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RESEARCH ARTICLE OPEN ACCESS

Prevalence of Methicillin-Resistant and Methicillin Sensitive *Staphylococcus aureus* Nasal Carriage and their Antibiotic Resistant Patterns in Kirkuk City, Iraq

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Abstract

The carriage of community-acquired methicillin-resistant *S. aureus* (CA-MRSA) has become a source of community-associated infections, particularly in the anterior nares of people worldwide. This study was aimed at determining the prevalence of community-acquired *S. aureus* (CA-SA) and CA-MRSA nasal carriage among healthy individuals of various ages in Kirkuk City and evaluating the susceptibility of isolates to various antibiotics. A total of 597 healthy individuals were recruited in the community between December 1, 2021, and December 30, 2022. Nasal swabs obtained from participants were taken to the laboratory, where bacteria were isolated and identified using phenotypic characteristics. The MRSA isolates were identified by applying the modified Kirby Bauer disc diffusion technique. The results showed that the prevalence of CA-SA and CA-MRSA nasal carriers was 16.6 and 4.5%, respectively. The average age of the participants was 33.2 years, with a male-to-female ratio of 1.1: 1. The highest resistance of the isolates was observed against oxacillin (27.3%), followed by penicillin G (24.3%), amoxicillin (15.2%), erythromycin (12.1%), and tetracycline (6.1%). There was a 9.1% resistance rate to clindamycin, rifampin, gentamycin, and ciprofloxacin. However, all CA-MRSA isolates were multi-drug resistant. However, all the isolates were sensitive (100 %) to vancomycin, linezolid, and mupirocin. The findings of the present study highlight the potential for CA-SA and CA-MRSA acquisition in this population, which may be related to antibiotic abuse or overuse as well as poor hygiene. To lessen the impact of community-associated strains of MRSA nasal carriage, this necessitates the probable need for infection prevention measures and adequate antibiotic therapy.

Keywords: Prevalence, *Staphylococcus aureus*, MRSA Nasal Carriage, Antibiotic Resistant, CA-MRSA

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INTRODUCTION

Staphylococcus aureus is a human skin and mucosa commensal.[1] It is a major pathogen found in both the community and hospitals that cause a variety of diseases,[2] including recurrent tonsillitis,[3] pneumonia, soft tissue infections, urinary tract infections, and bloodstream infections.[2] Although *S. aureus* can colonize various parts of the human body, the anterior nares are the principal ecological niche for this bacterium. About 20-30% of people are permanent carriers, and about 30 % are transient carriers.[4] *Staphylococcus aureus* has become resistant to some types of antibiotics. Methicillin-resistant *S. aureus* (MRSA) strains are spread worldwide and they were found to be resistant to various Beta-lactam (β-lactam) antibiotics such as penicillin, cephamycins, and cephalosporin,[5] except ceftaroline (fifth-generation cephalosporins), which is used for treating MRSA infection.[6] Also, the species of *Staphylococcus* often developed resistance to different classes of an antibiotic such as quinolones, macrolides, and aminoglycosides.[5]

The mecA gene, which encodes a penicillin-binding protein with a low affinity (PBP2a), is the genetic determinant of methicillin resistance in MRSA. The staphylococcal cassette chromosome mec (SCCmec) types IV and V, which are frequently carried by CA-MRSA, are the mobile genetic elements by which the mecA gene is transported.[6] Since the 1990s, there has been an increase in CA-MRSA infections in the general population, and the incidence of *S. aureus* and MRSA is also rising rapidly.[7,8] The nasal carriage rate of *S. aureus* has been frequently employed as an indicator for determining the antibiotic resistance of *S. aureus* and MRSA in the community because it is a significant risk factor for a wide range of staphylococcal infections.[9] A research in Kirkuk, Iraq, found that 16% of 100 restaurant employees had MRSA nasal carriage.[10] Additional research on CA-MRSA nasal carriage has been conducted in other regions of Iraq among a variety of populations, including healthy individuals in Diyala City,[11] intermediate students in Muthanna Province,[12] healthy children in Basrah City,[13] Syrian refugees in Duhok City,[14] and secondary school students at Duhok City.[15] They demonstrated relative prevalence rates of 21.5, 24, 41.2, 35, and 2.04%, respectively. Similarly, a greater prevalence of MRSA nasal carriage (55/181) among the healthy population has been recorded in other countries, such as west Iran.[16] Additionally, in a community-based study conducted in Arak, central Iran,[17] Turkey,[18] Jordan,[19] and Saudi Arabia,[20] the nasal colonization rates of MRSA were reported to be 4.5, 9, 40.9, and 25%, respectively. Screening enables the identification of nasal carriers, which enhances the application of decolonization and other preventive measures to reduce postoperative infection in carriers and the use of antibiotics.[21,22]

Therefore, the present study was conducted to determine the prevalence of CA-SA, including CA-MRSA nasal carriage, among a healthy population of all ages in Kirkuk City, Iraq. The antibiotic susceptibility of the test isolates was also detected.

MATERIALS AND METHODS

This study was conducted between 1st December 2021 and 30th December 2022 among healthy people living in Kirkuk City. Ethical approval was obtained from the Ethics Committee of the College of Nursing, University of Kirkuk. A total of 597 individuals of both genders, with ages ranging from 10 to 70 years, participated in the study. An anonymous questionnaire that asked about socio-demographic information like age, gender, and whether or not the respondent had ever used antibiotics or been hospitalized within the previous three months was created. The study's participants were chosen from a pool of community members. After giving their consent, approved by each participant or, in the case of minors (< 18 years old), by their parents on behalf of the minor, they were enrolled at their workplaces or homes.

Sample collection

Sterile cotton swabs were used for a biological sample collection from the anterior nares of all participants. The procedure was repeated after a month to demonstrate a persistent carrier of *S. aureus*, according to Azis et al.[23] The samples were rapidly transported using Cary Blair transport media to the laboratory at the Department of Medical Laboratory Techniques, College of Health and Medical Techniques,

Northern Technical University, Kirkuk, Iraq.

Microbiological analysis

The samples were cultured on 5% sheep blood agar and incubated at 37°C for 24-48 hr. The isolates were sub-cultured on mannitol salt agar, and nutrient agar, and then incubated at 37°C for 24-48 hr. Identification based on characteristics of colony features, gram staining reactions, and biochemical analysis including catalase, and coagulase tests (slide and tube methods) were used to identify *S. aureus* isolates according to previously described procedures.[24] More so, the API-Staph system (Biomerieus, France) was employed for a confirmatory test.

Antimicrobial susceptibility testing

A suspension colony of each confirmed *S. aureus* isolate was made in sterile normal saline and cultured at 37°C for 15 minutes. The concentration was adjusted to 0.5% McFarland Standard. Then, using the modified disc diffusion (Kirby- Bauer) technique, the sensitivity of the test isolates was assessed against a variety of antibiotics, including penicillin (P; 10 µg), amoxicillin (AM; 10µg), oxacillin (OX; 1 µg), gentamycin (Gen; 10 µg), clindamycin (CD; 2 µg), rifampin (RM; 5 µg), erythromycin (E; 15 µg), ciprofloxacin (CIP; 5 µg), tetracycline (TE; 30 µg), linezolid (LZ; 30 µg), mupirocin (200; µg), and vancomycin (VA; 10 µg) obtained from Bioanalysis Company. The results of the antibiotic susceptibility patterns were interpreted according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI).[25] All oxacillin-resistant isolates were assessed as MRSA by their ability to grow on Muller Hinton agar containing (6 mg/ml) of oxacillin and 4% Nacl.[26] *Staphylococcus aureus* strain (ATCC 25923) was used as a positive control to evaluate the effectiveness of microbiological and antibiotic sensitivity testing.[27] D-test was used for detecting inducible clindamycin-resistant bacteria by placing clindamycin and erythromycin discs (Bioanalysis Company) on Muller-Hinton agar plates at a distance of 15 mm from each other and were incubated at 37°C for 24 hr. Clindamycin resistance is thought to be inducible when there is a flattening or D-shaped zone between the clindamycin and erythromycin discs.[28] All tests were repeated three times.

Statistical analysis

All the data were collected and tabulated. The people who had recently taken antibiotics were removed. The association between the variables was examined using the Chi-Square test, and $p < 0.01$ was considered significant. The prevalence of nasal carriage of *S. aureus* as well as MDR-SA was calculated as a percentage of all participants who were positive at the anterior nares during the specific period of the study. Also, the percentage of resistant bacteria for each antibiotic was calculated.

RESULTS

A total of 597 healthy individuals were examined for *S. aureus* carriage using nasal swabs. Their ages ranged from 10 to 70 years, with a mean of 33.2 years. The ratio of male to female was 1.1:1. There was no statistical association between gender and CA-SA nasal carriage. Additionally, 99/597 (16.5%) persistent *S. aureus* carriers were identified because they produced positive results during both the first and second sample collection events, as shown in Tables 1 and 2. MRSA and MSSA were found to be present in 27/99 (27.3%)

Table 1. Events of sample collection (n=597)

Sampling event	No. of CA-Nasal carriage CA-MRSA *S. aureus*/Total	%	No. of nasal carriage/ Total	%
1st Sampling	102/597	17.1%	27/597	4.5
2nd Sampling	111/597	18.6%	31/597	5.2
The positive samples at both 1st and 2nd sample collection event	99 /597	16.6%	27/597	4.5

%: Percentage

Table 2. Distribution of *S. aureus* and CA-MRSA nasal carriage based on age group and sex

Variable	No. (%)	*S. aureus* nasal carrying No. (%)		P-value	CA-MRSA nasal carriage No. (%)		P-value
		Positive	Negative		Positive	Negative	
Age group (years)							
10-20	240(40.20)	18 (3.52)	222 (37.19)	0.00	03 (0.50)	237 (39.70)	0.001
21-30	171(28.64)	22 (3.69)	149 (24.96)		07 (1.17)	164 (27.47)	
31-40	75 (12.51)	20 (3.35)	55 (9.21)		05 (0.84)	70 (11.73)	
41-50	36 (6.03)	15 (2.52)	21(3.52)		03 (0.50)	33 (5.53)	
51-60	45 (7.54)	20 (3.35)	25 (4.19)		05 (0.84)	40 (6.53)	
61-70	30 (5.03)	04 (0.67)	26 (4.36)		04 (0.67)	26 (4.36)	
Total	597	99	498(83.45)		27(4.5)	570 (95.48)	
Gender							
Male	315(52.76)	54 (9.04)	261(43.72)	0.697	15 (2.52)	300 (50.25)	0.766
Female	282 (47.24)	45(7.54)	237 (39.7)		12 (2.01)	270 (45.23)	
Total	597 (100)	99(16.6)	498 (83.42)		27 (4.5)	570 (95.48)	

M: Male; F: Female; No.: Number: %: Percentage

and 72/99 (72.7%) of the participants, which constitute 4.5 and 12%, respectively. The majority of CA-SA and CA-MRSA nasal carriages, which account for 3.69 and 1.17%, respectively, of all individuals, were also found among participants aged 21 to 30 years (Table 2). This result indicates a statistical association between age and CA-MRSA nasal carriage rate.

As shown in Table 3, oxacillin resistance was found in 27/99 (27.3%) of the isolates, followed by penicillin G resistance in 24/99 (24.3%), amoxicillin resistance in 15/99 (15.2%), erythromycin resistance in 12/99 (12.1%), and tetracycline resistance in 6/99 (6.1%), and there was a 9/99 (9.1%) resistant rate to each of clindamycin, rifampin, gentamycin, and ciprofloxacin. When compared to methicillin-sensitive *S. aureus* (MSSA) isolates, it was observed that MRSA isolates have a greater resistance rate. Comparatively, all examined isolates were 100% (99/99) sensitive to vancomycin, linezolid, and mupirocin. Fifteen (2.51%) of the investigated isolates, including 9 MRSA and 3 MSSA, showed induced clindamycin resistance. Additionally, 27/99 (27.3%) of the isolates were MRSA, and 33/99 (33.3%) of them were multi-drug resistant. Multi-drug resistance, as used in this study, was defined as being resistant to three or more of the antimicrobial agents tested.

DISCUSSION

S. aureus is one of the most common opportunistic pathogens in the community and hospitals.[2] Clinically important infections are frequently caused by it, and their severity can range from mild skin infections to more dangerous invasive illnesses.[29] According to earlier studies, nearly 30% of healthy individuals are permanent nasal carriers of *S. aureus*, with a higher rate among children.[30] MRSA strains in particular pose a significant risk for the development and subsequent insidious staphylococcal infections.[31] In the current study, the prevalence of persistent nasal carriage of *S. aureus* among the population in Kirkuk City was 99/597 (16.6%). This observation is in line with that of other investigators in Marrakesh, who found that *S. aureus* was present in the anterior nares of 16.3% of 300 healthy children.[32] The adult population of nine European countries was studied, and the prevalence of *S. aureus* was found to be greater (21.6%), ranging from 12.1% in Hungary to 29.4% in Sweden.[33] Another study from the Iraqi province of Diyala also found a higher carriage rate than what was obtained in the present study, which was 21.5% out of 1186 healthy people.[11] Moreover, the value obtained in the current study was found to be lower compared

Table 3. Antibiotic resistant profile of nasal carriage *S. aureus* (n=99)

No.	Antibiotics	MRSA (n=27)	%	MSSA (n=72)	%	Total (n=99)	%
1.	Penicillin G	24	24.3	36	36.4	60	60.6
2.	Oxacillin	27	27.3	0	0	27	27.3
3.	Amoxicillin	15	15.2	09	9.1	24	24.2
4.	Erythromycin	12	12.1	06	6.1	18	18.2
5.	Tetracycline	06	6.1	06	6.1	12	12.1
6.	Clindamycin	09	9.1	03	3.0	12	12.1
7.	Rifampin	09	9.1	0	0	06	6.1
8.	Gentamycin	09	9.1	0	0	06	6.1
9.	Ciprofloxacin	09	9.1	03	3.0	06	6.6
10.	Linezolid	0	0	0	0	0	0
11.	Mupirocin	0	0	0	0	0	0
12.	Vancomycin	0	0	0	0	0	0

%: Percentage

with previous studies in other areas of the world, such as Turkey,[18] northwest Ethiopia,[28] Iran,[16] Italy,[34] Nepal,[35] which documented a carriage rate of 17, 23, 30.6, 42, 44, and 66%, respectively. In addition, the nasal carriage prevalence of *S. aureus* among specific groups, including healthcare workers in Erbil City,[36] healthy children in Basrah City,[13] secondary school students in Duhok City,[15] workers in restaurants in Kirkuk City,[10] were 24.5, 14.24, 18.4, and 30%, respectively. The variation of nasal carriage of *S. aureus* in the different investigations could be attributed to differences in geographical distribution, properties of the studied participants, sampling methodologies, preservation, and diagnostic methods.[28] It has been reported that the mucin layer in the anterior nares is related to *S. aureus* colonization in this area of the body through an interaction of mucin carbohydrates with staphylococcal protein.[37]

MRSA nasal carriers were present in 4.5% of the study's participants. This result is consistent with a community-based study from Arak, Iran.[17] It was greater than the prevalence rate of CA-MRSA nasal carriage among secondary school children in Duhok City, Iraq, which was reported by previous researchers to be 2.04%.[15] In other studies undertaken in Iraq, the nasal carriage prevalence of CA-MRSA among healthy children in Basrah City,[13] intermediate students at Muthanna Province,[12] and healthy workers in restaurants at Kirkuk City,[10] were 41.2, 24, and 16%, respectively. Furthermore, the result of the current study is consistent with the reports from Italy,[34] India,[38] and Marrakesh,[32] which showed that the prevalence of MRSA nasal carriage was 3, 3.17, and 4%, respectively. As opposed to reports from Egypt (32%), Saudi Arabia (25%),[20] Africa (15%),[28] Northwest Ethiopia (9.79%),[28] and Jordan (40.9%),[19] the prevalence of MRSA carriage in the present study was lower. The differences in the nasal carriage rate among these populations might be attributed to the initiation of antimicrobial therapy before sample collection.[39] Furthermore, some research has discovered a link between *S. aureus* resistance to methicillin and the use of a beta-lactam combination.[40]

Concerning gender, the result of the current study showed that the percentage of male carriers was higher than that of females, but there was no statistical association between gender and MRSA carriage rate ($p<0.01$). This result is consistent with that reported by Abdelmalek et al.[19] However, there is a significant statistical association between age and the CA-MRSA nasal carriage rate, as most CA-SA, and CA-MRSA nasal carriers were among the ages of 21 to 30 years, which constitute 3.69, and 1.17%, respectively, of all the participants. This observation might be attributed to attendance at a fitness center, as this practice is becoming very common in Iraq, particularly among males. This result is consistent with a recent study conducted in Jordan that examined the link between *S. aureus* carriage and gym attendance.[19]

Regarding antibiotic susceptibility, the majority of the studied isolates were resistant to oxacillin (27.2%), followed by penicillin G, 24/99 (24.3%), and amoxicillin, 15/99 (15.2%). The values obtained in this result were lower than those of other studies involving healthy individuals from the community, which showed high resistance to penicillin of about 65%,[23] resistance to penicillin in Europe (87.1%),[41] and resistance to penicillin in Nepal and Ohio (100%), with only 5.8 and 1.1% resistant rates to oxacillin in Nepal and Ohio, respectively.[35] The resistance of *S. aureus* isolates to penicillin was determined within a decade after its introduction in 1940.[42] As a result, new beta-lactam antibiotics were produced, which were related to the appearance of *S. aureus* strains that produce beta-lactamase.[43] Since methicillin was developed to treat infectious disorders brought on by microbial strains resistant to penicillin, the occurrence of resistant *S. aureus* to methicillin has resulted in a decrease in the effectiveness of antibiotic therapy.[44] Lower resistance of isolates was shown to exist against erythromycin (18.1%) and tetracycline (12.1%). Other studies also reported a low resistance (<12%) to both tetracycline and erythromycin, which is in line with the results obtained in the present study.[23] Other investigators showed in a comparative study of two cities in Egypt and Saudi Arabia that a higher resistance rate to erythromycin was observed in selected MRSA and MSSA isolated from outpatients. Values of 55 and 24% were recorded for MRSA and MSSA, respectively, in Egypt and 58 and 25%, respectively, in Saudi Arabia.[20] the percentage of resistance to clindamycin, rifampin, gentamycin, and ciprofloxacin was 9.1%. This observation was in line with other studies that reported low resistance of their *S. aureus* isolates and recorded high sensitivity of 94, 98, 90.2, 84 and 93% to clindamycin, gentamycin, cefoxitin, cotrimoxazole, and ciprofloxacin, respectively.[28]

Comparatively, all studied isolates (100%) were sensitive to vancomycin, linezolid, and mupirocin, indicating that these are appropriate treatments for MRSA infection and decontamination of MRSA carriers. This result is consistent with other findings in Basra, Iraq, that reported 100% susceptibility to vancomycin.[45] In addition, other researchers from Erbil, Iraq, demonstrated that all isolates of MRSA and MASSA were susceptible to linezolid and mupirocin.[36] The results also revealed that MRSA accounted for 27.3% of the isolates of *S. aureus* from healthy people's anterior nares, which were 33.3% multi-drug resistant. This observation is slightly higher than another study, which reported 30.8%.[28] Onanuga and Temedie, on the other hand, found that 52.5% of their isolates were multi-drug resistant community-associated *S. aureus* infections,[46] of which were higher than the result of the present study. Moreover, 2.51% of isolates showed an inducible clindamycin-resistant phenomenon. This observation was lower than that reported by Baguma, who discovered that 42% of the test isolates showed this phenomenon.[47] There are many recommendations for applying the PCR techniques in the diagnosis of Methicillin-resistant *Staphylococcus aureus*[48,49] due to their accurate results and short time. The polymerase chain reaction (PCR) was applied in the detection of pathogenic microorganisms,[50-60] and genetic-related diseases.[61-64]

CONCLUSION

The findings from this study reveal that there is a chance that the population under study will develop community-associated *S. aureus*, MRSA, and multidrug resistance isolates, which may be caused by improper or excessive use of antibiotic therapy and poor hygiene. Additionally, it has been observed that MRSA isolates had a greater rate of antibiotic resistance than MSSA isolates during the investigation. This calls for prospective surveillance among local populations in various parts of the country, as well as molecular characterization of nasal carriage CA-MRSA to ascertain the prevalence and profile of antibiotic resistance among local communities across the country. Also, applying antimicrobial stewardship will help reduce the burden of antibiotic-resistant bacteria and community-associated MRSA nasal carriage infections.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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None.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

This study was approved by the Ethics Committee of the College of Nursing, University of Kirkuk, Kirkuk, Iraq.

INFORMED CONSENT

Written informed consent was obtained from the participants before enrolling in the study.

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