicates that an organism must have originated from an environment where there are no strict regulations concerning prescriptions and usage of antibiotics (Paul *et al.*, 1997; Olayinka *et al.*, 2004). The presence of multiple drug resistant strains of *S. aureus* among the isolates may be attributed to antibiotic misuse arising from self-medication in suspected bacterial infections (Newman *et al.*, 2006). Self-medication prevents early reporting of patients to hospitals at the onset of disease symptoms, except where complications have occurred. Also, some other factors such as unnecessary prescriptions and substandard antibiotics could lead to the emergence of antibiotic resistance among organisms (Newman *et al.*, 2006).

CONCLUSION

From the results obtained in this study, the Henad Medical Centre was found to have had high prevalence rate of *Staphylococcus aureus* than Alpha Hospital and the Polytechnic Clinic. It is obvious in this work that, isolates of *S. aureus* were resistant to most commonly prescribed antibiotics except for ciprofloxacin, gentamicin, rifampicin, chloramphenicol, and levofloxacin to which the isolates were observed to be highly susceptible. High levels of antimicrobial resistance to other antibiotics amoxicillin, ampicillin and erythromycin used were observed. Notably, isolates from the three hospitals seems to be highly susceptible to ciprofloxacin, gentamicin, rifampicin, chloramphenicol and levofloxacin.

Recommendations

1. Personal cleanliness and environmental sanitation measures should be put in place in hospitals in order to prevent Staphylococcal contamination.
2. Disinfection of surfaces with potent disinfectants in order to break the chain of transmission of pathogens should be encouraged.
3. There is need to legislate and enforce laws to limit the prescription and dispensing of antibiotics and other drugs to only qualified professionals.
4. Proper health education regarding the menace of indiscriminate and over-the-counter use of antibiotics should be put in place. Also, antibiotics sensitivity testing, as well as adherence to prescription and dosage of antibiotics will help in no small measure in curtailing the menace of antimicrobial resistance by pathogens.

5. Also, antibiotic sensitivity tests should be conducted on isolates from patients suffering from bacterial infections before antibiotics are prescribed.

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