Tropical Journal of Pharmaceutical Research April 2022; 21 (4): 825-831
ISSN: 1596-5996 (print); 1596-9827 (electronic)

Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v21i4.20

Original Research Article

Molecular characterization of selected nasal isolates of methicillin-resistant *Staphylococcus aureus* (MRSA) from healthy students of a tertiary institution

Clement Oliseloke Anie[1], Emmanuel Chinedum Ibezim[2], Charles Okechukwu Esimone[3], Matthew Ikhuoria Arhewoh[4]*

[1]Department of Pharmaceutical Microbiology, Faculty of Pharmacy, Delta State University, Abraka, Delta State, [2]Department of Pharmaceutical Microbiology, Faculty of Pharmacy, University of Nigeria, Nsukka, [3]Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmacy, Nnamdi Azikiwe University, Awka, [4]Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

For correspondence: **Email: arhewoh@uniben.edu; **Tel:** +234-8055306846*

Sent for review: 5 May 2021 *Revised accepted: 26 March 2022*

Abstract

Purpose: *To investigate the incidence of methicillin-resistant Staphylococcus aureus (MRSA) and carry out molecular characterization in selected nasal isolates from healthy students in Abraka, Nigeria.*
Methods: *Three hundred (300) samples were obtained from apparently healthy 150 female and 150 male students and were cultured in suitable media for identification. The MRSA were detected by means of oxacillin antibiotic sensitivity disk. Antibiotics susceptibility pattern of MRSA isolates was carried out in accordance with the guidelines of Clinical Laboratory Standard Institute. Tests were carried out to determine the presence of penicillin binding protein2a (PBP2a). Molecular characterization of twenty (20) MRSA representatives was done to establish the existence of the mecA genes among the isolates.*
Results: *The incidence of MRSA colonization amongst apparently healthy students in the community was 68 (22.7 %). The sensitivity pattern was: amoxicillin, 40 (58.8 %); amoxicillin/clavulanate, 15 (22.1 %); chloramphenicol, 15 (22.1 %); ciprofloxacin, 19 (27.9 %); co-trimoxazole, 9 (13.2 %); gentamicin, 9 (13.2 %); ofloxacin, 8 (11.1 %); and streptomycin, 30 (44.1 %). All the twenty (20) isolates subjected to molecular characterization possessed penicillin binding protein2a (PBP2a) while only one possessed the mecA gene.*
Conclusion: *The MRSA is present among healthy individuals in Abraka, Delta State. It is also possible not to detect the existence of the mecA gene even when penicillin binding protein2a is present. Since most of the MRSA isolates are multi-drug resistant, there is a tendency for clinical antibiotic therapy failure in this area.*

Keywords: *Abraka, Resistance, Staphylococcus aureus, Antibiotic therapy failurre, MRSA, MecA*

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, International Pharmaceutical Abstract, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

The carriers of *Staphylococcus aureus* are a significant number in community-acquired methicillin-resistant *S. aureus* (CAMRSA) and hospital-acquired methicillin-resistant *S. aureus* (HAMRSA) also called nosocomial infection. Though *S. aureus* might be found on the skin of

hosts, a greater percentage of its carriage is normally via the nasal passages of the anterior nares and could be found in the ears [1]. *Staphylococcus aureus* bacteria are commensals, and could asymptomatically colonize more than 30 % of human population [2]. *Staphylococcus aureus* is known to cause infective bacteraemia and endocarditis [2]. The wide-spread infections of *S. aureus* occur during contact with infected wounds, skin contact with persons that are infected, or contact with previously infected materials such as clothing sheets, towels and athletic kit. Prosthetic joints are capable of putting humans at high risk of septic arthritis, pneumonia and staphylococcal-associated endocarditis [2]. In fact, *Staphylococcus aureus* has been reported to be common in people with atopic dermatitis [2].

It has been reported that in the United States, carriers of *Staphylococcus aureus* are up to one third of her population [3]. The MRSA is also referred to as oxacillin-resistant *S. aureus* (ORSA) and are known to cause a lot of difficult-to-treat disease conditions in humans [4]. This strain of *S. aureus* emerged, in the course of developing natural selection and also resistance to beta-lactam antibiotics such as penicillin and cephalosporin [5]. The emergence of the strains of MRSA in some parts of Africa have also been reported [6,7]. Penicillin-binding proteins (PBPs) are classes of proteins usually labelled according to their known binding affinity. They are usually constituents of bacterial and the given name portrays the method by which these proteins were discovered [8]. Antibiotic susceptibility profile is used by prescribers in deciding the choice of antibiotics that is prescribed during infectious disease conditions. This study was aimed to investigate the incidence of MRSA and the antibiotic susceptibility profile in apparently healthy individuals in Abraka, Delta State. Molecular characterization to establish the association of *mecA* genes among the isolates was also done.

EXPERIMENTAL

Materials

***Media*:** Nutrient agar, nutrient broth and peptone water (Titan Biotech media Limited, India) were purchased from Pyrex-IG Scientific Limited, Nigeria. Mannitol salt and Mueller-Hinton agar were purchased from Oxoid Limited, England.

***Antibiotics discs*:** Amoxicillin (30 µg), gentamicin (10 µg), co-trimoxazole (30 µg), oxacillin (1 µg), ofloxacin (10 µg), ciprofloxacin (10 µg), amoxicillin – clavulanate (30 µg), chloramphenicol (30 µg) and erythromycin (10 µg) were procured from Oxoid Ltd, UK.

Study area and sample size

The study was conducted among healthy students of Delta State University, Abraka. No specific sampling technique was used except that the university students were informed by one of the researchers about the nature and goal of the study and those that were willing to participate by allowing nasal swap to be collected from them freely volunteered and signed the consent forms before samples were collected. The process continued until a total of 300 specimens were collected. This comprised 150 specimens each from male and female students.

Ethical consideration

Informed consent was obtained from participants, after approval to proceed with the research was sought and obtained from the institutional ethical committee (approval no. 76714); the study also followed international guidelines for human studies.

METHODS

Sample collection

The nasal samples of apparently healthy students were obtained in Delta State University, Abraka campus with sterile swab sticks, which were carefully and gently inserted into the inner area and rubbed over the anterior nares of both nostrils.

Bacteria isolation

The samples collected were streaked immediately on solidified nutrient agar. The inoculum on the plate was streaked out for discreet colonies following standard procedures. The agar plates were inoculated and then placed in the incubator for 24 h at 37 ^{o}C. Colonies obtained were further inoculated in nutrient agar to obtain pure strains. Discreet colonies were cultured on a mannitol salt agar. The discreet colonies obtained were stored in agar slants for proper identification. Identification tests were done using standard microbiological methods including Gram staining, growth on selective media, catalase, and coagulase tests [9,10].

Phenotypic detection of MRSA with oxacillin disc

Susceptibility test for the isolates of *S. aureus* was carried out using the method of agar

screening with oxacillin (1 µg) disc on Muller-Hinton agar sensitivity disc. The McFarland standards of 0.5 of the isolates was obtained. The standardized suspension of the isolates was spot inoculated aseptically onto the Muller-Hinton agar containing plates. The culture plates were then placed in incubator for the period of 24 h at 30 °C. Various zones of inhibition of each isolate were measured with a meter scale and the reading was categorized into susceptible, intermediate and resistant following specified standard [9].

Antibiotic susceptibility testing

The antibiotic sensitivity of the isolates was carried out in accordance with the Guidelines of Clinical Laboratory Standard Institute [10] with the use of antibiotics discs. Precisely 0.2 mL of the standard inoculum test organism was aseptically pipetted into a sterilized petri dish and thereafter; 19 mL of molten Mueller-Hinton agar was added. A uniform spread of the test organism was done by rocking the petri dish gently but firmly. The antibiotic disc was gently removed from the cartridge and firmly dropped on the surface of the agar plate using a sterile pair of forceps. Agar plates were kept for 1 h at room temperature to permit the antibiotics to diffuse into the agar properly. The agar containing plates were placed in the incubator for 24 h at 37 ºC. At the end of 24 h, the inhibition zones were measured with metre rule and categorized as sensitive, intermediate or resistant [9].

Penicillin binding protein (PBP2) latex agglutination test

Extraction procedure for PBP2

Extraction reagent (4 drops) was added to the microcentrifuge tube. This was achieved with aid of a sterile 5 µL 1oop in order to obtain enough growth that will fill the loop internal diameter. The test culture was suspended in the microcentrifuge tube and vortexed. A very turbid suspension was obtained. The tube was placed inside boiling water at 95 ºC and heated for 3 min. The microcentrifuge tube was removed and then kept at room temperature to cool. A drop of extraction reagent was placed into the tube and then centrifuged for 5 min at 300 rpm. The supernatant was then used to carry-out the test.

Preparation of culture for PBP2

Colonies were tested using Mueller-Hinton Agar. The fresh culture (18 - 24 h old) was used.

Latex agglutination procedure

For the individual supernatant to be tested, test card circle was named with the test latex and the other with the control latex. The latex reagents were thoroughly mixed by inversions and a single drop for each labelled circle (a drop of control latex or test latex) was added.

A 500 µL volume of the supernatant was carefully dropped into the test circle and control to avoid pellet. Latex and the supernatant in every circle were carefully mixed with a mixing stick. The card was rocked gently until agglutination occurred under normal lighting conditions. After the experiment, the reaction card was safely discarded.

Detection of *MecA* gene

DNA extraction

Discreet colonies were obtained after incubating in the nutrient agar plate overnight. The cells were then suspended after centrifuging for 5 min at 4,500 rpm and 4 ºC. Washing of cells pellets was done with 1 mL of Tetrasodium ethylene diamine tetracetic acid (TE) (10 mM tris pH 8; 10 mM ethylene diamine tetracetic acid) and re-suspended in 100 µL of TE. The 50 µL of 10 % sodium dodecyl sulphate (SDS), was incubated at 65 ºC for 30 min, then lysate was centrifuged, and supernatant was eventually taken away. The microtubes, thereafter, were put into a microwave oven and then heated for three consecutive times at 750 watts. The pellets obtained were in 200 µL of TE and then extracted with same quantity of chloroform / Isoamyl / alcohol 24 : 25 : 1 for 15 min. The aqueous phase was then recovered by centrifuging for 20 min, then precipitated with acid of ethanol and re-suspended in 50 µL of TE.

Preparation of PCR reaction mixture

The PCR reaction mixture was prepared in a reaction volume of 25 µL containing PCR mix of 12.5 µL of PCR mix (Promega, USA), 8.5 µL sterile distilled deionised water, 0.5 µL primers for forward and backward and 2 µL of amplification DNA.

DNA amplification

The isolated DNA was amplified with primer 756-bp (Inqaba Biotechnical Company Pty, South Africa), while the primers MecA1 and MecA2 was used to determine methicillin resistance using *mecA* gene (Table 1). Each individual PCR was expected to yield a fragment of the expected

sizes of 756 and 310 bp for the 16S rRNA, and *mecA* genes, respectively. The PCR was performed in a thermal cycler (A & E Laboratories UK, Version 7.0) with the reaction cycles consisting of an initial denaturation at 94 ºC for 4 min; 30 cycles at 94 ºC for 30 sec, 50 ºC for 45 min and 68 ºC for 1 min. A final extension step at 68 ºC was continued for another 10 min. The PCR products were analysed on 1.5 % agarose gels containing 0.5 µg/mL ethidium bromide and visualized on UV transilluminator (Edvotek, USA).

Statistical analysis

Statistical analysis was done using Graph Pad In-stat R version 2.05. Statistical comparisons were applied in order to determine the significance of the differences in the resistance levels using analysis of variance, with $p < 0.05$ considered statistically significant.

RESULTS

Characterization studies revealed that from the three hundred (300) nasal samples collected from the study location and screened, a total of 208 (69 %) of the isolates contained *S. aureus*. The incidence of *S. aureus* among females and males as shown in Table 1, were 110 (80 %) and 98 (65.3 %), respectively. The total incidence of MRSA colonisation of the *S. aureus* isolated was 68 (22.7 %) (Table 2).

Antibiotic sensitivity of MRSA

Table 3 show the result of the MRSA antibiotic sensitivity. The resistant pattern was highest with amoxicillin (58 %) and lowest with amoxicillin-clavulanate (7.4 %). The reverse was also the case with the percentage of susceptible isolates.

Plates: 1 and 2 showed that one (5 %) out of the twenty representative MRSA isolates collected from the study area and screened for the presence of *mecA* gene, contain *mecA* gene while the remaining nineteen (95 %) failed to produce the band that is specific for the *mecA* gene.

DISCUSSION

The result of this study showed that females have a higher number of nasal carriages of *S. aureus* compared to males which is in conformity with the observation of by Oyetunji *et al* [12]. Methicillin resistant SA has been known as a regular pathogen in several communities and healthcare facilities globally. It is well known that the presence of *S. aureus* in nostrils present a great risk as a source for consequent *S. aureus* infections [13]. Nevertheless, in Delta State of Nigeria, to the best of our knowledge no data exist as regards to MRSA carriage.

Table 1: Primer sequence

Primer	Forward (5′-3′)	Reverse (5′-3′)
756-bp	AAC TCTGTT ATT AGG GAA GAA CA	CCA CCT TCC TCC GGT TTG TCA CC
MecA1	GTAGAAATGACTGAA CGTCCGATAA	
MecA2	CCAATTCCACATTGT TTCGGTCTAA	

Table 2: Frequency of isolates and incidence of methicillin resistant *S. aureus*

Source	Number of samples collected	Number positive	Percent (%)	Frequency of MRSA	Percentage
Male	150	98	65.3	30	30.6
Female	150	110	80	38	34.5
Total	***300***	***208***	***69***	***68***	***22.7***

Table 3: Antibiotic susceptibility pattern of MRSA isolates from nasal swab

Antibiotic	Number resistant (n = 300)		Number susceptible (n = 300)	
	Number	*Percentage*	*Number*	*Percentage*
Amoxicillin	40	58.8	28	41.2
Amoxicillin-clavulanate	5	7.4	63	92.6
Chloramphenicol	15	22.1	53	77.9
Ciprofloxacin	19	27.9	49	72.1
Co-trimoxazole	9	13.2	59	86.8
Erythromycin	22	32.4	46	67.7
Gentamicin	9	13.2	59	86.8
Ofloxacin	8	11.8	60	88.2
Streptomycin	30	44.1	38	55.9

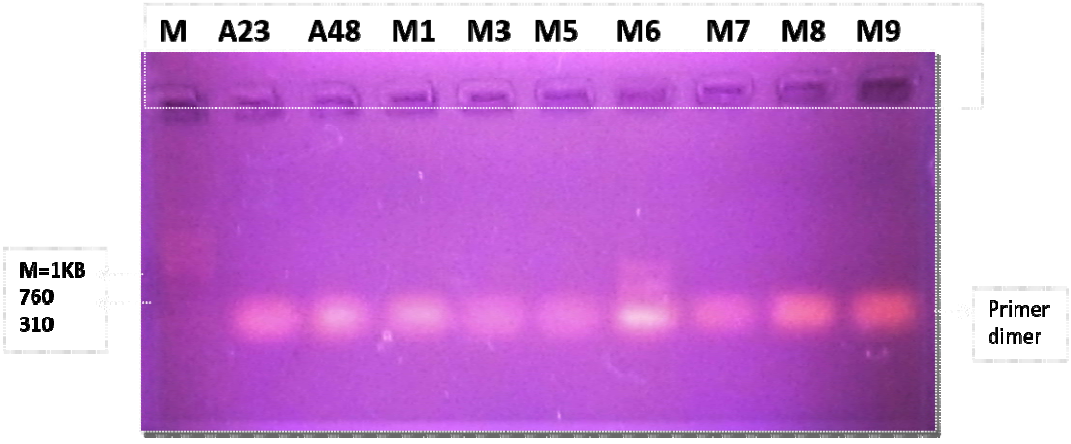

Plate 1: Amplification of *Staphylococcus* genus-specific 16S rRNA and *mecA* genes. **Key:** Lane M; 1Kb DNA Ladder; Lane M6 showed positive amplifications at 756 bp for the *Staphylococcus* genus-specific rRNA gene and amplification of *mecA* gene showing expected sizes, of 310 bp. Lanes A23, A48, M1, M3, M5, M6, M7, M8, M9 show no amplification at the expected band sizes

Plate 2: Amplification of *Staphylococcus* genus-specific 16S rRNA and *mecA* genes. Lane M; 1 Kb DNA Ladder; Lanes Y1 – Y19 show no amplification at the expected band sizes of 756 bp and 310 bp

The incidence of 208 (69 %) of *S. aureus* isolates from nasal samples obtained from healthy students was recorded. This finding is in support with reports of Onanuga *et al* [14]. The female to male incidence of *S. aureus* were 80 and 65.3 %, respectively. This is in harmony with results of Oyetunji *et al* [12]. The *S. aureus* colonization in male was insignificant ($p > 0.05$).

This study recorded high MRSA resistance to amoxicillin (58.8 %), streptomycin (44.1 %), but low resistance to erythromycin (32.4 %), ciprofloxacin (27.9 %) and chloramphenicol (22.1 %) among the tested antibiotics. The highest resistance among MRSA isolates was to amoxicillin (58.8 %) which agrees with the findings of Weems [15]. The MRSA isolates however showed very low resistance to amoxicillin-clavulanic acid. This could be attributed to the presence of β-lactamase inactivator, which is lacking in amoxicillin [14].

This study report that majority of the MRSA was sensitive to ofloxacin, cotrimoxazole and gentamicin. Sixty isolates (88.2 %) out of 68 MRSA isolates were susceptible to ofloxacin, while only eight (11.8 %) were resistant. Very high percentage of MRSA isolates were susceptible to gentamicin (86.8 %) and co-trimoxazole (86.8 %) meanwhile nine 9 (13.2 %) isolates were resistant to both [13]. The high sensitivity to ofloxacin (88.2 %) and gentamicin (86.8 %) is in line with earlier reports [17]. The isolates that were methicillin-resistant but did not contain *mecA* gene agrees with what was earlier reported [18]. Locating *mecA* gene in MRSA is

usually the main proof for the discovery of MRSA isolates. This assertion has been accepted by many researchers in several countries such as Sudan [19]. Nevertheless, the results in this study showed low *mecA* incidence (5 %). This finding brings up the need to look out for innate factors that could in addition to *mecA* gene be able to produce resistance phenomenon in MRSA as observed in this study. Conversely, the non-existence of mecA gene inside methicillin-resistant *Staphylococcus aureus* isolates has been reported as well [6]. Earlier studies reported absence of *mecA* genes [6,7]. Five major *Staphylococcal* cassette chromosome mec (SCCmec) types, even the PBP2a gene in MRSA isolates that were MRSA phenotype suggest the tendency of over synthesis of beta-lactamase that led to this occurrence. The SCC*mec* is referred to as a genomic island whose origin is not known containing the antibiotic resistance gene *mecA*. It is also known to contain other genes which are responsible for resistance. As observed previously, the *mecA* gene is customarily a component of this unique and mobile genetic part called SCC*mec*. These structures may possess other genes that could cause insertion sequence even antimicrobial resistance, and genes of unknown functions [20].

At present, six unique types of SCC*mec* which range from 21 – 67 kb have been recognized; and named types I - VI and they are differentiated by their distinction in *ccr* gene and *mec* complexes. Due to SCC*mec* elements size and the restrictions associated with transfer of gene horizontally, an inadequate clone number are considered to be accountable for the MRSA infectious spread. This observation implies that there could be MRSA where there is absence of *mecA* gene in the PCR amplification of gene and agarose gel electrophoresis, and this could be as a result of the different types of *Staphylococcus* cassette chromosome referred to as SCCmec [20].

CONCLUSION

This study has established that most of the MRSA isolates from the nasal specimens are multidrug-resistant which signifies that there would be a high probability of antibiotic therapy failure when they are used. It is obvious from the results that it is possible not to detect the occurrence of *mecA* gene even when PBP2a is present. The results show that there is presence of methicillin-resistant *Staphylococcus aureus* (MRSA) among healthy tertiary school students in Delta State University, Nigeria.

DECLARATIONS

Acknowledgement

Authors are grateful to the students for accepting to participate in the study

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. C.O Esimone initiated the topic and supervised the research work. Clement O Anie did the bench work, while Emmanuel C Ibezim and Matthew I Arhewoh equally supervised and guided the result analysis. All authors read through the manuscript and approved it for publication.

REFERENCES

1. *Quinn GA, Cole AM. Suppression of innate immunity by a nasal carriage strain of Staphylococcus aureus increases its colonization on nasal epithelium. Immunol 2007; 122: 80–89.*
2. *Tong SY, Davis JS, Eichenberger E, Holland TL, Fowler VG. Staphylococcus aureus infections: epidemiology, pathophysiology, clinical manifestations and management". Clin Microbiol Rev 2015; 28: 603–661.*
3. *American Academy of Dermatology. Five things physicians and patients should question, choosing wisely: an initiative of the ABIM Foundation (American Academy of Dermatology), Assessed 2016/09/08*
4. *McDougal LK, Steward CD, Killgore GE, Chaitram JM, McAllister SK, Tenover FC. Pulsed-field gel electrophoresis typing of oxacillin-resistant Staphylococcus aureus isolates from the United States: Establishing a national database. J Clin Microbiol 2003; (41): 5113–5120.*

5. Yan M, Pamp SJ, Fukuyama J, Hwang PH, Cho DY, Holmes S, Relman DA. Nasal micro-environments and interspecific interactions influence nasal microbiota complexity and S. aureus carriage. Cell Host Microb 2013;14: 631–640.
6. Ugwu MC, Odimegwu DC, Ibezim EC, Esimone CO. Antibiotic resistant pattern of Staphylococcus aureus isolated from nostrils of healthy subjects in a southeastern Nigerian locality. Macedonian J Medical Sci 2009; 12: 294-300
7. Ehinmidu JO. Antibiotic susceptibility patterns of urine bacterial isolates in Zaria, Nigeria. Trop J Pharm Res 2003; 2: 223-228
8. Nguyen-Distèche M, Leyh-Bouille M, Ghuysen JM. Isolation of the membrane-bound 26 000-Mr penicillin-binding protein of Streptomyces strain K15 in the form of a penicillin-sensitive D-alanyl-D-alanine-cleaving transpeptidase. Biochem J1982; 207: 109–115.
9. Cheesbrough M. Distric laboratory practice in tropical countries part 2 (2nd edition) Cambridge University press. 2006
10. Clinical and Laboratory testing: Performance standards for antimicrobial susceptibility. Twenty-third international supplement edition. Standards Institute. CLSI Document. Mi00-S23, Wayne, Pa, USA: CLSI: 2013.
11. Baker FJ, Silverton RE, Pallister CJ. Baker and Silverton's introduction to Medical Laboratory Technology.7th Ed. Nigerian Ed. Sam–Adex Printer, Ibadan 2001: 323.
12. Oyetunji IA, Ikenna OO, Olumide OA. Prevalence of methicillin-resistant Staphylococcus aureus from healthy community individuals' volunteers in Jos South, Nig J Microbiol Biotech Food Sci 2012: 6: 1389-1405
13. Rasamiravaka T, Rasoanandrasana S, Zafindraibe NJ, Rakoto A, Alson O, Rasamindrakotroka A. Evaluation of methicillin-resistant Staphylococcus aureus nasal carriage in Malagasy patients. J Infect Developing Countries 2016; 7: 318-322.
14. Onanuga A, Oyi AR, Onaolapo AJ. Prevalence and susceptibility pattern of methicillin resistant Staphylococcus aureus isolates among healthy women in Zaria, Nigeria. Afri J Biotechnol 2005; 11(4): 1321-1324.
15. Weems JJ. The many faces of Staphylococcus aureus infections. Postgrad Med 2001; 110(4): 24-36.
16. Hafez EE, Sohaimy SA, Saadani ME. The effect of the mecA gene and its mutant form on the response of S. aureus to the most common antibiotics. Inter J Immunol Studs 2009; 1: 106–122.
17. Meshref AA, Omer MK. Detection of (mecA) gene in methicillin resistant Staphylococcus aureus (MRSA) at Prince A/Rhman Sidery. J Medical Genetics Genomics 2011; 3: 41–45.
18. Olayinka BO, Olayinka AT, Obajuluwa AF, Onaolapo JA, Olurinola PF. Absence of mecA gene in methicillin-resistant Staphylococcus aureus isolates, Afri J Infect Dis 2009; 3(2): 49–56.
19. Elhassan MM, Ozbak HA, Hemeg HA, Elmekki MA, Ahmed LM. Absence of the mecA Gene in Methicillin Resistant Staphylococcus aureus Isolated from Different Clinical Specimens in Shendi City. Sudan BioMed Res Int 2015; Article ID 895860, 5 http://dx.doi.org/10.1155/2015/895860
20. Del-Valle O, Trincado P, Mrtino MT, Gomez E, Cano A, Vindel A. Prevalence of methicillin-resistant Staphylococcus aureus among hospitals. Enfermed Infect Microbiol Clín 1999; 17: 498–505.