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Bacteriological and Molecular Detection of *Staphylococcus Aureus* and its Resistance to Methicillin among Specimens from Kirkuk Community

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Abstract
Background: The nasal cavity is the main colonization site of Staphylococcus aureus (S. aureus) in human body. Nasal carriage may be a strong risk factor for some serious infection.
Methods: One thousand anterior nasal swabs were collected,700 from healthy adult individuals in Kirkuk university which include 100 swabs from science staff , 300 from science students , 150 swabs from medical and 150 swabs from nursing students and 300 from health care workers with other 50 clinical samples from burn and surgery wound patients in Azadi teaching hospital in Kirkuk city\Iraq the samples were collected by cotton swabs and diagnosed by standard tests in addition to molecular diagnosis and mecA gene detection for the isolates.
Results :(%22.7) 159of isolates from community students and 91 (%30.3) from HCWs were recorded as S.aureus and 3(1.9%) with 4(4.4%) were identified as CA-MRSA and HA-MRSA respectively depending on the standard tests of *S.aureus* identification, According to the age (18-28) and (51-60) age group were demonstrated the higher rate of S.aureus and MRSA carriage 181 (72.4) and 3 (1.2) respectively. According to sex the male 131 (52.4) and 3 (1.2) were recorded the higher range of *S.aureus* and MRSA carriage respectively. Also 50 clinical samples were collected by which 25 samples were from burn patients and 25 samples from surgery wound patients : the higher rate of S.aureus and MRSA isolates were from burn patients 13 (%54.2) and 11(%45.8) respectively.all S.aureus and MRSA isolates were demonstrated high resistant towards beta-lactam antibiotics except oxacillin because in present study 10mg of oxacillin was used, while the isolates were recorded low resistant towards non beta-lactam antibiotics and all of them were sensitive towards ciprofloxacin and vancomycin. All the isolates (n = 20) molecularly diagnosed as *S.aureus*, also all of them expressed specific sequence gene of mecA gene that confirmed all the isolates were MRSA
Conclusions: The continuous monitoring of nasal *S.aureus* is needed to control MRSA-related infections

Keywords: *Staphylococcus aureus*, Nasal carriage, Antimicrobial susceptibility, MRSA ,Community , Healthcare workers , PCR , mecA gene.

Introduction

Staphylococcus aureus is a frequent cause of dangerous health complications increasing rate of morbidity and mortality. It is a part of normal flora colonizes the nasal cavity and skin. About 25–30% of healthy persons in community are nasal carriers for *S.aureus* [1, 2]. *S.aureus* invades tissues in case of epithelial barriers damaging by trauma or surgical interventions [3, 4].
In recent years, *S.aureus* gradually increased its resistance to various antibiotics. misuse and/or overuse of antimicrobial drugs increased the resistant strains of *S.aureus* [5].
It causes both community and hospital infections. The Methicillin resistant *S.aureus* (MRSA) is considered as commonest causes of hospital acquired infection and considered as common factor in causing failure of management [6]. MRSA strains have appeared in community, not only in hospitals, called as community acquired MRSA (CA-MRSA),these strains have spread among healthy persons

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in communities and entered the hospitals [7].
Cross contamination of Community acquired-MRSA and Hospital acquired MRSA may happened by Healthcare workers (HCWs)[8]. In developing countries, HCWs considered as the source of nosocomial infections [9, 10].
The mecA gene is carried by cassette chromosome mec(SCCmec) causes methicillin resistance[11].

This study done to determine the nasal carriage rate of *S.aureus* and MRSA among community persons' and HCWs in addition to clinical samples and to determine antibiotic susceptibility pattern of the isolates and molecular diagnosis with mecA gene detection of the isolates.

Materials and methods

Samples

From January 2012 to May 2012,one thousand anterior nasal swabs were collected,700 from healthy adult individuals in Kirkuk university which include 100 swabs from science staff , 300 from science students , 150 swabs from medical and 150 swabs from nursing students and 300 from health care workers with other 50 clinical samples from burn and surgery wound patients in Azadi teaching hospital in Kirkuk city\Iraq the samples were collected by cotton swabs and then transferred to laboratory ,cultured on mannitol salt agar and blood agar media , all culture plates incubated at 37c for 24hr.

Diagnosis of *Staph. aureus*

Staph. aureus isolates were identified by using standard microbiological methods which include colony morphology on blood and mannitol salt agar , Gram stain , Catalase , DNAase and Coagulase test(slide and tube method).
The collected isolates were stored at 20c in brian-heart infusion containing 15% glycerol utile use [12,13].

Detection of MRSA

Detection of MRSA isolates was carried out by using disk diffusion test . A sterile swab was dipped in an *Staph. aureus* suspension (McFarland standard 0.5) and plated on to muller – hinton agar, methicillin/ oxacillin disks (10mg from Bioanalyes company)

Drug Susceptibility Tests. The antimicrobial susceptibility of S. aureus isolates was assessed by disk-diffusion tests, according to the Clinical Laboratory Standards Institute guidelines [14]. The following antimicrobial discs were used: Antibiotic susceptibility test was done on Mueller-Hinton agar (Oxoid) by Kirby– Bauer method. 19 antibiotic discs, Ampicillin, Penicillin, Oxacillin (10mg), Amoxicillin, Cephalothin ,Cefoxitin ,Cloxacillin ,Cefotaxime ,Cefexime, Erythromycin, Lincomycin ,Trimethoprim , Chloramphenicol, Tetracycline Amikacin, Rifampicin, Vancomycin, Gentamycin and Ciprofloxacin
S. aureus ATCC 25923 was used as the internal control in each run of the test.

Data analysis

It made by version 6 of Graph Pad Prism software.

Genetic tests

MRSA chromosomal DNA isolation

Total genomic DNA was extracted from only *S.aureus* isolates were identified as ethecillin/oxacillin resistant by disk diffusion using Genomic DNA Mini kit (Geneaid;USA).The DNA concentration has

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been determined by measuring absorbance of the sample at 260 nm using spectrophotometer ,then resolved by gel electrophoresis in 0.8% agarose gel in 1xTBE buffer at 60 volt for 30 min. ,visualized under Uv light and photographed using a high resolution digital camera [15].

PCR amplification

Primer selection

Table -1 list the primers were selected to detect the 16SrRNA gene and mecA gene (Bioneer: USA), according to [16].

Table-1 : primers used in this study

Primers	Sequence	Target gene	Size)bp(
Sauf 234 Saur 1501	5-CGA TTC CCT TAG TAG CGG CG-3 3-CCA ATC GCA CGC TTC GCC.5	Staphylococcus aureus 16SrRNA	1267
MR1 MR2	5-GTG GAA TTG GCC AATACA GG-3 3-TGA GTT CTG CAG TAC CGG AI-5	mec A gene	1339

PCR protocol (Bioneer:USA) PCR premix kit: Table-2 list the PCR premix component

Table-2: PCR component volume

components	Reaction size
Taq DNA Polymerase (dNTPs) (pH9.0) Tris- Hcl Kcl mgcl2	1U 250mM 10mM 30mM 1.5mM

PCR mixture reaction

According to the kit procedure a mixture of a total of 20 *uL* reaction volume was prepared as in table-3

Table-3:PCR reaction mixture

Final reaction content	Volume *ul*	Final concentration
Master mix	3ul	50 pmole
Forward primer	2ul	50 pmole
Reverse primer	2ul	50 ng
Template DNA	5ul	
Sterile de ionized water	8u	
Total	20ul	---

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The amplification of DNA were performed in thermocycler programmed as in table-4

Table-4:Steps of thermo cycler program

Steps	Temperature	Time	No.of cycles
Initial denaturation	94 c	5min	1
Denaturation	94 c	30 sec	30
Anneding	55 c	30 sec	
Extended	72 c	45 sec	
Extra incubation	72 c	5 min	1

The amplification products were resolved by electrophoresis in 1.5% agarose gel at 90 volt for 50 min , gels stained with ethidium bromide visualized under UV light which appeared as light fluorescent band and photographed using a high resolution digital camera.

RESULTS

700 samples were collected from community students and 300 samples from healthcare workers at Azadi teaching hospital in Kirkuk city, Iraq.(%22.7) 159 of isolates from community students and 91 (%30.3) from hospital staff were identified as *S.aureus* and 3(1.9%) with 4(4.4%) were identified as CA-MRSA and HA-MRSA respectively depending on standard tests of *S.aureus* identification (Table 5).

Table5 : Distribution of Nasal carriage *S.aureus and MRSA isolates* among community and HCWs samples

Groups	Total number	NO. of nasal carriage *S.aureus*(%)	NO. of nasal carriage MRSA(%)
Community persons'	700	(%22.7) 159	(%1.9) 3
Health care workers	300	(%30.3) 91	(%4.4) 4
Total	1000	(%25) 250	(%2.8) 7

The higher rate of nasal carriage of *S.aureus* were recorded among medical students40 (%26.6) and the CA-MRSA were higher among science college staff 2(%9.5) (Table 6)

Table 6 : Distribution of Nasal carriage *S.aureus and MRSA isolates* among community groups

Community groups	SEX	NO. of nasal carriage *S.aureus*(%)	NO. of nasal carriage MRSA(%)

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Science college students	male 150 female150	%59 %41	36 25	0 0	
Total	300	%20.3	61	0	
Medical college students	male 71 female 79	%52.5 %47.5	21 19	1 0	
Total	150	%26.6	40	%2.5	1
Nursing college students	male 51 female 99	%31.6 %68.4	12 26	0 0	
Total	150	%25.3	38	0	
Science college staff	male 54 female 46	%52.3 %47.6	11 10	1 1	
Total	100	%21	21	%9.5	2
Total Number	male 326 female 374	%50.3 %50	80 79	%1.3 %0.6	2 1
	700	%22.7	159	%1.9	3

while the nurses were recorded the higher carriage rate of *S.aureus* among health care workers 47 (%31.5) and the laboratory technicians were demonstrated the higher carriage of HA-MRSA 2 (%10) (Table 7).

Table 7 : Distribution of Nasal carriage *S.aureus* and MRSA among HCWs

Health Care Workers groups (HCW)	**SEX**	**NO. of nasal carriage *S.aureus*(%)**		**NO. of nasal carriage MRSA(%)**	
Doctors	male5 female5	%50 %50	1 1	- -	
Total	10	%20	2		
Laboratory technicians	male30 female38	%55 %45	11 9	1 1	
Total	68	%29.4	20	%10	2
Nurses	male78 female71	%55.3 %44.7	26 21	1 1	
Total	149	%31.5	47	%4.3	2
Clean workers	male40 female33	%59.1 %40.9	13 9	- -	
Total	73	%30.1	22	-	
Total Number	male153 female 147	%56 %44	51 40	2 2	
	300	%30.33	91	%4.41	4

According to the age (18-28) and (51-60) age group were demonstrated the higher rate of *S.aureus*

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and MRSA carriage 181 (72.4) and 3 (1.2) respectively.(Table 8)

Table8 : Prevalence of *Nasal carriage S.aureus and MRSA isolates* according to their age

Age groups	Total number	NO. of nasal carriage *S.aureus*(%)		NO. of nasal carriage MRSA(%)	
18- 28 year	730	(72.4)	181	(0.8)	2
29-39years	114	(9.6)	24	(0.8)	2
40-50 years	115	(11.6)	29	0	
51 - 60 years	41	(6.4)	16	(1.2)	3
Total	1000	(25)	250	(2.2)	7

According to sex the male 131 (52.4) and 3 (1.2) were recorded the higher range of *S.aureus* and MRSA carriage respectively. (Table 9)

Table9 : Prevalence of Nasal carriage *S.aureus and MRSA isolates* according to their sex

Gender	Total number	NO. of nasal carriage *S.aureus*(%)		NO. of nasal carriage MRSA(%)	
Male	479	(52.4)	131	(1.6)	4
Female	521	(47.6)	119	(1.2)	3
Total	1000	(25)	250	(2.8)	7

In the present study, also 50 clinical samples were collected by which 25 samples were from burn patients and 25 samples from surgery wound patients : the higher rate of *S.aureus* and MRSA isolates were from burn patients 13 (%54.2) and 11(%45.8) respectively. (Table 10)

Table 10 : Distribution of *Nasal carriage S.aureus and MRSA isolates* among clinical samples

Clinical samples	Total number	NO. of nasal carriage *S.aureus*(%)		NO. of nasal carriage MRSA(%)	
Burn patients	25	(%54.2)	13	(%33.3)	8
Surgery wound patients	25	(%45.8)	11	(%20.8)	5
Total	50	(%48)	24	(%54.1)	13

all *S.aureus* and MRSA isolates were demonstrated high resistant towards beta-lactam antibiotics except oxacillin because in present study 10mg of oxacillin was used, while the isolates were recorded low resistant towards non beta-lactam antibiotics and all of them were sensitive towards

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ciprofloxacin and vancomycin. (Table 11,12).

Table11 : Nasal carriage *S.aureus* resistance towards antibiotics according to their source

CLINICAL ISOLATES	NASAL ISOLATES		Source of *S.aureus* ANTIBIOTICS
Burn & surgery wound patients n=24	HCW n=91	Community Persons n=159	
(%100)24	(%100)91	(%88)140	Penicillin
(%100)24	(%87,9)80	(%83)132	Ampicillin
(%100)24	(%87,9)80	(%76,1)121	Amoxicillin
(%100)24	(%100)91	(%100)159	Carbincillin
(%54,2)13	(%4,3)4	(%1,8)3	Oxacillin
(%83,3)20	(%80,2)73	(%69,1)110	Cloxacillin
(%100)24	(%100)91	(%61,6)98	Cephalothin
(%83,3)20	(%28,5)26	(%21,3)34	Cetoxitin
(%100)24	(%100)91	(%100)159	Cefexime
(%100)24	(%100)91	(%100)159	Cefotaxime
(%20,8)5	(%10,9)10	%0	Gentamycin
(%37,5)9	(%8,7)8	(%6,9)11	Trimethoprim
(%29,2)7	(%7,6)7	(%5,6)9	Chloram phenicol
(%58,3)14	(%21,9)20	(%11,9)19	Erythromycin
(%20,8)5	(%19,7)18	(%7,5)12	Tetrayetin
(%50)12	(%12)11	(%7,5)12	Refampin
(%20,8)5	(%12)11	(%5,6)9	Lincomycin
(%16,6)4	(%7,6)7	(%13,8)4	Amikacin
———	———	———	Ciprofloxacin
———	———	———	Vancomycin

Table 12: Nasal carriage MRSA resistance towards antibiotics according to their source

	NASAL ISOLATES		Source of MRSA ANTIBIOTICS
Community Persons n=13	HCW n=4	Community Persons n=3	
(%100)13	(%100)4	(%100)3	Beta lactam antibiotics

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(%30,7)4	(%25)1	%0	Gentamycin
(%38,5)5	(%25)1	(%33,3)1	Trimethoprim
(%46,1)6	(%25)1	%0	Chloramphenicol
(%46,1)6	(%50)2	(%33,3)1	Erythromycin
(%30,7)4	(%25)1	%0	Tetracycline
(%38,5)5	%0	%0	Rifampin
(%30,7)4	%0	%0	Lincomycin
(%15,4)2	%0	%0	Amikacin
%0	%0	%0	Ciprofloxacin
%0	%0	%0	Vancomycin

All the isolates (n = 20) molecularly diagnosed as *S.aureus*, also all of them expressed specific sequence gene of mecA gene that confirmed all the isolates were MRSA(Fig.1,2).

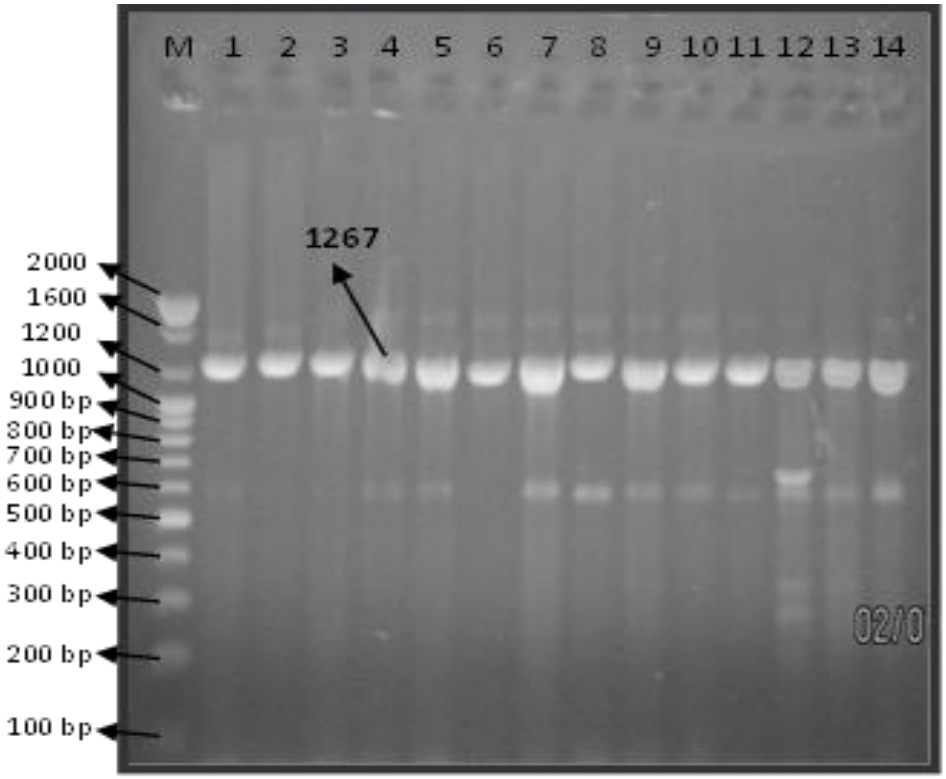

Figure 1- PCR product of ((Sauf 236 , SauR 1501) gene for S.aureus identification by gel electrophoresis. Lane M: 100bp DNA ladder. Lane 1-3: CA-MRSA 4-7: HA-MRSA 8-14: Clinical isolates.

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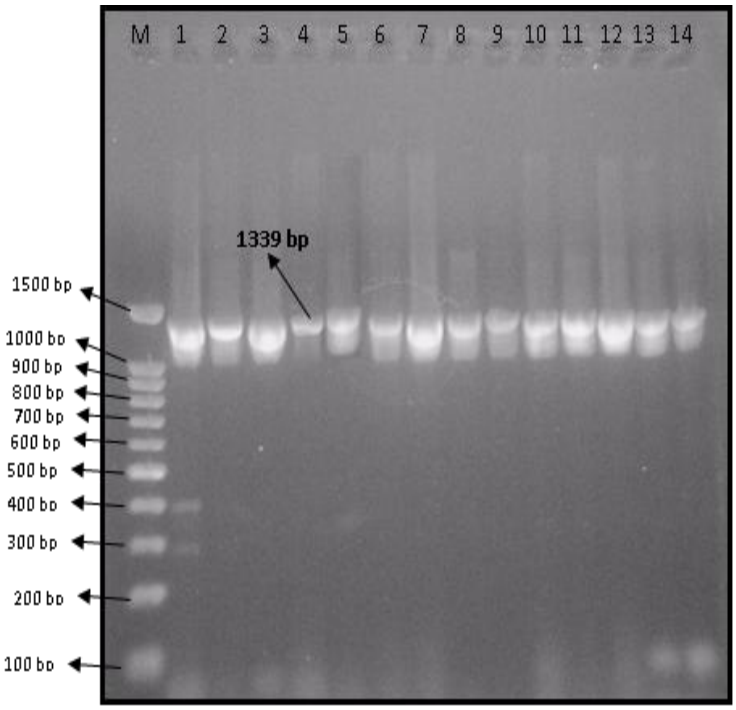

Figure 2- PCR product of (MR2 , MR1) gene for mecA gene detection by gel electrophoresis.

Lane M: 100bp DNA ladder. Lane 1-3: CA-MRSA 4-7: HA-MRSA 8-14: Clinical isolates.

DISCUSSION

The distribution of *S.aureus* and MRSA among community persons' and HCWs were (22.7% , 30.3%) and (1.9% , 4.4%) respectively.
These findings agreed with many studies ; in a study in China ,prevalence of *S.aureus* and MRSA carriage in community were 24.7%and (1.4%) respectively.[17]
In another study in Nepal ,prevalence of MRSA nasal carriage was 4.6 % among HCWs [3]. Other studies in Nepal have demonstrated 20.37 – 43.8 % nasal carriage rate of *S.aurues* among HCWs [18–21] and agreed with study in Iraq in which the prevalence of MRSA was 4.2% [22]. In contrast ,some studies didn't agree with the present study , in Jordan ,rate of nasal MRSA was 10.1% among HCWs [23], 73% among healthcare workers from Saudi Arabia [24]. in Northern China (16.5%), of which 0.3% were MRSA and in adults in community settings in Taiwan (22.1%) [25, 26]. These variations in the rate of *S.aureus* nasal carriage among countries may be because of the differences in culturing, geographical distribution, diagnostic techniques and sampling used in studies.[27-30]

The prevalence of *S. aureus and MRSA* were higher in male than in female and in 18-28 , 51-60 age group respectively , these findings agreed with many studies [31, 32].

The rate of *S. aureus and MRSA* were higher in burn patients than in surgery wound patients ,this agreed with many studies .[33,34].

In present study , *S. aureus and MRSA* were demonstrated higher resistance towards "Beta-lactam antibiotics" group than "non Beta-lactam" group, by β-lactamase and penicillin-binding proteins

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production and mutations effect on the permeability of bacterial outer membrane toward these antibiotics also through alterations in the targets of drugs.[27-29],with exception in oxacillin because in present study oxacillin 10mg was used,this agreed with study of Norfarid et al [35], ciprofloxacin and vancomycin were the most affective antibiotics in present study ,no resistance demonstrated towards both of them , many studies demonstrated various rate of bacterial resistant like Olayemi etal , Norfarid et al ,Nagi et al and Carvalho et al [35-38] ,the differentiations among the studies may be because of hygienic culture of studied population and their practices with having these antibiotics and kind of clinical samples that were taken in each studies [27-29].

All the isolates (n = 20) molecularly diagnosed as *S.aureus*, also all of them expressed specific sequence gene of mecA gene that confirmed all the isolates were MRSA ,molecular detections consider as most sensitive techniques at both genus and species level of *S.aureus* detection with 100% accuracy in MRSA detection, when compared with the classical tests in *S.aureus* identification. [39].

Conclusion

To control the nasal colonization of *S.aureus* and MRSA among healthcare workers, continuous screening program ,infection control measures and treatment of MRSA positive healthcare workers in addition to control in the frequency of their exposure with the vulnerable patients.

Such studies can provide information on the spread or rate of nasal colonization of *S.aureus* and MRSA and detect new strains in hospitals and communities. Active surveillance of MRSA in health care settings is highly recommended.

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