British Microbiology Research Journal
14(6): 1-8, 2016, Article no.BMRJ.25360
ISSN: 2231-0886, NLM ID: 101608140

SCIENCEDOMAIN *international*
www.sciencedomain.org

Isolation and Antibiogram of *Staphylococcus aureus* from Patients Admitted at Specialist Hospital Okitipupa, Ondo State, Nigeria

E. Nmema Eucharia[1*]

[1]Department of Biological Sciences, Ondo State University of Science and Technology (OSUSTECH), PMB 353, Okitipupa 350002, Ondo State, Nigeria.

Author's contribution

The sole author designed, analyzed and interpreted and prepared the manuscript.

Article Information

DOI: 10.9734/BMRJ/2016/25360
Editor(s):
(1) Rashedul Islam, Department of Biological Sciences, Inha University, South Korea.
Reviewers:
(1) Cucunawangsih, University of Pelita Harapan, Indonesia.
(2) Jarosław Bystron, Wroclaw University of Environmental and Life Sciences, Poland.
(3) Masaaki Minami, Nagoya City University, Japan.
(4) Monthon Lertcanawanichakul, Walailak University, Thailand.
(5) Paul E. Imade, University of Benin, Nigeria.
Complete Peer review History: http://sciencedomain.org/review-history/14648

Original Research Article

Received 29th February 2016
Accepted 26th April 2016
Published 16th May 2016

ABSTRACT

Aims: To isolate and determine the antimicrobial resistance and susceptibilities of *Staphylococcus aureus* from patients hospitalized at State Specialist Hospital, Okitipupa, Ondo State, Nigeria.
Study Design: Prospective cross sectional.
Place and Duration of Study: Department of Biological Sciences, Ondo State University of Science and Technology, Okitipupa, between March and April 2015.
Methodology: Forty three (43) samples were obtained from nasal cavity, skin, wound, or umbilical cord of 20 patients aged 4 days to 80 years. Isolates were identified by cultural characteristics on Mannitol Salt Agar, Nutrient agar and biochemical tests. Antibiotic susceptibility testing was performed by the disk diffusion method (Kirby-Bauer). Multiple antibiotic resistance (MAR) index for each *S. aureus* was determined.
Results: Seventeen *S. aureus* isolates were identified. Nine *S. aureus* strains recovered from 18 samples from male patients was higher than 8 strains recovered from 25 samples from females. 21-30 age-group yielded the highest number of *S. aureus* isolates (7 strains), followed by 31-40

**Corresponding author: E-mail: ukaria2003@yahoo.com;*

age group (5 strains). Eleven (64.7%) strains showed multiple resistances ranging from 55.6% to 88.8%, four (23.5%) strains showed multiple resistances ranging from 33.3% to 44.4%, one (5.9%) strain (N1) showed 100% resistance, while one (5.9%) strain (O1) showed 100% sensitivity. 70.6% of the isolates had a MAR index above 0.5 indicating that they probably originated from an environment where antibiotics are frequently used or abused. The highest resistance was offered to the β-lactam antibiotics, including oxacillin (88.2%), cloxacillin (82.4%) and amoxicillin/clavulanic acid (76.5%); and the cephalosporins: cefuroxime (70.6%) and ceftazidime (64.7). Resistance to ceftriaxone (cefuxitin) was the lowest (23.5%). Resistance to erythromycin was high (70.6%), but gentamicin and ofloxacin were offered relatively low resistance (41.2%) respectively.

Conclusion: The study revealed multiple antibiotic resistances in *S. aureus* isolated from hospitalized patients. The preponderance of multiple antibiotic *S. aureus* in the hospital environment continues to present challenges to the health sector. The high rates of resistance to the β-lactams – oxacillin, cloxacillin, amoxicillin-clavulanic acid and cephalosporins highlight the decreasing importance of β-lactams in the therapy of *S. aureus* infections.

Keywords: Staphylococcus aureus; antibiogram; MAR index; hospital environment; Nigeria.

1. INTRODUCTION

Staphylococcus aureus is a Gram-positive, facultative anaerobic, unencapsulated cocci which occur in clusters. The bacterium occurs as a commensal on the human anterior nares and on the skin (and less commonly in other locations) of the human body [1,2]. It is a normal inhabitant of the healthy lower reproductive tract of women [3,4]. *S. aureus* colonizes approximately 20% to 30% of the human population [5], and is reported to be found in 25% to 27% of health workers [6]. The presence of *S. aureus* does not always indicate infection. *S. aureus* can survive from hours to weeks, or even months, on dry environmental surfaces, depending on strain [7].

Though the bacterium occurs as normal flora, it can infect tissues when the skin or mucosal barriers have been breached. This can lead to many different types of infections ranging from furuncles and carbuncles to life-threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome (TSS), bacteremia, and sepsis [8,9]. *S. aureus* is still one of the five most common causes of nosocomial infections and is often the cause of postsurgical wound infections [10]. Each year, some 500,000 patients in United States' hospitals contract a staphylococcal infection [11]. The control of infections caused by *S. aureus* relies heavily on intensive use of antibiotic drugs. However, there is increasing evidence of drug resistance by this microorganism [12].

The treatment of choice for *S. aureus* infection is penicillin, a β- lactam. Penicillin is bactericidal by inhibiting the formation of peptidoglycan cross-linkages that provide the rigidity and strength in a bacterial cell wall. In most countries, but penicillin resistance is extremely common. This is because many members of the genus *Staphylococcus* produce penicillinase (a β- lactamase), which inactivate penicillins, making them naturally resistant to these antibiotics [13]. Following the emergence of penicillin resistance, penicillinase-resistant β-lactam antibiotics such as oxacillin or flucloxacillin were developed and used as first-line therapy. However resistance to penicillinase-resistant β-lactams soon emerged in many bacterial strains and has become a global health problem [14].

Staphylococcus aureus continues to offer challenges to medical science in the area of resistance to chemotherapeutic agents leading to treatment failures using common antibiotics [15]. Adejuwon et al. [16] reported the presence of *S. aureus* in pus from acne which was sensitive to gentamicin, tetracycline, amoxicillin, amoxicillin/clavulanic acid, chloramphenicol and sulphamethoxazole but resistant to ampicillin, erythromycin, cloxacillin, cotrimoxazole, streptomycin and penicillin.

Attempts to control diseases caused by *S. aureus* through the use of antibiotics have resulted in increased prevalence of resistant strains of the organism [17,18]. Therefore, in order to effectively treat infections caused by *S. aureus*, culture and antibiotic sensitivity tests must first be performed. Once culture and sensitivity results confirm the type of bacterial infection and sensitivity pattern, treatment may be modified [19].

An antibiogram is the result of an in vitro sensitivity test of an isolated bacterial strain to different antibiotics. The antibiogram shows the profile of the antimicrobial resistance and susceptibility of a particular microorganism. The correlation of *in vitro* to *in vivo* is often high enough for the test to be clinically useful. In the hospital, antibiograms are commonly used to help guide empirical antimicrobial treatment and are an important component of detecting and monitoring trends in antimicrobial resistance [20].

Strategies for monitoring antibiotic susceptibility patterns should include periodic isolation and testing of isolates for antibiotic susceptibility in all localities, using a state-owned hospital as a sampling center for a cross sectional study. Results of cross sectional studies usually provide a good overview of situations in the entire community.

The aims of the present study were to isolate and determine the profile of antimicrobial resistance and susceptibilities of *S aureus* isolated from patients hospitalized at State Specialist Hospital, Okitipupa, Ondo State, Western Nigeria. No previous study of this nature has been carried out in Okitipupa L.G.A in Ondo South senatorial district. This study seeks to provide data on the resistance pattern for *S. aureus* isolated from the hospital in Okitipupa.

2. MATERIALS AND METHODS

2.1 Samples Collection, Handling, Culture and Identification

Samples were collected from State Specialist Hospital, Okitipupa from March to April 2015. Sterile cotton swabs moistened in sterile distilled water were used to collect samples from the skin, nostrils, wound exudates or umbilical cord excision sites of hospitalized patients. The swabs sticks were inserted into their tubes and transported immediately into the Microbiology laboratory for analysis. Prior to sample collection, permission was obtained from the Hospital Authorities through a written application. The patients also had to give their consent before samples were collected from them.

All swab samples were directly inoculated onto Nutrient agar and Mannitol salt agar (MSA) plates. The plates were incubated at 37℃ for 24 h, and then examined for colony characteristics. Colonies that were white, round, smooth, substantial and opaque on Nutrient agar, and colonies producing a golden-yellow pigment on MSA were presumptively considered *S. aureus* and subsequently sub-cultured on nutrient agar to obtain pure, discreet colonies. Seventeen pure colonies (named A1, B1, B2, C2, G2, H2, I3, N1, O1, O2, P1, P2, Q1, Q2, T2, V2, V2) that yielded Gram positive clustered cocci, and reacted positively to coagulase and catalase tests were identified as *S. aureus* [21,22].

2.2 Antimicrobial Susceptibility Testing

The isolates were screened using the disc diffusion method [23]. Gram positive antibiotic discs (Rapidflex) included ceftazidime 30 µg, cefuroxime 30 µg, ceftriaxone 30 µg, cloxicillin 5 µg, amoxicillin/clavulanic acid 30 µg, gentamicin 10 µg, erythromycin 30 µg, ofloxacin 5 µg. Oxacillin (1 µg) obtained from Oxoid Ltd Britain was also screened.

Antimicrobial susceptibility tests were performed for all isolates according to the criteria of the Clinical and Laboratory Standards Institute (CLSI) [24]. Bacterial suspensions were prepared, adjusted to the 0.5 McFarland Standards, and inoculated onto Mueller-Hinton agar (Oxoid) by surface swabbing. Using sterile forceps, the antibiotic-containing discs were placed aseptically on the inoculated plates and left on the table for 1 hour for proper diffusion to occur. The plates were iincubated in an inverted position, at 35℃ for 16-18 hours and thereafter examined for clear zones of inhibition. Inhibition zone diameters (IZD) around each antibiotic disk (if any), were measured using a transparent ruler, and recorded in millimeters (mm). A standardized table was used to determine if the bacterium was “Resistant”, “Intermediate” or “Sensitive”.

2.3 Determination of Multiple Antibiotic-Resistance (MAR) Index

Multiple antibiotic-resistance (MAR) index of each *S. aureus* isolate was calculated using the formula: MAR Index = a/b, where a is number of antibiotics the isolate is resistant to; and b is number of antibiotics tested.

3. RESULTS

3.1 Isolation of *S. aureus*

A total of 43 samples were collected from skin, nares, wound or unbilical cord scar of twenty

patients and analyzed. Seventeen (17) *S. aureus* isolates were recovered from the samples. The skin samples yielded 8 (47.06%) of the *S. aureus* isolates, nasal samples yielded an equal number of *S. aureus* isolates: 8 (47.06%), while a traumatic wound yielded 1 (5.88%). Sex-based analysis revealed that for male patients, nine *S. aureus* isolates were recovered from eighteen samples (9/18), while for females, eight *S. aureus* isolates were recovered from twenty-five samples (8/25). Age-based analysis showed rates of *S. aureus* isolation among the age groups were: 21-30 years (7 isolates), 31-40 years (5 isolates), 4 days -10 years (2 isolates), 71- 80 years (2 isolates), and 51-60 years (1 isolate). The presence of *S. aureus* from the skin of 4 day old infants and not from their umbilical cord wounds implies that skin of infants as young as 4 days can be colonized by *S. aureus* in consonance with previous views [2].

3.2 Antibiotic Susceptibility Testing of *S. aureus* Isolates

3.2.1 Percentage of resistances, intermediates and sensitivities of *S. aureus* isolates

The seventeen (17) *S. aureus* isolates showed varying degrees of susceptibilities and resistances to the antibiotics. The percentage resistances, intermediates and sensitivities of each *S. aureus* isolate to the antibiotics are represented by a stacked column chart (Fig. 1).

The chart in Fig. 1 clearly depicts the degrees of resistances, intermediates and sensitivities shown by each *S. aureus* isolate to the antibiotics. Strain N1 showed resistance to all the antibiotics used (100% resistance), and strain O1 showed sensitivity to all the antibiotics used (100% sensitivity). Eleven (64.7%) isolates showed multiple resistances ranging from 55.6% to 88.8%. Four (23.5%) isolates showed multiple resistances ranging from 33.3% to 44.4%.

3.2.2 Multiple Antibiotic-Resistance (MAR) indices *S. aureus* isolates

Multiple antibiotic-resistance (MAR) index was calculated using the formula: MAR Index = a/b, where a is number of antibiotics the isolate is resistant to; and b is number of antibiotics tested. MAR Indices of the isolates are presented in Table 1.

Table 1. Multiple Antibiotic Resistance (MAR) indices of *S. aureus* isolates

S. aureus isolate (Code number)	MAR index
A1	0.89
B1	0.56
B2	0.56
C2	0.67
G2	0.67
H2	0.67
I3	0.67
N1	1.00
O1	0.00
O2	0.44
P1	0.44
P2	0.89
Q1	0.44
Q2	0.33
T2	0.89
V1	0.67
V2	0.78

MAR index shows the level of multiple antibiotic-resistance attained by a particular bacterial strain. From data presented in Table 1, MAR Indices of the isolates varied from 0.00 (not resistant to any antibiotic tested) to 1 (resistant to all antibiotics tested). 70.6% of the isolates had a high MAR index above 0.5.

3.2.3 Resistance profile of *S. aureus* isolates

The number and percentage of *S. aureus* isolates resistant to each antibiotic used is presented on Table 2.

Table 2. Number (Percentage) of *S. aureus* isolates resistant to each antibiotic

Antibiotics	Number (%) of resistant strains
CAZ	11 (64.7)
CRX	12 (70.6)
CTR	4 (23.5)
OX	15 (88.2)
CXC	14 (82.4)
AUG	13 (76.5)
GEN	7 (41.2)
ERY	12 (70.6)
OFL	7 (41.2)

Key: CAZ- ceftazidime, CRX- cefuroxime, CTR- ceftriaxone, OX- oxacillin, CXC- cloxacillin, AUG- amoxicillin/clavulanic acid, GEN- gentamicin, ERY- erythromycin, OFL- ofloxacin

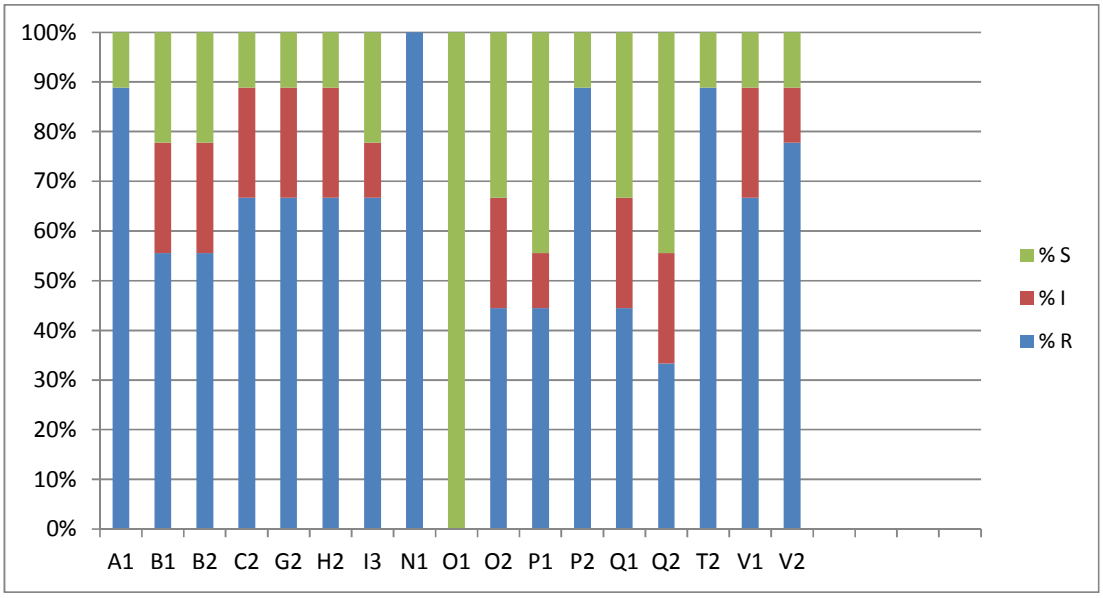

Fig. 1. Percentage resistances (R), intermediates (I) and sensitivities (S) shown by *S. aureus* isolates (A1 –V2) to the antibiotics Axis-Y: percentage of susceptibility. Axis-X: isolate number

The Figure shows the overall susceptibility of each isolate to nine antibiotics. E.g isolate B1 was resistant to 5 (55.6%) antibiotics, sensitive to 2 (22.2%) and intermediate to 2 (22.2%)

Table 2 shows how each antibiotic fared with the isolates. For instance, to ceftazidime (CAZ) 64.7% of all the isolates were resistant.

The highest resistance was offered to the β-lactam antibiotics, including oxacillin (88.2%), cloxacillin (82.4%) and amoxicillin/clavulanic acid (76.5%); and the cephalosporins: cefuroxime (70.6%) and ceftazidime (64.7). However, resistance to ceftriaxone (cefuxitin) was the lowest (23.5%). Resistance to erythromycin was high (70.6%), but gentamicin and ofloxacin were offered relatively low resistance (41.2%).

4. DISCUSSION

The recovery of *S. aureus* from patients varied among the sexes and age groups. Sex-based analysis revealed that for male patients, nine *S. aureus* isolates were recovered from eighteen samples (9/18), while for females, eight *S. aureus* isolates were recovered from twenty-five samples (8/25). Age-based analysis showed rates of *S. aureus* isolation among the age groups were: 21-30 years (7 isolates), 31-40 years (5 isolates), 4 days -10 years (2 isolates), 71- 80 years (2 isolates), and 51-60 years (1 isolate).

Results from the present study revealed multiple antibiotic resistance (MAR) in *S. aureus* strains isolated from Specialist Hospital, Okitipupa. 70.6% of the isolates had a MAR index above 0.5 indicating that they probably originated from an environment where antibiotics are frequently used or abused. Multiple resistance in *S. aureus* seems to be a longstanding problem yet to be solved, since a similar finding was reported from Zaria, Nigeria in 2003 [25].

The resistance profile showed a high rate of resistance to β-lactam antibiotics including oxacillin (88.2%), cloxacillin (82.4%), amoxicillin/clavulanic acid (76.5%), and the cephalosporins: cefuroxime (70.6%) and ceftazidime (64.7). However, resistance to ceftriaxone (cefuxitin) was the lowest (23.5%). Resistance to β-lactam antibiotics in this study may be due to hyper-production of β-lactamase enzymes which inactivate this class of antibiotics [13,14]. Resistance to erythromycin was high (70.6%), but gentamicin and ofloxacin were offered relatively low resistance (41.2%). Findings in this study agree with previous workers who reported high rates of antibiotic resistance in *S. aureus* [15,16,26]. However, the findings differ from reports from another part of Nigeria where phenotypic resistance to oxacillin was 40.3%, cefuxitin 46.5%, gentamicin 11.5% and erythromycin 21.2% [27].

The findings of this study are in consonance with reports that resistant microbes abound in the hospital environments because of antibiotic

treatment regimes administered to hospital patients. In hospitals, inappropriate use of antimicrobial agents tends to create a selective pressure that promotes the emergence of resistant strains and predisposes patients to colonization with such organisms. The carriage of *S. aureus* on the bodies, especially hands of 25%-27% of health workers reported by previous workers [6] is an important factor in transferring resistant microbes to patients hospitalized in the wards.

The dominance of antibiotic resistant pathogens in the hospital environment is a phenomenon that must be approached in a more proactive manner. More emphasis should be placed on proper and regular hand washing by health workers, sterilization/disinfection of instruments, materials and surfaces, as well as maintenance of sanitary conditions in the wards. All these will greatly reduce the occurrence and transfer of resistant microbes in the hospitals. Regular washing of bed clothes with soaps and disinfectants, and use of sterile hand gloves when handling patients will ensure that antibiotic resistant microbes do not survive and are not transferred from patient to patient.

Research efforts to develop alternative treatments for resistant bacterial infections should attract more funds from the government and stakeholders on the private sector. Special attention should be focused on medicinal plant extracts which have been used as antimicrobials for centuries with no development of resistance. Vaccine development is another area that should not be undermined in the search for solution for antimicrobial resistance in bacterial pathogens. More research effort is needed in the area of DNA vaccines, which is reported to be effective, with no adverse effects in experimental animals [28,29].

5. CONCLUSIONS

High MAR indices above 0.5 shown by 70.6% of *S. aureus* strains isolated from Specialist Hospital, Okitipupa, reveals the challenges in the area of empirical therapy for diseases caused by *S. aureus.* The high rates of resistance to the β-lactam antibiotics including oxacillin (88.2%), cloxacillin (82.4%), amoxicillin/clavulanic acid (76.5%), and the cephalosporins: cefuroxime (70.6%) and ceftazidime (64.7) highlights the decreasing importance of these drugs in antimicrobial therapy for *S. aureus* infections. Antibiotic resistance is highest where a majority of the population is poor and ignorant of appropriate use of antibiotics as is the case with the study area in this investigation. The authorities in the health sector should mount public enlightenment programs to educate the masses on the dangers of abusing antimicrobials, as well as the appropriate ways to use them. Development of and strict adherence to sound antibiotic policies in the health sector will help slow the emergence of drug resistance.

COMPETING INTERESTS

Author has declared that no competing interests exist.

REFERENCES

1. Kluytmans J, van Belkum A, Verbrugh H. Nasal carriage of *Staphylococcus aureus*: epidemiology, underlying mechanisms, and associated risks. Clin. Microbiol. Rev. 1997;10(3):505–20.
2. Cole AM, Tahk S, Oren A, Yoshioka D, Kim YH, Park A, Ganz T. Determinants of *Staphylococcus aureus* nasal carriage. Clin. Diagn. Lab. Immunol. 2001;8(6): 1064–9.

   DOI: 10.1128/CDLI.8.6.1064-1069.2001
3. Senok AC, Verstraelen H, Temmerman M, Botta GA. Probiotics for the treatment of bacterial vaginosis. Cochrane Database Syst. Rev. 2009;7(4):CD006289.

   DOI: 10.1002/14651858.CD006289.pub2.
4. Hoffman BL. Williams gynecology, 2nd edition. New York: McGraw-Hill Medical; 2012.
5. Tong SY, Davis JS, Eichenberger E, Holland TL, Fowler VG. *Staphylococcus aureus* infections: Epidemiology, patho-physiology, clinical manifestations, and management. Clin. Microbiol. Rev. 2015;28(3):603–61.

   DOI: 10.1128/CMR.00134-14
6. Imanifooladi AA, Sattari M, Peerayeh SN, Hassan ZM, Hossainidoust SR. Detection of *Staphylococcus aureus* producing enterotoxin isolated from skin infections in hospitalized patients. Pakist. J. Biol Sci. 2007;10:502-5.

   DOI: 10.3923/pjbs.2007.502-505

7. Cimolai N. MRSA and the environment: Implications for comprehensive control measures. Eur. J. Clin. Microbiol. Infect. Dis. 2008;27(7):481–93.
   DOI: 10.1007/s10096-008-0471-0
8. Taiwo SS, Onile BA, Akanbi AA. Methicillin-resistant *Staphylococcus aureus* (MRSA) isolates in Ilorin, Nigeria. Afr. J. Clin. Exp. Microbiol. 2004;5(2):189–97.
9. Chantratita N, Wikraiphat C, Andhavanant S, Wongsuvan G, Ariyaprasert P, Suntornsut P, et al. Comparison of community-onset *Staphylococcus argenteus* and *Staphylococcus aureus* sepsis in Thailand: A prospective multicentre observational study. Clin. Microbiol. Infect; 2016. (In press).
   DOI: 10.1016/j.cmi.2016.01.008
   [Epub ahead of print]
10. Holmes A, Ganner M, McGuane S, Pitt TL, Cookson BD, Kearns AM. *Staphylococcus aureus* isolates carrying Panton-Valentine leucocidin genes in England and Wales: Frequency, characterization, and association with clinical disease. J. Clin. Microbiol. 2005;43(5):2384–90.
11. Bowersox J. Experimental staph. Vaccine Broadly Protective in Animal Studies; 1999. NIH. Archived from the original on 5 May 2007.
12. Lowy FD. Antimicrobial resistance: The example of *Staphylococcus aureus*. J. Clin. Invest. 2003;111:1265-73.
13. Laurence DR, Benneth PN, Brown MJ. Chemotherapy of infections. In: Clinical pharmacology. 8th ed. Edinburgh. London, N.Y: Churchill Livingstone; 1999.
14. Chambers HF, Deleo FR. Waves of resistance: *Staphylococcus aureus* in the antibiotic era. Nat. Rev. Microbiol. 2009;7:629–41.
   DOI: 10.1038/nrmicro2200
15. Adu F, Sixtus BBMS, Agyare C, Gbedema SY, Boamah VE, George DF. Antibiotic resistance patterns of *Staphylococcus aureus* isolated from patients in three hospitals in Kumasi, Ghana. J. Bacteriol. Res. 2013;5(3):35-40.
16. Adejuwon AO, Ajayi AA, Akintunde OO, Olutiola PO. Antibiotics resistance and susceptibility pattern of a strain of *Staphylococus aureus* associated with acne. Int. J. Med. Medic. Sci. 2010;2(9): 277-80.
17. Levy S. Antibiotic resistance: Consequences of inaction. Clin. Infect. Dis. 2001;33(3):124-29.
18. Crowder MW, Spencer J, Vila AJ. Metallo-β-lactamases: Novel weaponry for antibiotic resistance in bacteria. Acc. Chem. Res. 2006;39(10):721-8.
19. Onanuga A, Oyi AR, Olayinka BO, Onaolapo JA. Prevalence of community-associated multi-resistant *Staphylococcus aureus* among healthy women in Abuja, Nigeria. Afr. J. Biotech. 2005;4:942-5.
20. Pakyz AL. The utility of hospital antibiograms as tools for guiding empirical therapy and tracking resistance. Insights from the Society of Infectious Diseases Pharmacists. Pharmacotherapy. 2007; 27(90):1306-12.
21. Cheesbrough M. Medical laboratory manual for tropical countries. Volume 11: Microbiology Tropical Health Technology, Cambridgeshire; 1994.
22. Cowan ST, Steel KJ. Manual for the identification of medical bacteria. Barrow GI, Feltham RKA. 3rd ed. Cambridge University Press; 1993.
23. Bauer AW, Kirby WMM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. American Journal of Pathology. 1966;45:493–6.
24. Clinical Laboratory Standards Institute (CLSI) M100-S20. Performance standards for antimicrobial disk susceptibility tests. Informal Supplement; 2010.
25. Olayinka BO, Olayinka AT. Methicillin resistance in staphylococcal isolates from clinical and asymptomatic bacteriurea specimens: Implications for infection control. Afr. J. Clin. Exp. Microbiol. 2003;4(2):79-90.
26. Nmema EE. Peculiar pattern of antibiotic resistance in bacteria isolated from various sources in South-East Nigeria and the implications in health and economy. J. Appl. Sci. Environ. Manage. 2013;17(4): 529-34.
   Available:www.ajol.info
   Available:www.bioline.org.br/ja
27. Olowe OA, Olayinka OK, Taiwo SS, Ojurongbe O, Opaleye OO, Bolaji OS, et al. Phenotypic and molecular characteristics of methicillin-resistant *Staphylococcus aureus* isolates from Ekiti State, Nigeria. Infect Drug Resist. 2013;6:87-92.
   DOI: 10.2147/IDR.S48809

28. Naval Medical Research Center U.S.A. Silver Spring, Maryland. Immunology Genetic Immunization against Biothreat Agents; 2005.
Available:http://www4.nationalacademies.org/rapNsfTitle/56.61.05.B5382?opendocument

29. Martin T, Parker SE, Hedstron R, Le T, Hoffman SL, Norman J, Hobart P, Lew D. Plasmid DNA malaria vaccine: The potential for genomic integration after intramuscular injection. Hum – Gene-Ther. 1999;10(5):759-69.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/14648