Distribution of Methicillin Resistant *Staphylococcus aureus* in Iraqi patients and Healthcare Workers

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Abstract

One hundred and six *S. aureus* were isolated from 250 Nasal swabs of Healthcare workers and patients at Al- Kadhamia teaching Hospital and Al-Numan hospital, Baghdad, Iraq. The study was undertaken over a period of ten months between August 2011 and June 2012. *S. aureus* isolates were diagnosed based on phenotypic traits and biochemical tests. Antibiotics sensitivity to 11 antibiotics, revealed that *S.aureus* is totally resistant to Pencillin G (100%), highly resistant to Cefoxitin (alternative to Methicillin) (94.3%) While there are varied resistance percentage for the rest of antibiotics: Erythromycin (37.7%), Tetracycline (34.9%), Gentamicin (29.3%), Trimethoprim/sulfamethoxazole (50%), Ciprofloxacin (29.2%), and showed highly sensitive to Rifampin (96.2%), Clarithromycin (78.3%) and Clindamycin (73.6%),Whereas Vancomycin intermediate *S. aureus* (VISA) was 32.1% and 3.8% was Vancomycin resistant *S. aureus* (VRSA). The incidence of MRSA among *S. aureus* was 94.3%. It is concluded that *S. aureus* nasal carriage is a common health problem all over the world and Methicillin resistant *S. aureus* is an emerging subject even in our community, which requires further attention and support.

Key words: MRSA, healthcare workers, Antibiotics sensitivity.

انتشار العنقوديات الذهبية المقاومة للمثيسللين بين المرضى وعمال الصحة العراقيين.

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الخلاصة

تم عزل ١٠٦ (42.2%) عزله من بكتيريا *S. aureus* من ٢٥٠ مسحه انفيه من عمال و مرضى راقدين في مستشفى الكاظميه التعليمي ومستشفى النعمان ، بغداد ،العراق . بدأت الدراسه من اب ٢٠١١ ولغاية حزيران ٢٠١٢. شخصت العزلات اعتمادا على الصفات المظهرية والفحوصات الكيموحياتية .

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بينت دراسه اختبار الحساسيه ل١١ مضاد حياتي بأن بكتريا *S. aureus* ابدت مقاومه كليه لمضاد بنسلين (جي) 100% في حين كانت مقاومتها لمضاد السيفوكستين(البديل عن الميثيسللين) 94.3% بينما تباينت نسب مقاومتها لبقية المضادات على النحو الاتي: الارثرومايسين 37.7% ، التتراسايكلين 34.9%، الجنتامايسين 29.3% ،الترايميثوبرايم/ سلفاميثاكزازول 50% و السبروفلكساسين 29.2%. في حين ابدت بكتريا *S. aureus* تحسس عالي تجاه مضاد الريفامبين 96.2% ، الكلارثرومايسين 78.3% و الكلندامايسين 73.4%. بينما كانت المكورات العنقوديه الذهبية متوسطة المقاومة للفانكومايسين (VISA) 32.1% والمكورات العنقوديه الذهبية المقاومة للفانكومايسين(VRSA) 3.8% ، كان انتشار المكورات العنقوديه المقاومه للمثيسللين(MRSA) 94.3% بين *S. aureus*. خلصت الدراسه الى ان الاشخاص الحاملين لبكتريا *S. aureus* في الانف وبالتحديد تلك المقاومه للمضاد المثيسيللين (MRSA) يشكلون مشكله صحيه شائعه حتى في مجتمعنا، الأمر الذي يتطلب مزيدا من الاهتمام والدعم من اجل السيطره على انتشارها .

Introduction

The anterior nares are the primary ecological reservoir of *Staphylococcus aureus* in humans, and *S. aureus* nasal carriage is a major risk factor for a variety of infections [1]. Several bacterial factors involved in *S. aureus* nasal colonization: These include sortase A, the wall-teichoic acid, clumping factor B, capsular polysaccharides, Iron-regulated surface determinant A, alkyl hydroperoxide reductase, catalase, and the autolysin SceD [2, 3]. Recent evidence has been shown that polymicrobial interactions likely play a role in *S. aureus* nasal colonization [4, 5].Furthermore, host factors [6, 7] as well as environmental factors are recognized determinants of the *S. aureus* nasal carrier state [1]. There are three human nasal *S. aureus* carriage patterns can be distinguished: persistent carriage, intermittent carriage, and noncarriage [8]. *S. aureus* density in the anterior nares is higher in persistent carriers [9], which may partly explain their increased risk for *S. aureus* infections [10] an acquisition of *mecA* gene by *S. aureus* allows it to resist methicillin and other β-lactam antibiotics[11]. The first isolate of methicillin-resistant *Staphylococcus aureus* (MRSA) was reported in 1961 in England [12] Recently, MRSA has become a major public health problem worldwide [13].The burden of MRSA continues to rise and the rising colonization rates lead to the increasing of infection rates in the community and in hospitals. The consequence to the health care system is longer hospital stays and greater costs, which approximately double the expenditure per patient [14]. Large outbreaks of MRSA in other institutions, such as correctional facilities [15] and among otherwise healthy individuals in the community [16], raise the concern that this organism is spreading outside of its traditional role as a health care-related pathogen. MRSA are of particular clinical significance because they are resistant to all β-lactam antibiotics and has cross-resistant to other antibiotics with high ability to be transmitted among hospitalized patients so called epidemic MRSA [1, 17]. Nasal carriage is a major risk factor for MRSA infection and may disperse the organism into the air [18]. Therefore, Screening for colonization with methicillin-resistant *Staphylococcus aureus* (MRSA) is a key aspect of infection control to limit the nosocomial spread of this organism.

The aim of the present study is the detection of MRSA from nasal carrier in patients and healthcare workers and determining their susceptibility to some antimicrobial agents.

Materials and Methods

Specimen collections

Anterior nares swabs were taken from healthcare workers as well as patients, sterile swab was moistened with sterile normal saline and was rotated at least 5 times in one nares, then was placed in the transport media, using standard methods [19, 20, 21].

Specimens processing

All specimens were directly inoculated from transport media, brain-heart infusion (BHI) broth (Oxoid, England) onto plates of Mannitol Salt Agar (MSA) and incubated at 37°C for 24 hr. All colonies from primary cultures were purified by subculture on BHI agar (Oxoid, England) and then re-inoculated onto MSA and

incubated at 37°C for 24 hr. [22]**.** *S. aureus* were identified depending on the morphological features on culture media and biochemical tests according to Bergey's manual [23], Mastastaph latex Kit and Analytic profile index system (API-staph) were followed for further confirmation.

Antibiotic susceptibility test

All *S. aureus* isolates were tested for detection of susceptibility of the isolates for the commonly used antimicrobial agents by Kirby-Bauer method on Muller-Hinton agar (MHA) (Hi-media) [24]. Plates were incubated at 37°C for 18 h. Following the incubation, the diameter of inhibition zone was used as parameter for determination of sensitivity as compared with to a standard zone of growth inhibition table 1. Vancomycin susceptibility performed in broth dilution test, the results were compared with standard break points values; sensitive (≤ 2 µg/ml), intermediate (4-8 µg/ml) and resistant (≥ 16 µg/ml) according to Clinical and Laboratory Standards Institute (CLSI) recommendations [25].

Table 1-** Interpretation of zone inhibition using Kirby and Bauer method [disc diffusion method]

Antimicrobial agent	Code	Disc potency µg /Disc	Diameter of zone inhibition[mm]		
			Resistant	Intermediate	Sensitive
penicillin G	PCN	10 units	≤ 28	-	≥ 29
Cefoxitin	FOX	30 µg	≤ 21	-	≥ 22
Tetracycline	TE	30 µg	≤ 14	15-18	≥ 19
Ciprofloxacin	CIP	5 µg	≤ 15	16-20	≥ 21
Rifampin	RA	5 µg	≤ 16	17-19	≥ 20
Trimethoprim/sulfamethoxazole	SXT	1.25/23.75 µg	≤ 10	11-15	≥ 16
Gentamicin	CN	10 µg	≤ 12	13-14	≥ 15
Clindamycin	DA	2 µg	≤ 15	16-18	≥19
Clarithromycin	CLR	15 µg	≤10	11-12	≥13
Erythromycin	E	15 µg	≤ 13	14-22	≥ 23

****Adopted from CLSI (2012).**

Detection of Methicillin Resistant *S. aureus* (MRSA)

Cefoxitin 30µg disc was used as a alternative Methicillin; report Methicillin [25]**.** A 0.5 McFarland standard suspension of the isolate was made and lawn culture done on MHA plate. Plates were incubated at 37℃ for 18 h and zone diameters were measured. An inhibition zone diameter of ≤ 19 mm was reported as Methicillin resistant and ≥20 mm was considered as Methicillin sensitive.

Detection of Penicillin-resistant *S. aureus* (PRSA)

Penicillin-resistant, Methicillin susceptible strains are resistant to penicillinase-labile penicillins but susceptible to other penicillinase-sTable Penicillins, β-lactam/β-lactamase inhibitor combinations, relevant Cephems, and Carbapenems. Cefoxitin-resistant staphylococci are resistant to all currently available β-lactam antibiotics. Thus, susceptibility or resistance to a wide array of β-lactam antibiotics may be deduced from testing only Penicillin and Cefoxitin [25].

Results and Discussion

A total of 250 nasal swabs from patients and Healthcare workers (HCWs) were screened for, 106 individuals (42.4%) were identified as nasal carriers of *S. aureus.* Table 2 shows the distribution of participants in the study. The total response rates (the number of people who answered the survey divided by the number of people in the sample. It is usually expressed in the form of a percentage) [26] are 70% and 30% for patients and HCWs respectively. 32.4% and 37.6% of participating patients were male and female respectively, whereas 18% and 12% of participating HCWs were male and female respectively. The incidence of *S. aureus* varied among collected specimens. *S. aureus*

distributed in nasal accounted 44.7%, 46.9%, 33.3% and 35.6% among Female patients, Male patients, Female HCWs and male HCWs respectively. Patients had higher nasal carriage rate (45.7%) than HCWs (34.66 %). However in the general the range of *S. aureus* carriage rates was 42.4% table 2.

Table 2- Distribution of staphylococci between patients and HCWs**.**

Patients 175(70%)				HCWs 75(30%)			
Male (81)		Female (94)		Male (45)		Female (30)	
COPS	CONS	COPS	CONS	COPS	CONS	COPS	CONS
38 (46.9%)	43 (53.1%)	42 (44.7%)	52 (55.3%)	16 (35.6%)	٢٩ (64.4%)	10 (33.3%)	٢٠ (66.7%)

- **HCWs = Healthcare workers**
- **COPS = Coagulase Positive Staphylococci (*S. aureus*)**
- **CONS = Coagulase Negative Staphylococci**

While the incidence of MRSA varied among patients and HCWs. MRSA distributed in nasal accounted (100%) and (97.4%) for male and female patients respectively and (81.3%), (80%) for male and female HCWs, respectively. However, the nasal carriage of MRSA among *S. aureus* was 94.3% table 3.

Table 3- MRSA and MSSA nasal carriage among Patients and HCWs**.**

Total patients N=80				Total HCWs N=26			
Male		Female		Male		Female	
38*		42*		16*		10*	
37R (97.4%)	1S (2.6%)	42R (100%)	0S (0%)	13R (81.3%)	3S (18.7%)	8R (80%)	2S (20%)

- ***= *S. aureus***
- **R= MRSA**
- **S= MSSA**

In this study, the incidence of PRSA among *S. aureus* is 100%. However, by testing 9 additional antibiotics from different groups to 106 *S. aureus* isolates, the results showed that *S. aureus* isolates were resistant to Tetracycline 34.9%, Ciprofloxacin 29.2% , Rifampin 3.8% ,Trimethoprim/sulfamethoxazole 50% , Gentamicin 29.3%, Clindamycin 9.4%, Clarithromycin 14.2%, Erythromycin 37.7% and Vancomycin 3.8%. The highest frequency of sensitivity was observed with Rifampin 102 96.2% followed by Clarithromycin 83 78.3% figure 1

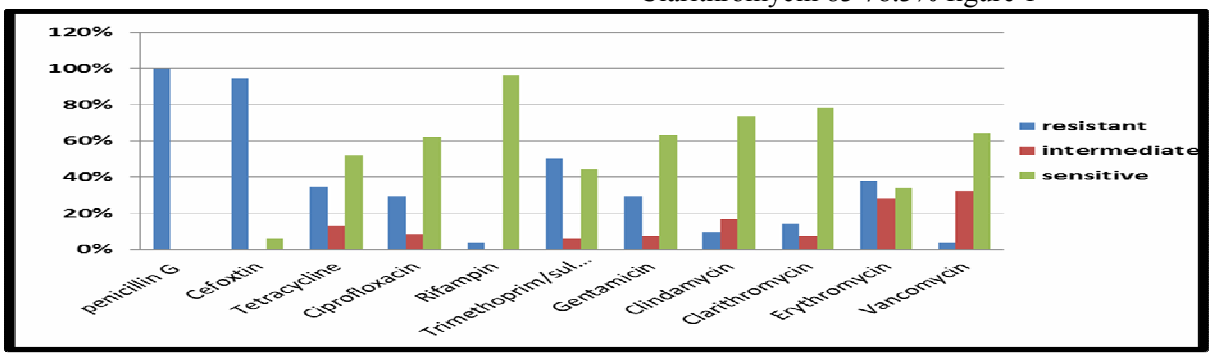

Figure 1- Antibiotic susceptibility of *S. aureus* isolates

Carriage of *S. aureus* appears to play a key role in the epidemiology and pathogenesis of infection [27]. However its prevalence have been severally reported in healthy populations; 43.2 % of *S. aureus* in nasal cavity of adults in Iraq, 17.3% in nasal cavity of Turkish children, 36% in nares of Japanese adults and 32.4% in nasal cavity of adults in USA[28,29,30,31]. Pant and Rai's [32] findings revealed higher *S. aureus* nasal colonization rate 43.8% in staffs of teaching hospital in Nepal. Also, in Abia state of Nigeria, Chigbu and Ezeronye [33] reported 50% nasal colonization in both hospital and non-hospital subjects. Chatterjee *et al.* [34] showed that the overall prevalence of *S. aureus* nasal colonization was 52.3 %. Whereas Onanuga and Temedie [35] showed that 33.3% *S. aureus* isolates were obtained from 120 nares specimens screened. Whilst, Adesida *et al.* [36] reported a much lower 14.0% nasal colonization in medical students in Lagos, Nigeria. These variations may be attributed to the characteristics of the population under study. A population that is on antibiotics as at the time of sampling may yield a much lower prevalence of *S. aureus* while a population from hospital settings may yield a much higher prevalence because of the high prevalence of infectious patients in that environment. Other factors that can cause variations may be sampling and culture techniques. *S. aureus* isolates of the present study appeared to be high resistance to Methicillin (Cefoxitin). The resistant rate was 94.3%. Such findings are in agreement with Al-Geobory [37] who found a resistant percentage of 90.9%. Fey *et al.*[38] stated that the resistancy to Methicillin was 81%, while Jain *et al.*[٢١] observed about 75.26% of isolates were Methicillin resistant, these observed differences may due to the variation in the geographic area, sources of clinical specimens, genetic background and the collection site of isolates[21]. The reason behind continuous increasing in resistant to β-lactam antibiotics may attribute to misuse of these antibiotics by the people. Different class of antibiotics such as Vancomycin, Linezolid, Quinupristin/ Dalfopristin (Streptogramin) and newer fluoroquinilones used for treatment of severe MRSA infection caused by multidrug resistant strain [39]. However, since 1996, MRSA strains with decreased susceptibility to Vancomycin (MIC, 8-16 μg/ml) and strains fully resistant to Vancomycin (MIC ≥ 32 μg/ml) have been reported [40].

To date, over 530 β-lactamases have been reported [41], This may explain why *S. aureus* isolates in the present study were totally resistance to Pencillin G 100% PRSA, This data agreed with Khorvash *et al*. [42], Al-Geobory [37], Al-Jundiy [43] and Zeidan [44], the percentage of penicillin G resistant in these studies were, 88%, 100%, 90%, 90.5%, respectively. Our results are compatible with those obtained by Brady *et al*. [45] as they observed that all isolates were resistant to penicillin and other β-lactam antibiotics. Our study results agreed with a study from Iran done by Aghazadeh *et al.* [46]. Likewise, our results are compatible with those obtained by Brady *et al*. [4٤] as they observed that all isolates were resistant to penicillin and other β-lactam antibiotics. However, the present study showed notorious creation in resistant to Vancomycin which represented by increasing in Vancomycin intermediate resistant *Staphylococcus aureus* (VISA) rate which was in this study 34 out of 106 (32.1%). With 4 out of 106 (3.8%) *S. aureus* isolates showed fully resistance to Vancomycin. This increase in rates of VRSA and VISA which may be due to increasing the usage of Vancomycin in hospitals and this suggests nasal carriers of *S. aureus*, represent important risk factor for infection and airborne dispersal of MRSA and VRSA in the hospital. This study agreed with Al-Geobory [37] in that the rate of resistant to Vancomycin was 2.27%. Where the VRSA isolate among *S. aureus* is isolates 4 out of 50 (8%) [47]. While Al-Hossainy [48] showed that the 20% VRSA among *S. aureus*. This suggests nasal carriers of *S. aureus*, represent important risk factor for infection and airborne dispersal of MRSA and VRSA in the hospital. Low levels of resistance revealed in this study to other antibiotics which is commonly used, these antibiotics include Erythromycin, Tetracycline, Gentamycin, Ciprofloxacin and Rifampin, the resistance percentages were 37.7%, 34.9%, 28.5%, 29.2% and 3.8%, respectively. Al-Geobory [37] demonstrated that the rates of resistant to Erythromycin, Tetracycline, Gentamycin, Ciprofloxacin and Rifampin were 34.09%, 31.81, 20.45%, 13.63% and 13.63%

respectively. This study found that 10 isolates (9.5%) were resistant to Clindamycin, this results were very closely similar to results obtained by Moran *et al.* [49] and Johnson *et al,* [50], in contrast, Al- Jundiy [43] found that 73% of *S.aureus* isolates were resistant, also Sauer *et al.*[51] observed that of 60% isolates were resistant to Clindamycin these differences in results may due to variation in geographic areas, sources of isolates, variations in isolates number and site of collection. The study also showed highly activity of Clindamycin 9.4% and Rifampcin 3.8% against both Methicillin resistant and sensitive *S. aureus* isolates [MRSA & MSSA], highly efficacy of Clindamycin and Rifampcin may belong to low rate of usage among patients inside or out hospitals, this result making Clindamycin and Rifampcin the unique chose in treatment. Finally, the study showed that all MSSA (6 isolates) were sensitive to all antibiotics except PenicillinG (100% resistant) and only one isolate was intermediate to Vancomycin (VISA).

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References

1. van den Akker, E. L.; Nouwen, J. L.; Melles, D. C.; van Rossum, E. F.; Koper, J. W.; Uitterlinden, A.G.; Hofman, A.; Verbrugh, H. A.; Pols, H. A.; Lamberts, S. W.; and van Belkum, A. **2006**. Staphylococcus aureus nasal carriage is associated with glucocorticoid receptor gene *polymorphisms. J. Infect*. Dis. **194**,pp:814-818.
2. Weidenmaier, C.; Kokai-Kun, J.F.; Kulauzovic, E.; Kohler, T.; Thumm, G.; Stoll, H.; Götz, F. and Peschel, A. **2008**..Differential roles of sortase-anchored surface proteins and wall teichoic acid in Staphylococcus aureus nasal colonization. *Int. J. Med. Microbiol*. **298**,pp: 505–513
3. Stapleton, M.R.; Horsburgh, M.J.; Hayhurst, E.J.; Wright, L.; Jonsson, I.M.; Tarkowski, A.; Kokai-Kun, J.F.; Mond, J.J.; and Foster, S.J. **2007**.Characterization of IsaA and SceD, two putative lytic transglycosylases of Staphylococcus aureus. *J. Bacteriol*. 189,pp: 7316–7325.
4. Iwase, T.; Uehara, Y.; Shinji, H.; Tajima, A.; Seo, H.; Takada, K.; Agata, T.; and Mizunoe Y. **2010**. Staphylococcus epidermidis Esp inhibits Staphylococcus aureus biofilm formation and nasal colonization. Nature 465,pp: 346–349.
5. Park, B.; Nizet, V.;and Liu, G.Y. **2008**. Role of Staphylococcus aureus catalase in niche competition against Streptococcus pneumoniae. *J. Bacteriol*. 190,pp: 2275–22.
6. Peacock, S.J.; and de Silva, I.; and Lowy, F.D. **2001**. What determines nasal carriage of Staphylococcus aureus? *Trends Microbiol.* 9,pp: 605–610.
7. Krebes, J.; Al-Ghusein, H.; Feasey, N.; Breathnach, A.; and Lindsay, J.A. **2011**. Are nasal carriers of Staphylococcus aureus more likely to become colonized or Infected with Methicillin-Resistant Staphylococcus aureus on Admission to a Hospital?. *J. Clin. Microbiol*. 49(1),pp: 430–432.
8. Wertheim, H.F.; Melles, D.C.; Vos, M.C.; van Leeuwen, W.; van Belkum, A.; Verbrugh, H.A.; and Nouwen, J.L. **2005**. The role of nasal carriage in Staphylococcus aureus infections. *Lancet. Infect. Dis*. 5,pp:751–62.
9. Nouwen, J. L.;Ott, A.;VandenBergh, M. F. Q.; Boelens, H. A. M.; Hofman, A.; van Belkum,A.; and Verbrugh, H. A. **2004**. Predicting the Staphylococcus aureus nasal carrier state: derivation and validation of a "culture rule". *Clin. Infect. Dis*. **39**,pp:806-811.
10. Bruun, J. N. **1970**. Post-operative wound infection: predisposing factors and the effect of a reduction in dissemination of staphylococci. *Acta. Med. Scand*. **514**,pp:1-89.
11. Deurenberg, R.H.; and Stobberingh, E.E. **2009**. "The molecular evolution of hospital- and community-associated methicillin-resistant Staphylococcus aureus". Current molecular medicine 9 (2),pp: 100–15.
12. Rolinson G.N. **1998**. Forty years of beta-lactam research. *J Antimicrob Chemother.* 41,pp:589-603.
13. Jarvis W.R.; Schlosser J.; Chinn, R.Y.; Tweeten, S.; and Jackson, M. **2007**. National prevalence of methicillin-resistant Staphylococcus aureus in inpatients at US health care facilities. Am. J. Infect. Control**.35**,pp: 631-637.
14. Kim, T.; Oh, P.I.; and Simor, A.E. **2001**. The economic impact of methicillin resistant Staphylococcus aureus in Canadian hospitals. Infect. *Control Hosp. Epidemiol*. 22,pp: 99-104. 13.

15. Centers for Disease Control and Prevention(CDC). **2003**. Methicillin-resistant Staphylococcus aureus infections in correctional facilities—Georgia, California, and Texas, 2001–2003. *MMWR Morb. Mortal*. Wkly. Rep.pp: 52:992–6.
16. Francis, J.S.; Doherty, M.C.; Lopatin, U.; Johnston, C.P.; Sinha, G.; Ross, T.; Cai, M.; Hansel, N.N.; Perl, T.; Ticehurst, J.R.; Carroll, K.; Thomas, D.L.; Nuermberger, E.; and Bartlett, J.G. **2005**. Severe community-onset pneumonia in healthy adults caused by methicillin-resistant Staphylococcus aureus carrying the Panton-Valentine leukocidin genes. Clin. Infect. Dis. 40(1),pp:100-7.
17. Lafi, M.A. **2008**. Incidence of methicillin-resistance and macrolide-lincosamidest-reptograminB resistance in a clinical sample of Staphylococcal isolates: a pharmacodynamic *study. J Fac Med* Baghdad .50 (4),pp:1-4.
18. Mohammed S.M. **2011**. Use of Cefoxitin as indicator for detection of Methicillin Resistant Staphylococcus aureus. *Baghdad Science Journal*. 8 (4),pp:947-955.
19. Kloos, W. E.; and Bannerman, T. L. **1999**. Staphylococcus and Micrococcus. In Manual of Clinical Microbiology, 7th edn, pp. 264–282. Edited by *P. R.* Murry, E. J. Baron, M. Pfaller, A. F. C. Tenover & R. K. Yolken. Washington, DC: *American Society for Microbiology*.
20. Monsen, T.; Rönnmark, M.; Olofsson, C.; and Wiström, J. **1998**. An inexpensive and reliable method for routine identification of staphylococcal species. *Eur. J. Clin. Microbiol. Infect.* Dis. **17**,pp: 327–335.
21. Jain, A.; Agarwal, A.; and Verma, R.K. **2008**. Cefoxitin disc diffusion test for detection of meticillin-resistant staphyloc-occi. *J. Med. Microbiol*. **57**,pp: 957-61.
22. Talan, D.A.; Staatz, D.; Staatz, A.; and Overturf, G.D. **1989**. Frequency of Staphylococcus intermedius as human nasopharyngeal flora. *J. Clin. Microbiol*. 27: ,pp:23-93.
23. Holt, J.G.; Krieg,N.R.; Sneath, P.H.A.; Staley, J.T.; and William, S.T. **2004**. Bergey's Manual of Determinative Bacteriology. 9th Edn., Willams and Wilkins, Baltimore, MA., USA.
24. Baur, A.W.; Kirby, W.M.; Scherris, J.C.; and Torch, M. **1966**. Antibiotic susceptibility testing by standardized single methods. *AM. J.eli. Path* 45,pp:493-496.
25. Clinical and Laboratory Standards Institute (CLSI). **2012**. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-second Informational Supplement. CLSI document M100-S22. CLSI, Wayne, PA.
26. Keeter, S.; Kennedy, C.; Dimock, M.; Best, J.; and Craighill, P. **2006**. Gauging the Impact of Growing Nonresponse on Estimates from a National RDD Telephone Survey. *Public Opinion Quarterly*. 70(5),pp: 759-779.
27. Kuehnert, M.J.; Kruszon-Moran, D.; Hill, H.A.; McQuillan, G., McAllister, S.K.; Fosheim, G.; McDougal, L.K.; Chaitram, J. .; Jensen, B.; Fridkin, S.K.; Killgore, G.;and Tenover, F.C. **2006**.Prevalence of Staphylococcus aureus Nasal Colonization in the United States. *The Journal of Infectious Diseases.*193,pp:172–179.
28. Moellering, R.C. **1998**.Problems with antimicrobial resistance in gram-positive cocci. *Clin Infect Dis*.26,pp:1177–1178.
29. Uemura, E.; Kakinohana, S.; Higa, N.; Toma C.; Nakasone, N. **2004**. Comparative characterization of Staphylococcus aureus isolates from Throats and Nose of Healthy Volunteers. *Japanese J. Infect. Dis*. 57,pp :21–24.
30. Soysal, A.; Sahin, H.; Yagci, A.; Barlan, I.; and Bakir, M. **2006**. The low rate of Methicillin-Resistant Staphylococcus aureus in Turkish Children. *Japanse J Infect Dis*. 59,pp:195–196.
31. Onanuga, A.; and Onalapo, J.A. **2008**. Antimicrobial susceptibility of community associated Staphylococcus aureus isolates from healthy women in Zaria. *Tropical Journal of Pharmaceutical* Research. 7(1),pp:929–939.
32. Pant, J.; and Rai, S.K. **2007**. Occurrence of Staphyloccous aureus in Hospital Environment and Staffs in Teaching Hospital in Katmandu, *Nepal. J. Nepal Assoc*. Medi. Lab. Sci. 8,pp:72–73.
33. Chigbu, C.O.; and Ezeronye, O.U. **2003**. Antibiotic resistant Staphyloccous aureus in Abia State of Nigeria. *Afr. J. Biotech*. 2(10),pp:374–378. .
34. Chatterjee, S.S.; Ray, P., Aggarwal, A.; Das, A.; and Sharma, M. **2009**. A community-based study on nasal carriage of

Staphylococcus aureus. *Indian. J. Med*. Res. 130(6),pp:742-8.

35. Onanuga, A.; and Temedie, T.C. **2011** .Nasal carriage of multi-drug resistant Staphylococcus aureus in healthy inhabitants of Amassoma in Niger delta region of Nigeria. *Afr Health Sci*. 11(2),pp:176-81.
36. Adesida, S.A.; Abioye, O.A.; Bamiro, B.S.; Brai, B.I.C.; Smith, S.I.; Amisu, K.O.; Ehichioya, D.U.; Ogunsola, F.T. ; and Coker A.O. **2007**. Associated risk factors and pulsed field gel electrophoresis of nasal isolates of Staphylococcus aureus from medical students in a tertiary hospital in Lagos, Nigeria. *Braz. J. Infect. Dis*. 11(1): ,pp:63-69.
37. Al-Geobory, H. A. **2011**. Comparative study between Methicillin resistant Staphylococcus aureus (MRSA) and Methicillin sensitive Staphylococcus aureus (MSSA), and detect the antimicrobial effects of some plant extracts on them. Msc. Thesis. College of Science/ Baghdad University. Iraq
38. Fey, P. D.; Said-Salim, B.; Hinrich, S. H.; Boxrud, D. J.; Davis,C. C.; Kreiwirth, B. N.; and Schlievert, P. M. **2002**. Comparative molecular analysis of community or hospital-Acquired Methicillin-Resistant Staphylococcus aureus. *J. Antimicrob. Chemother*. 2003,pp: 196-203.
39. Boyce, J.M. **2003**.Update on resistant *Staphylococcus aureus* infections. National foundation for infect dis. VI. Issue 2.
40. Sonavane, A.D.A.; and Mathur, M. **2007**. Screening for Vancomycin intermediate resistant Staphylococcus aureus among clinical isolates of MRSA. *Indian J. Med Microbiol* .,pp:25:79-80.
41. Babic, M.; Hujer, A.; and Bonomo, R. (2006). What's new in antibiotic resistance? Focus on beta-lactamases. Drug. Resist. Upd., 9: 142-156. Humphreys H **2009**. Preventing surgical site infection. Where now? *J. Hosp. Infect*.,pp: 73: 316-322.
42. Khorvash, F.; Abdi, F.; Ataei, B.; Neisiani H.F., Kashani H.H.;and Narimani T. **2012**.Nasal carriage of *staphylococcus aureus:* Frequency and antibiotic resistance in healthy *adults. J Res Med Sci*; 17(5) Special Issue 2,pp: S230-S233.
43. AL-Jundiy, A. S. **2005**. Immunological Study on TSS-1 toxin extracted from Staphylococcus aureus Isolated from infected wounds. Ph. D. Thesis. College of Science/ AL-Mustansyria University. Iraq.
44. Zeidan, I. A. **2005**. Genetic and bacteriologic study to Staphylococcus aureus isolated from clinical specimens and resistant to Vancomycin antibiotic. Msc. Thesis. College of Science/ Baghdad University. Iraq.
45. Brady, J. M.; Stemper, M. E.; Weigel, A.; Chyou, P-H.; Reed, K. D.; and Shukla, S. K. **2007**. Sporadic "Transitional" Community-Associated Methicillin- Resistant *Staphylococcus aureus* Strains from Health Care Facilities in the *United States. J. Clin. Microbiol*.**45**,pp:2654-2661.
46. Aghazadeh, M.; Rahbar, M. ; Monnavar, M. K.; and Moghadam, F.S. **2009**.Sensitivity Pattern of Methecillin Resistant and Methecillin Sensitive *Staphylococcus aureus* Isolates, Against Several Antibiotics Including Tigecycline in Iran : A hospital Study . *Pak. J. Med. Sci*.25 (3),pp:443-446.
47. Mohammed S.M. **2010**. Use of Cefoxitin as indicator for detection of Methicillin Resistant Staphylococcus aureus. *Baghdad Science Journal* 8(4),pp: 947-955.
48. Al-Hossainy D.J.M. **2007**. Isolating and diagnosis staphylococcus aureus bacteria from patients infection of urinary tract infection in Al-Diwanyia city. *Al-Qadisiyah Journal for Science Veterinary Medicine*.6 (1),pp:52-57.
49. Moran, G. J.; Krishnadasan, A.; Gorwitz, R. J.; Fosheim, G. E.; McDougal, L. K.; Carey, R. B.; and Talan, D. A.**2006**. Methicillin-Resistant *S. aureus* infections among patients in the emergency department. N. Engl. Med. 355,pp: 666-674.
50. Johnson, P. D. R.; Howden, B. P.; and Bennett, C. M. **2006**. Staphylococcus aureus : a *guide for the perplexed. M. J*. a. 184,pp: 374-375.
51. Sauer, P.; Sila, J.; Stosova, T.; Vecerova, R.; Hejnar, P.; Vagnerova, I.; Kolar, M.; Raclavsky, V.; Petrzelova, J.; Lovecova, Y.; and Koukalova, D. **2008**. Prevalence of genes encoding extracellular virulence factors among methicillin resistant Staphylococcus aureus isolates from the university hospital, Olomuc, Czech republic. *J. Med. Microbiol*. 57,pp: 403-410