British Microbiology Research Journal
11(5): 1-7, 2016, Article no.BMRJ.18278
ISSN: 2231-0886, NLM ID: 101608140

SCIENCEDOMAIN *international*
www.sciencedomain.org

Molecular Detection of Panton Valentine Leukocidine Gene among Beta-lactamase Producing *Staphylococcus aureus* Isolated from Clinical Samples in Ondo State, Nigeria

C. O. Esan[1], O. M. David[2] and O. Famurewa[2*]

[1]Medical Microbiology Laboratory, Health Centre, Ekiti State University, P.M.B. 5363, Ado-Ekiti, Nigeria.
[2]Department of Microbiology, Ekiti State University, P.M.B. 5363, Ado-Ekiti, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author OF designed the study. Author COE wrote the protocol and wrote the first draft of the manuscript. Authors COE, OMD and OF managed the analyses of the study and literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BMRJ/2016/18278
Editor(s):
(1) Luis Martinez-Sobrido, University of Rochester, School of Medicine and Dentistry, NY, USA.
(2) En Tao Wang, Departamento de Microbiología, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, México and State Key Laboratory of Agrobiotechnology and College of Biological Sciences, China Agricultural University, Beijing, China (invited professor).
Reviewers:
(1) Gonsu Kamga Hortense, University of Yaounde, Cameroon.
(2) Nnamdi Oli, Nnamdi Azikiwe University, Nigeria.
Complete Peer review History: http://sciencedomain.org/review-history/12313

Original Research Article

Received 13th April 2015
Accepted 8th October 2015
Published 16th November 2015

ABSTRACT

Background: In recent time *Staphylococcus aureus* has been implicated not only in hospital-acquired but also in community-acquired infections. Some clonal types of *S. aureus* that produce β-lactamase have evolved. Panton-Valentine leukocidine (*pvl*) gene among β-lactamase-producing *S. aureus* from clinical samples in Ondo State, Nigeria has not been reported; hence the objective of this study.
Methods: Clinical samples were collected from secondary and tertiary hospitals in the study area. Amplification and detection of *nuc* gene was used to confirm the identity of the isolates while polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) of the coagulase gene and pulsed field gel electrophoresis (PFGE) were further carried out on the

**Corresponding author: E-mail: ofamurewa@gmail.com;*

isolates. Production of β-lactamase was also detected in the isolates using iodometric method while disc diffusion method was used to determine the sensitivity of the isolates to selected antibiotics. The presence of *pvl* genes among the β-lactamase producers was detected by PCR.
Results: A total of 58 isolates were biochemically confirmed as *S. aureus* out of which only 21 were β-lactamase producers. Out of the 21 β-lactamase-producing isolates 90.48% (n=19) possessed the *nuc* gene. Molecular screening of the isolates showed that only one out of the isolates did not possess the coagulase gene. Nine out of the isolates possessed *pvl* gene among β-lactamase-producing *S. aureus.* All the isolates were resistant to penicillin and tetracycline while 33.3% and 19.04% were resistant to chloramphenicol and erythromycin respectively. Oxacillin, cefoxitin, clindamycin and fusidic acid completely inhibited the growth of the isolates. Gentamicin and ciprofloxacin were effective on only 4.76% of the isolates.
Conclusion: The prevalence of oxacillin resistance among *S. aureus* is very low.

Keywords: Antibiotics; β-lactamase; community-acquired infections; PCR-RFLP; pvl gene; resistance; Staphylococcus aureus.

1. INTRODUCTION

Staphylococcus aureus is a Gram-positive, facultative anaerobic, catalase- and coagulase-positive, coccus shaped bacterium. Though a normal microbiota of human skin, it is one of the most commonly isolated human bacterial pathogens. *Staphylococcus aureus* causes various infections ranging from mild to life-threatening ones. The organism has been implicated in different local and systemic infections [1] acquired from both the community and hospital environments globally. *Staphylococcus aureus* colonises the skin and mucosal surfaces of humans. The anterior nares of the nose are the most consistent carriage site in humans [2]. Death rate as a result of *S. aureus* infection ranges between 13% and 34% [3,4]. Among other things age, comorbid conditions, and high bacterial infection burden have been identified as the main risk factors responsible for high mortality due to the infection of this difficult-to-treat human pathogen [4-6].

The recent increase in antibiotic-resistant bacteria has increased pressure on both health care facilities and workers as a major public health problem. Cross-resistance, mutation and transfer of genetic materials have been reported to account for the upsurge in the resistance. The occurrence of multiple antibiotic resistance among *S. aureus* has been reported to increase from 2% in 1974 to 64% in 2004 [7] with ever increasing tendencies particularly in the low-income nations.

Wide varieties of the virulence factors contribute to the severity of infections caused by *S. aureus.* The ability to alter the virulence factors and the ease of antibiotic resistance acquisition make this organism a re-emerging human bacterial pathogen. The interface between antibiotic resistance in *S. aureus* and virulence has been reported to increase morbidity and mortality and makes the treatment of this pathogen a serious clinical challenge [8]. The expression of *pvl* gene has been associated with community-acquired methicillin resistant *S. aureus* (CA-MRSA). Wang et al. [9] reported more than 100% increase in CA-MRSA. High antimicrobial resistance has also been reported in CA-MRSA. Poor or no health care policies and infection control measures have been identified to be responsible for the high incidence of *pvl* genes among isolates of the organism [10].

Antibiotic resistance and detection of pathogenic genes in *S. aureus* from clinical samples have been grossly underreported in Nigeria. The aim of this study was to evaluate the presence of *pvl* gene among *S. aureus* isolated from clinical samples in Ondo State, Nigeria.

2. MATERIALS AND METHODS

2.1 Sources of Samples and Isolation of *S. aureus*

The sources of samples included nasal swabs from the apparently healthy subjects (n=24) and health care personnel (n=11) while samples from clinical sources included: Urine (n=4), wound (n=25), blood (n=8), pus/discharge (n=12), semen (n=5), vagina swab (n=6) and throat swab (n=3). The samples were inoculated on mannitol salt agar (Oxoid, Basingstoke, and Hampshire, UK). Colonies with brilliant yellow pigment were sub-cultured onto nutrient agar and the pure discrete colonies were transferred onto slants for further examination. The Gram's reaction and

biochemical tests were conducted on isolates. Coagulase (using rabbit plasma) and catalase tests were conducted. Also the isolates were cultured in thioglycollate broth and incubated anaerobically. The ability of the isolates to ferment maltose, mannose, lactose, sucrose and trehalose was also determined according to the methods of Fawole and Oso [11] and Olutiola et al. [12]. The results were interpreted according to Holt et al. [13].

2.2 Detection of β-lactamase Production by Iodometric Method

The solution of penicillin was dispensed in 0.5 ml into a test tube and colonies of the 18 h culture of the *S. aureus* was dispensed into the penicillin solution to make a density of at least 10^4 CFU/ml. The suspension was left at room temperature for one hour after which two drops of starch indicator was added. This was immediately followed by a drop of iodine reagent. Development of blue colour that disappeared immediately was taken to be a positive reaction while persistence of blue colour for longer than 10 minutes constituted a negative test [14]. The β-lactamase-producing strains were selected for further studies.

2.3 Molecular Detection of the *nuc* Gene by PCR

The forward and reverse primers of *coa* gene used for this work were: F: 5'-CGA GAC CAA GAT TCA ACA AG-3' and R: 5'-AAA GAA AAC CAC TCA CAT CA-3' according to Esan et al. [15]. Each PCR assay was made up of the following: 25 µl of mastermix (Sigma), containing 1.5 units of Taq DNA polymerase, 10 mM Tris-HCl, 50 mM KC1, 1.5 mM $MgCl_2$, 0.001% gelatin and 0.2 mM dNTPs, 1 µl (20 pmol) of the forward and reverse primers and 5 µl of template DNA. Sterile distilled water was added to make a final volume of 50 µl. The thermocycler was programmed with the following parameters: 40 cycles of denaturation at 95ºC for 30 seconds, annealing at 55ºC for 2 minutes and extension at 72ºC for 4 minutes. *Staphylococcus aureus* ATCC 25923 served as the positive control in each PCR assay. The PCR products were detected by gel electrophoresis using 1.5% w/v agarose (Seakem, Whittaker USA) and run in 1x TBE (0.089 M Tris, 0.089 M boric acid, EDTA disodium 0.002 M) buffer (pH 8.3) at 80 V for 2 hours. The gels were stained with ethidium bromide and visualized under UV light.

2.4 PCR-RFLP of the Coagulase Gene

Restriction fragment length polymorphisms (RFLPs) of the amplicons were determined by digestion with alµl (Fermentas, UK). The reaction mixture was made up of 10 µl of the PCR product, 1.0 µl enzyme and 20 µl. It was then incubated at 37ºC for 2 hours. The restriction DNA digests were detected by electrophoresis in 2% w/v agarose (Seakem, Whittaker USA) in 1x TBE buffer (0.089 M Tris, 0.089 M Tris 0.089 M boric acid, EDTA disodium 0.002 M) buffer (pH 8.3) at 80 V for 2 hours. The gels were stained with ethidium bromide and visualized under UV light.

The sizes of the PCR products and of the restriction DNA digests (RFLPs with respect to the overall number of 81-bp tandem repeats) were estimated by comparison with a 100 bp molecular size standard marker, visual inspection and analysis using the Gene tools program (SynGene Bioimaging System). The strains were grouped on the basis of three characteristics of their PCR products, i.e. the presence of the one of two PCR products, their size(s), and the *AluI* restriction digest patterns of the PCR products as described by Shittu and Lin [16].

2.5 Molecular Detection of Panton-Valentine Leukocidin (*pvl*) Genes

PCR amplification of *pvl* genes was performed on the *S. aureus* strains. PCR primers and the procedures used were those described previously by Jarraud et al. [17]. The PCR conditions used were as follows: Initial denaturation at 95ºC for 2 minutes, amplification at 94ºC for 30 seconds, annealing at 50ºC for 30 seconds and extension at 72ºC for 5 minutes. The PCR products were electrophorezed and visualized same as for the *nuc* gene detection described above.

2.6 Antibiotic Susceptibility Test

The isolates were grown at 37ºC in Mueller-Hilton broth (Oxoid) for 18 h and diluted to an optical density of 0.1 (0.5 McFarland Standard). The disc diffusion method was used for susceptibility testing as described by Clinical and Laboratory Standard Institute [18]. Each of the isolates was seeded on sterile Mueller-Hinton Agar (Oxoid) and the antibiotic discs gently place on the plate at equidistance and incubated at 37ºC for 18 hours. The zone of clearance around

each of the antibiotics was measure using venial caliper. The zones of inhibition (clearance) were compared to the standards of CLSI [18]. The commercial antibiotics used in this study with their concentrations in (µg) were cefoxitin (30), chloramphenicol (30), ciprofloxacin (10), ciprofloxacin (10), clindamycin (2), erythromycin (5), fusidic acid (2), gentamicin (10), oxacillin (5), penicillin (10 IU) and tetracycline (30).

3. RESULTS AND DISCUSSION

β-lactamase-producing *S. aureus* has been increasingly reported worldwide. Their occurrence in many clinical samples has been reported. In Nigeria there is no serious monitoring of community-acquired staphylococcal infections and their antimicrobial susceptibility pattern. In this study, a total of 58 isolates were recovered and identified to be *S. aureus.* The ability of the isolates to produce β-lactamase was detected by iodometric method in 21 out of the isolates. The resistance to methicillin and all other β-lactam antibiotics is developed in *S. aureus* due to the acquisition of the *mecA* gene [19].

Out of the 21 β-lactamase-producing isolates, 19 (about 90.5%) possessed the staphylococcal nuclease (*nuc*) gene and 20 possessed coagulase (*coa*) gene (Figs. 1, 2 and 3). Only nine out of the isolates possessed *pvl* gene. The occurrence of community-acquired staphylococcal infections is lower than that involved in nosocomial infections [20]. The gene *pvl* encodes a cytotoxic protein secreted by strains that possess the gene. The cytotoxin helps the pathogen to invade and destroy the

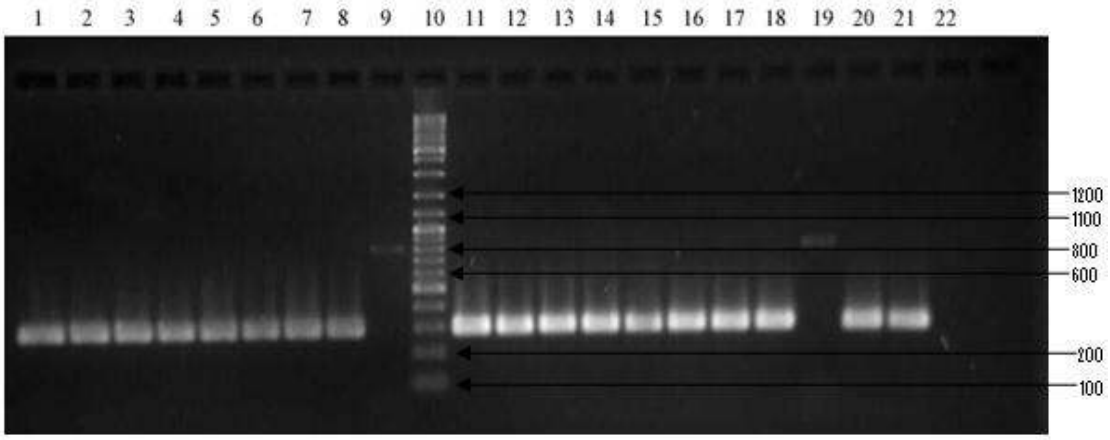

Fig. 1. Detection of *nuc* gene of *S. aureus* isolates from Ondo state

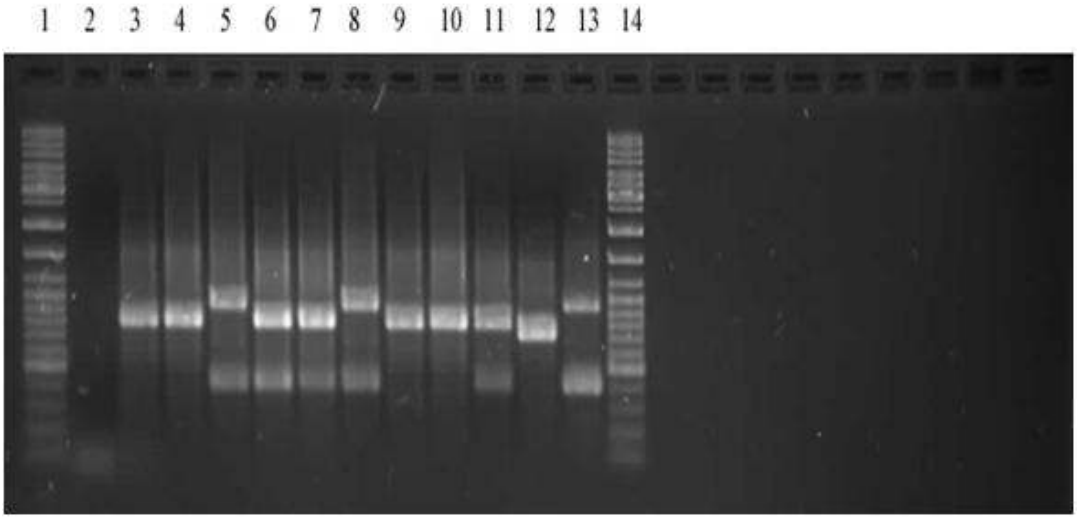

Fig. 2. PCR coagulase (*coa*) gene of *S. aureus* isolates from Ondo State (WW set)

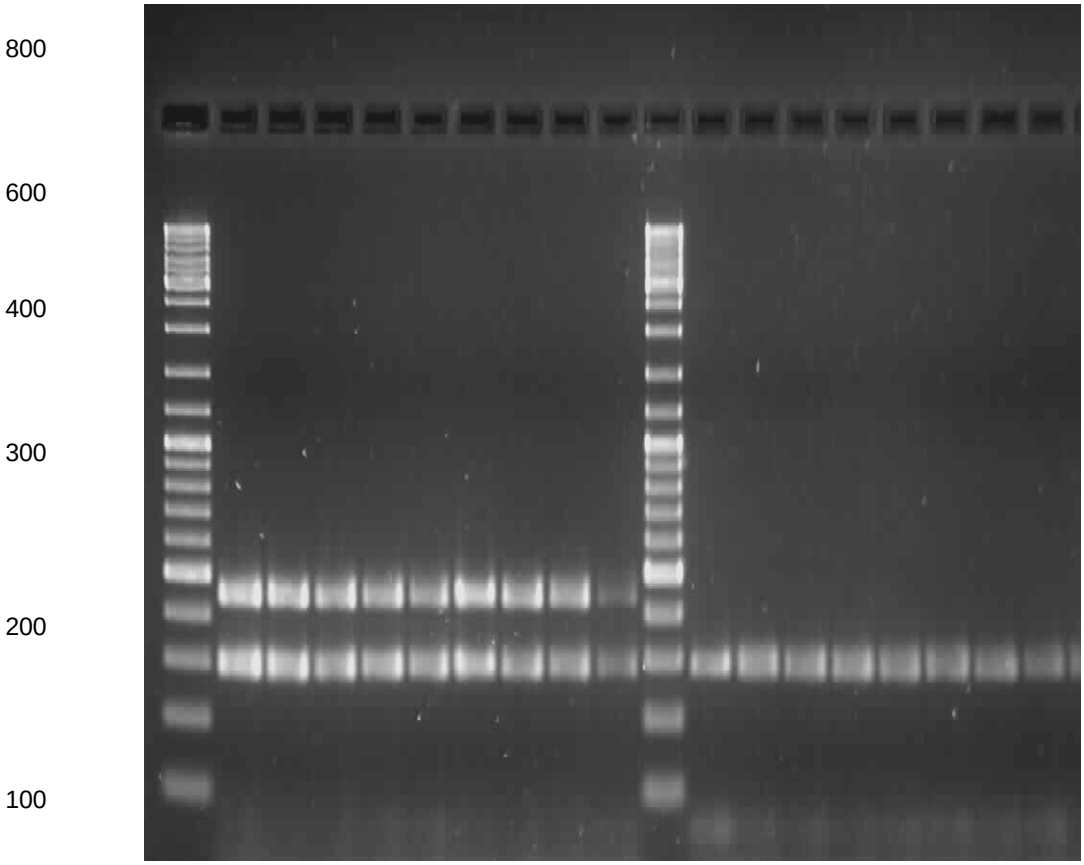

Fig. 3. PCR detection of *pvl* gene of *S. aureus* isolates from Ondo State

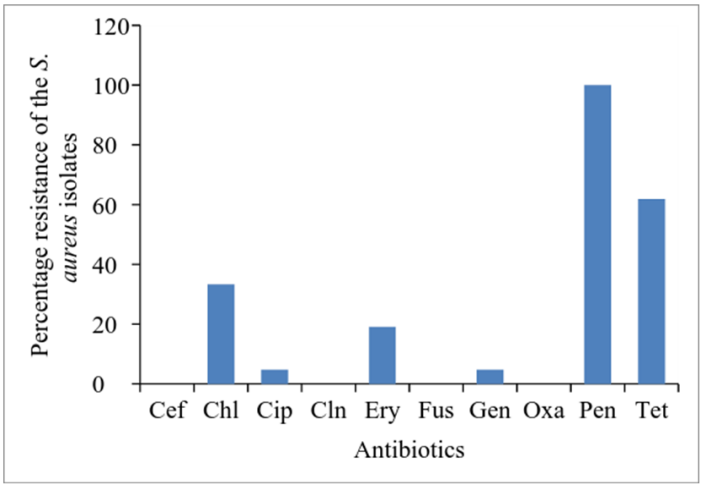

Fig. 4. The antibiotic resistance of *S. aureus* from the clinical samples

cefoxitin (Cef), chloramphenicol (Chl), ciprofloxacin (Cip), ciprofloxacin (Cip), clindamycin (Cln), erythromycin (Ery), fusidic acid (Fus), gentamicin (Gen), oxacillin (Oxa), penicillin (Pen) and tetracycline (Tet)

local cellular and structural elements of host tissue and organs [20-22]. Panton-Valentine leukocidin has been reported to be lethal to neutrophils and causes tissue necrosis by forming pores in cell membranes, and it is associated with skin and soft tissue infections

and severe necrotising pneumonia [23]. Contrary to the reports of Vandenesch et al. [24] that only MRSA can produce *pvl*, the *pvl* gene was detected among the methicillin- (oxacillin) susceptible *S. aureus* strains. Prophages and pathogenicity islands are regarded to contribute to the virulence of *S. aureus* [25].

In the present study, all the isolates were resistant to penicillin and tetracycline. Only 7 (33.3%) and 4 (19.04%) were resistant to chloramphenicol and erythromycin respectively. All the isolates were susceptible to oxacillin, cefoxitin, clindamycin and fusidic acid. Gentamicin and ciprofloxacin were effective against only 1 (4.76%) of the isolates. This was lower than the values recorded by Iroha et al. [26] who reported 18% and 30% resistance against gentamicin and ciprofloxacin respectively. The resistance of the isolates to antibiotics in this study was higher than those reported by Onelum et al. [27]. This is an evidence of inconsistent susceptibility profile of these organisms indicating level of exposure to other classes of antibiotics and antimicrobials due to attitudinal behaviours of the populace to antibiotic consumption.

4. CONCLUSION

This study provides the basic epidemiological data in the study area as well as giving an insight to the possibility of community-hospital transmission, and vice versa, of pathogens. Further studies should be conducted to determine the factors that encourage the transmission of this organism in the study area.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Tenover FC, Gorwitz RJ. The epidemiology of *Staphylococcus* infections, in gram-positive pathogens. Fiscetti VA, (Editor). ASM Press: Washington, D.C. 2006;526-534.
2. Wertheim HF, Melles DC, Vos MC, Van Leeuwen W, Van Belkum A, Verbrugh HA, Nouwen JL. The role of nasal carriage in *Staphylococcus aureus* infections. Lancet Infect Dis. 2005;5(12):751-62.
3. Jensen AG, Wachmann CH, Espersen F, Scheibel J, Skinhoj P, Frimodt-Moller N. Treatment and outcome of *Staphylococcus aureus* bacteremia: A prospective study of 278 cases. Arch Intern Med. 2002;162: 25–32.
4. Malani PN, Rana MM, Banerjee M, Bradley SF. *Staphylococcus aureus* bloodstream infections: the association between age and mortality and functional status. J Am Geriatr Soc. 2008;56:1485–9.
5. Shurland S, Zhan M, Bradham DD, Roghmann MC. Comparison of mortality risk associated with bacteremia due to methicillin-resistant and methicillin-susceptible *Staphylococcus aureus*. Infect Control Hosp Epidemiol. 2007;28:273–9.
6. Jenkins TC, Knepper, BC, Moore SJ, Saveli CC, Pawlowski SW, Perlman DM, McCollister BD Burman WJ. Microbiology and initial antibiotic therapy for injection drug users and non-injection drug users with cutaneous abscesses in the era of community-associated methicillin-resistant *Staphylococcus aureus*. Acad Emerg Med. 2015;22(8):993-997.
7. National Nosocomial Infections Surveillance System. (NNISS). Changes in the epidemiology of methicillin-resistant *Staphylococcus aureus* in intensive care units in US hospitals, 1992-2003. Clin Infect Dis. 2006;42:389–391.
8. Aijazi I, Abdulla FM, Ibrahim AE. Community acquired methicillin-sensitive *Staphylococcus aureus* cerebral abscess in a previously health gentleman mimicking signs of cavernous sinus thrombosis-a unique presentation. J Ayub Med Coll Abbottabad. 2015;27(1):245–7.
9. Wang J, Wang J, Fang C, Chie W, Lai M, Lauderdale T, Weng C, Chang S. Risk factors for mortality of nosocomial methicillin-resistant *Staphylococcus aureus* (MRSA) bloodstream infection: With investigation of the potential role of community-associated MRSA strains. J. Infect. 2010;61:449-457.
10. Anyanwu NCJ, Abdullahi IO, Ameh JB, Ella EE. Molecular detection of *pvl, msrA* genes and antibiotic susceptibility pattern of *Staphylococcus aureus* from skin and soft tissue infections in Zaria, Nigeria. Scientific J. Microbiol. 2013;2(2):43-52.
11. Fawole MO, Oso BA. Laboratory Manual of Microbiology. Spectrum Books Limited, Ibadan. 2001;127.
12. Olutiola PO, Famurewa O, Sonntag H-G. An introduction to general microbiology: A practical approach. Heidelberger

Verlagsanstalt und Druckerei GmbH, Heidelberg, Federal Republic of Germany. 2001;267.

13. Holt JG, Krieg NR, Sneath PHA, Staley JT, Williams ST. Bergey's Manual of Determinative Bacteriology 9th Edn. Williams & Wilkins, Baltimore; 1994.
14. Bethel CD, Boonlayengoor S. Beta-lactamase test. In: Clinical microbiology procedures handbook. Isenberg H. D. 2nd Edition. ASM Press. 2004;1:534-536.
15. Esan CO, Famurewa O, Lin J, Shittu AO. Characterization of *Staphylococcus aureus* isolates obtained from health care institutions in Ekiti and Ondo States, South-Western Nigeria. Afri. J. Microbiol. Res. 2009;3(12):962-968.
16. Shittu AO, Lin J. Antimicrobial susceptibility patterns and characterization of clinical isolates of *Staphylococcus aureus* in KwaZulu-Natal province, South Africa. BMC Infect. Dis. 2006;6(1):125.
17. Jarraud S, Mougel C, Thioulouse J, Lina G, Meugnier H, Fore F, Nesme X, Etienne J, Vandenesch F. Relationships between *Staphylococcus aureus* genetic background, virulence factors, agr groups (alleles), and human disease. Infect. Immun. 2002;70:631–641.
18. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing. 15th informational supplement. Wayne, PA: Clinical and Laboratory Standards Institute. 2012;M100-S15.
19. Deurenberg RH, Stobberingh EE. The evolution of *Staphylococcus aureus*. Infect Genet Evol. 2008;8(6):747-63.
20. Fankhauser C, Schrenzel J, Francois P, Pittet D, Harbarth, S. Secular trends of methicillin-resistant *Staphylococcus aureus* (MRSA) at Geneva University Hospitals (HUG) over a 14-year period. Antimicrob Resist Infect Control. 2015; 4(Suppl 1):9.
21. Diep BA, Carleton HA, Chang RF, Sensabaugh GF, Perdreau-Remington F. Roles of 34 virulence genes in the evolution of hospital- and community-associated strains of methicillin-resistant *Staphylococcus aureus*. J. Infect Dis. 2006;193(11):1495-503.
22. Gordon RJ, Lowy FD. Pathogenesis of methicillin-resistant *Staphylococcus aureus* infection. Clin Infect Dis. 2008; 46(Suppl 5): S350-9.
23. Labandeira-Rey M, Couzon F, Boisset S, Brown EL, Bes M, Benito Y, Barbu EM, Vazquez V, Hook M, Etienne J, Vandenesch F, Bowden MG. *Staphylococcus aureus* Panton-Valentine leukocidin causes necrotizing pneumonia. Science. 2007;315(5815):1130-1133.
24. Vandenesch F, Naimi T, Enright MC, Lina G, Nimmo GR, Heffernan H, Liassine N, Bes M, Greenland T, Reverdy ME, Etienne J. Community-acquired methicillin-resistant *Staphylococcus aureus* carrying Panton-Valentine leukocidin genes: Worldwide emergence. Emerg Infect Dis. 2003;9(8): 978-84.
25. Baba T, Bae T, Schneewind O, Takeuchi F, Hiramatsu K. Genome sequence of *Staphylococcus aureus* strain Newman and comparative analysis of staphylococcal genomes: Polymorphism and evolution of two major pathogenicity islands. J Bacteriol. 2008;190(1):300-10.
26. Iroha I, Okoh I, Ejikeugwu C, Nwakaeze E, Nwuzo A, Afiukwa N, Udu-Ibiam E. Prevalence of methicillin-resistant *S. aureus* (MRSA) among apparently healthy students in Afikpo, Ebonyi State, Nigeria. Bio Sci. Pharmaceut Res. 2015; 3(1):1-4.
27. Onelum O, Odetoyin B, Onipede A, Oyelese A. The role of methicillin-resistant *Staphylococcus aureus* in clinical infections in Obafemi Awolowo University Teaching Hospitals Complex, Ile-Ife, South western Nigeria. J Microbiol Exp. 2015; 2(2):1-6.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/12313