

PREVALENCE OF METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* AMONG FOOD HANDLERS IN DUHOK CITY

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Food handlers harbouring *S. aureus* on their noses or in hands are considered as the main source of food contamination. Antibiotic resistance in *S. aureus* is a serious issue. The study aimed to find out the prevalence of methicillin-resistant *S. aureus* among food handlers in restaurants of Duhok city. Nasal and hand swabs were taken from 200 food handlers. The collected swabs inoculated on mannitol salt agar and incubated at 37°C for 24-48h. Isolates identified as *S. aureus* underwent antimicrobial sensitivity testing to methicillin and vancomycin on Mueller Hinton agar. A total of 74 (37%) out of 200 cultures of food handlers were found to be colonized with *S. aureus*. 53 (26.5%) isolated from the nose, compared with 8 (4.0%) from hands and 13 (6.5%) from both. 27% of the food handlers were found to be MRSA carrier, and none of the isolates were resistant to Vancomycin. The study revealed a high prevalence of MRSA among food handlers in Duhok city. Food handlers in public places are required to go through regular screening for both nasal and skin carriage of *S. aureus* for the early detection and treatment of carriers. This is to protect the community from staphylococcal food poisoning and the spread of resistant *S. aureus* strains among the population.

KEYWORDS: MRSA, Food handlers; Prevalence; Duhok.**1. INTRODUCTION**

Staphylococcus aureus is a common commensal of the skin and mucosal membranes of humans, with estimates of 20–30% for persistent and 60% for intermittent asymptomatic carriers. The presence of these bacteria in food occurs frequently due to inappropriate manipulation of food by carriers (Alhashimi *et al.*, 2017). Globally, foodborne diseases are one of the main health problems and the World Health Organization (WHO) estimated that they caused 600 million foodborne illnesses and 420,000 deaths in 2010. The global burden of foodborne diseases was 33 million disability adjusted life years (WHO, 2015). Nasal carriage of *S. aureus* in food handlers is the major risk of staphylococcal food poisoning, but the skin is much less. Food handlers carrying enterotoxin-producing *S. aureus* in their noses or on their hands are concerned as the main source of food contamination, by either manual contact or through respiratory discharges (Ahmed and Mashat, 2014). Moreover, antibiotic resistance in *S. aureus* is a serious issue and the resistance occurred in different media and on different surfaces. Strains of methicillin-resistant *Staphylococcus aureus* (MRSA) are of special concern as they responsible for the substantial causes of morbidity and mortality worldwide. Methicillin-resistant *S. aureus* is resistant to all available penicillin and other lactam antimicrobial drugs. Data collected from 28 countries during 2009-2012 showed a significant increase in the percentage of MRSA isolates that reached up to 50% in some countries like Portugal and Romania (Castro *et al.*, 2015). The incidence of community-acquired *S. aureus* infections has been rising with the increasing emergence of MRSA, earlier MRSA infections were limited to the hospitals, but it has been steadily more reported in the community (Mainous *et al.*, 2006). Although, vancomycin is one of the first line antibiotics that used to treat patients with MRSA but recently cases of vancomycin resistance have been also recorded (Shokouhi *et al.*, 2017). Staphylococcal food poisoning outbreaks (SFPOs) are ranked in the last years as the first cause of food-borne illness outbreaks in many countries. *S.*

aureus has attributed to 23% of the food-borne disease outbreaks in 2014 (Mossong *et al.*, 2015). SFPOs are under-reported for many reasons such as the short incubation period 30 minutes to 6 hours, only 10% of SFPOs patients visit a health facility, the medical staff usually does not request a stool analysis and even if a stool analysis is carried out, the routine procedures frequently do not involve testing for the existence of enterotoxigenic *S. aureus* unless particularly requested by the medical staff (Mossong *et al.*, 2015).

The main objective of this study is to evaluate the prevalence of nasal and skin carriage of *S. aureus* and MRSA and to examine the vancomycin sensitivity pattern amongst food handlers working in different types of restaurants in Duhok city, Kurdistan Region, Iraq.

A cross-sectional study was adopted to achieve our objectives. The study was conducted from 1st November 2017 to 31st July 2018.

2. METHODS**2.1 Sampling**

At the time of the study, there were (314) different restaurants in Duhok city. The restaurants classified by Directorate of Preventive Health Affairs in Duhok into three categories casual dining (popular), fast foods and fine dining restaurants. The classification depended on according to certain criteria like type of services and total area (Directorate of Preventive Health Affairs, 2017). The available restaurants are divided into three types (strata), a stratified sampling procedure was used to enrol 40 restaurants from Duhok city through choosing proportionate number of restaurants from each strata (restaurants lists) by simple random sampling procedure (table1). These restaurants are distributed throughout all quarters, sectors and main malls in the city. Two hundred samples were taken from the selected restaurants.

Table 1. Number & percentage of restaurants stratified by types

Types of Restaurants	Total number of Restaurants		Number of selected Restaurants
	No.	%	
Casual dining (Popular)	219	69.7	27
Fine dining	41	13.1	6
Fast food	54	17.2	7
Total	314	100.0	40

2.2 Inclusion criteria

All persons who are serving in the enrolled restaurants and involved in the process of food preparation and introducing it like cooks, waiters and kitchen assistants are included in the study.

2.3 Exclusion criteria

Other restaurants' staffs such as administrative staff, owners and others who do not have contact with food preparation or serving are excluded from the study.

2.4 Isolation and identification of *S. aureus*

From each food handler two samples were taken one from nasal cavities and the other from their hands, and two different swabs were utilized for each of them. One sterile swab was used for both anterior nares (left and right) of the workers; the swab was inserted 1-2 cm inside the nasal cavity with rotating 2-3 seconds around the insides of each nostril. To inhibit any discomfort, the used swabs were moistened with sterile distilled water. Moreover, another sterile swab was used to swab both hands of the food handlers, involving the rest of both hands and in between fingers about five seconds for each hand. Then the swabs transferred into transport media then cultured in Mannitol salt agar (Oxoid) and incubated aerobically at 37°C for 24 to 48 hours. Then isolates were considered *S. aureus* by using gram staining, standard biochemical reactions (catalase and coagulase tests) and fermentation on mannitol salt agar.

2.5 Antibiotic susceptibility testing of *S. aureus*

Antimicrobial susceptibility was performed on Mueller-Hinton agar (LAB M, UK) using Kirby-Bauer disk diffusion method according to diameter interpretation standards charts. The bacterial suspension prepared by emulsifying in 5 ml of sterile normal saline until turbidity is approximately equivalent to that of the McFarland No. 0.5 turbidity standard. A sterile swab was dipped into the bacterial mixture; removed excess inoculum by squeezing on the wall of the test tube. Then, using this swab the surface of the agar was then uniformly and lightly inoculated. Then by sterile forceps two antibiotic disks (Methicillin 5µg and Vancomycin 30µg) [LAB M™, A Neogen® company, United Kingdom] were placed for each plate. The plates were incubated at 35°C for 24 hours. After incubation time finished, inhibition zones that observed on agar plate were measured according to standard chart (Vandepitte *et al.*, 2013; CLSI, 2017).

2.6 Data analysis

All data pertaining to demographic information, and laboratory results were entered and edited using Microsoft Word 2010 for windows 10. Data analysis was conducted with the Statistical Package for Social Science (SPSS) software version 23. Descriptive statistics was provided for the demographic data and questionnaire of the study. Chi-square test was utilized to estimate the association of the studied variables with MRSA. P-Values ≤ 0.05, was considered statistically significant.

2.7 Ethical considerations

Before starting the study, it was approved first by the Scientific Committee of Shekhan Technical College of Health in Duhok Polytechnic University. At the same time, this study was approved by the Research Ethical Committee of the Directorate General of Health of Duhok governorate was obtained.

3. RESULTS

3.1 Demographic characteristics

There were 200 food handlers working in 40 different restaurants, and all participants were males. The main age group of the subjects was 20-29 years. About two thirds of the participants were high school educated and illiterate, 37% and 35%, respectively, and only 3.5% of the participants were graduates of institute or university. Meanwhile about 36.5% of food handlers were involved in food preparation (chefs) followed by waiters and kitchen assistants (including cleaners and dishwashers) 35.5% and 24%, respectively. These characteristics are presented in table 2.

Table 2. Demographic characteristics of the food handlers.

Demographic Variables	Number (%)	
Age (years)	<20	29(14.5)
	20-29	80(40.0)
	30-39	38 (19.0)
	40 and more	53 (26.5)
Education level	Illiterate	70 (35)
	Primary school	49 (24.5)
	High school	74 (37)
	Institute or university	7 (3.5)
Job title	Cook	73(36.5)
	Waiters	71(35.5)
	Kitchen assistants	48(24)
	Others	8 (4)

3.2 Types of restaurants and number of food handlers

200 workers from 40 restaurants are included in the study. Casual dining (popular) restaurants represent the bulk of included restaurants 27 (67.5%) followed by fast food restaurants 7 (17.5%) and fine dining restaurants 6 (15%) respectively. Out of 200 food handlers included in the study, more than half of them 105 (52.5%) belonged to casual dining restaurants and 59 (29.5%) of them were from fine dining restaurants while the least number 36 (18%) were from fast food restaurants, as shown in table 3.

Table 3. Classification of restaurants and distribution of food handlers

Types of restaurants	Food Handlers	
	No. (%)	No. (%)
Casual dining (popular)	27(67.5)	105 (52.5)
Fast food	7 (17.5)	36 (18.0)
Fine dining	6 (15)	59 (29.5)
Total	40 (100.0)	200 (100.0)

3.3 Staphylococcus aureus carriers and age groups

The prevalence of *S. aureus* carriers among food handlers was 37% (74 out of 200 food handlers). The distribution of *S. aureus* carriers according to age groups are shown in table 4. There were no significant association between age group and *S. aureus* carriers.

Table 4. *Staphylococcus aureus* carriers and age group

Age group in years	<i>Staphylococcus aureus</i> carriers		Total (%)
	Positive (%)	Negative (%)	
<20	14 (18.9)	15 (11.9)	29 (14.5)
20-29	23 (31.1)	57 (45.2)	80 (40)
30-39	15 (20.3)	23 (18.3)	38 (19)
40 and above	22 (29.7)	31 (24.6)	53 (26.5)
Total	74 (100.0)	126 (100.0)	200 (100.0)

P-value=214

3.4 Prevalence of *S. aureus* carriers according to the anatomical site

Table 5 shows that in 71.6 % of carriers, the *S. aureus* was isolated from their noses only and in 10.8% was isolated from the hands (skin) only, while in 17.6% the *S. aureus* was isolated from both sites (nasal cavity and hand). There were highly significant differences between the anatomical sites of isolation (P -value < 0.001).

Table 5. Prevalence of *S. aureus* carriers according to the anatomical site

<i>S. aureus</i> carriers	All sample size (n.200)	Carriers (no.74)
	No. %	No. %
Nose	53 (26.5)	53 (71.6)
Hand	8 (4.0)	8 (10.8)
Nose and Hand	13 (6.5)	13 (17.6)
Total	74 (37)	74 (100.0)

P-Value < 0.001

3.5 Prevalence of *S. aureus* carriers and MRSA status

Table 6 shows that MRSA was detected among 54(73%) of the *S. aureus* carriers and it represents 27% of the studied population.

Table 6. Prevalence of *S. aureus* carriers and MRSA status

<i>S. aureus</i> carriers	MRSA status		Total No. (%)
	Positive (%)	Negative (%)	
Yes	54 (73)	20 (27)	74 (37)
No	Not applicable	126 (63)	126 (63)
Total No. (%)	54 (27)	146 (73)	200 (100.0)

3.6 Prevalence of *S. aureus* carriers and vancomycin resistant *S. aureus* status

No cases of Vancomycin Resistant *Staphylococcus aureus* (VRSA) were detected among all *S. aureus* food handlers' carriers.

4. DISCUSSION

S. aureus is involved in nearly all *Staphylococcal* food poisoning. Food handlers are usually considered as one of the primary sources of these organisms. However, it is difficult to identify the source of the strains implicated in *Staphylococcal* food poisoning outbreaks. The major source of food contamination via direct contact or through respiratory secretions is food handlers carrying enterotoxin-producing *S. aureus* in their noses or on their hands.

Moreover, food handlers have been accepted to play an important role in the transmission of foodborne diseases; imparting important to the global incidence and burden of diseases (Omololu-Aso *et al.*, 2017).

4.1 Demographic Characteristics of Food Handlers

In the present study, all the food handlers were male and this might be due to the reluctance of the female, the tradition and customs of the region that may prevent female from working in the restaurants or public places, and indeed, the owners of public places in Duhok recruit foreigner workers. A similar result was obtained by Simsek *et al.*, (2009) who found that (299) food handlers in Sanliurfa, in South-eastern Anatolia are males because the majority of women in Southern Anatolia are unemployed. Habiballah *et al.*, (2017) found that majority of workers (81.6%), who are working in tourist restaurants in Irbid, Jordan were males; they found that females are not eager to work in restaurants (Kasturwar and Shafee 2011). Other studies revealed that their samples are mixed near to equal percentages (Kasturwar and Shafee 2011; El-Shenawy *et al.*, 2014; Castro *et al.*, 2016). Regarding the educational levels, the majority of food handlers (96.5%) have high school or less educational levels, because less educated people have little chances to work in high ranking jobs. El-Shenawy *et al.*, (2014) mentioned that all of their study samples are either illiterate or high school educated, while Kasturwar and Shafee, (2011) found that about only 20% of food handlers having a degree above high school level. In this study, the percentage of cooks (36.5%) is slightly more than waiters (35.5%) in contrast to a study in Karimnagar, India, where the majority of food handlers were serving food (waiters) 60.2% and only 12% working in food preparations (cooks) (Kasturwar and Shafee, 2011). Due to the financial crisis in Kurdistan region that started in 2014, many companies and institutions reduced their manpower including restaurants in Duhok city. They reduced the number of waiters while kept their chefs and even many of the owners and their family members worked as waiters to ease the financial burden on them.

4.2 Prevalence of *S. aureus* carrier

The current study revealed that 37% of food handlers are carriers of *S. aureus*, while Saeed, (2015) found about 29.24% (n=106) of food handlers in restaurants and cafeterias of Duhok city are carriers of *S. aureus*. Another study was done in Tikrit, Iraq by Alamarai *et al.*, (2015) who found that 28% (n=100) of studied food handlers were carrier for *S. aureus*. Alhashimi *et al.*, (2017) revealed that the prevalence of nasal carriage of *S. aureus* among 332 food handles in Karbala city, Iraq was 30.1%. A further study was done in Turkey; in which *S. aureus* was lower than that of the current study 20% (Vatansever *et al.*, 2016). Mainous *et al.*, (2006) estimated that about 32.40% of the total United States population (86.9 million) was colonized with *S. aureus*. Meanwhile, another study among 200 participants working in three different food processing plants in Egypt that reported the prevalence rate of *S. aureus* 38% that are in accordance with our results (EL- Shenawy *et al.*, 2014), and the prevalence of *S. aureus* in Makkah city among food handlers working in city kitchens was 40.3% (Ahmed and Mashat, 2014). In addition to all of the previous studies, higher *S. aureus* carrier rates among food handlers were recorded in many studies such as the study done among 140 food handlers in public hospitals in Salvador city, Brazil, where the carrier rates were 50% (Ferreira *et al.*, 2014), while higher rate 57.7% detected in Gaborone, Botswana. (n=200) (Loeto *et al.*, 2017). The ability of *S. aureus* to colonize or infect its host is related to its ability to express virulence factors that facilitate their adherence to surfaces, cause damage or its ability to evade host's immune system (Udo *et al.*, 2009).

4.3 Prevalence of *S. aureus* carriers according to anatomical sites

Staphylococcus aureus is part of the commensal flora of human skin and mucosal surfaces. The main site for *S. aureus* in human carriers is the nose, which may be colonized during the first few days of life. Strains present in the nasal cavity may contaminate the hands and nasal carriers can thus readily become skin carriers (Hatakka *et al.*, 2000). Approximately 20% (range, 12% to 30%) of healthy people are persistent *S. aureus* nasal carriers, 30% (range, 16% to 70%) are intermittent carriers, and 50% (range, 16% to 69%) are non-carriers (Yan *et al.*, 2015). Persistent carriers usually carry the same strain for extended periods of time, whereas intermittent carriers tend to host different strains over time. The presence of *S. aureus* in the hands of the general population range from 8-28%, according to independent surveys (Hatakka *et al.*, 2000).

The prevalence of *S. aureus* nasal carriage varies by geographical location, age, gender, and ethnicity. The carriage is highest amongst newborns (up to 70 %) but steadily decreases with age (Aryee and Edgeworth, 2017). Rates of carriage have also been found to be higher in patients with certain diseases like diabetes mellitus, intravenous drug addicts, haemodialysis patients, surgical patients and AIDS patients. Despite the fact that *S. aureus* is found at multiple body sites, most studies have only focused on nasal carriage (Aryee and Edgeworth, 2017). The present study shows that the distribution of carriers according to the anatomical sites of swab collections was from the nasal cavity in 71.6 % of (53 out of 74) of carriers, 10.8% from the hands (skin) and in 17.6% the *S. aureus* was isolated from both sites (nasal cavity and hand). (Vatansever *et al.*, 2016) investigated 282 food workers in Kars city, Turkey. They took swabs from the nose, mouth, and hands. *S. aureus* was isolated from 56 (20%) of food workers which involving 32 (57 %) isolates from the nasal nares, 13 (23%) isolates from the mouth and 11 (20 %) isolates from the hands of food handlers respectively. In Portugal, the prevalence of *S. aureus* among the studied population was 24.7% (n=162), the distribution of carriers according to the sites of swab taking was 32(19.8%), 18 (11.1%) and 10 (6.2%) from nose, hands and both sites respectively (Castro *et al.*, 2016).

In contrast, in (Ferreira *et al.*, 2014) study *S. aureus* detected from the skin in 50% of carriers and less detected from the nasal cavity 29.3. In another study of the prevalence of *S. aureus* carriage among food workers at the cafeteria of the Federal University of Technology, Nigeria showed the *S. aureus* was isolated from 28 (51.85%) of the 54 food handlers examined, 21 (38.9%) harbour *S. aureus* in their nasal cavity, 11 (20.4%) in their throat and 6 (11.1%) in their both sites (Emeakaroha *et al.*, 2017). The distribution of the microorganism varied according to sites on the body, with the nasal cavity harbouring higher numbers of isolates (44.6%), while the hand was (30.9%) and face was (24.5%) (Loeto *et al.*, 2017).

4.4 Prevalence of MRSA

In our study, the prevalence of MRSA among the study population was 27%. This result was slightly different from other studies done in Duhok province. (Habeeb *et al.*, 2014) conducted a big study in Duhok province and they found that the MRSA rates had widely different between secondary school students' group (2 %) (10/489) and health care workers (12%) (61/510). Three separate studies conducted in Zakho city, the researchers found that the MRSA rates were relatively low among University students (4.2%), and 4% among primary school students, while higher rates 13.7% detected among health care workers (Hussein *et al.*, 2010; Assafi *et al.*, 2015). However, (Saeed, 2015) found that

22.5% of food handlers serving in restaurants and cafeterias in Duhok city were positive for MRSA. Our results were in line with two other studies (Alsamarai *et al.*, 2015) study who demonstrated the MRSA among 28% of food handlers in Tikrit, and (Ferreira *et al.*, 2014) study who demonstrated the MRSA among 28.6% of food handlers. The higher prevalence rate of MRSA was observed in a study done in Sweden among household contacts was (36.8%) (42 /114) (Johansson *et al.*, 2007). Also, a higher MRSA rate detected by (Wolde *et al.*, 2016), they found that 92.5% of food handlers of Jigjiga university student's cafeteria were MRSA carrier. Lower MRSA rate 9.8% was reported among food handlers working in Gondar university in Ethiopia (Dagneu *et al.*, 2012). (Shokouhi *et al.*, 2017) found that only 1.25% of the studied population in the tertiary outpatient center in Iran had MRSA. In contrary to our study, no cases of MRSA were detected by other studies (Castro *et al.*, 2016; Vatansever *et al.*, 2016). Many different factors contributed in the distribution of *S. aureus* and MRSA including crowded housing, bad hygienic practices, sharing of personal utensils, recurrent antibiotic usage, hospital admissions and intravenous drug addicts (Braga *et al.*, 2014; Assafi *et al.*, 2017).

4.5 Prevalence of VRSA

Vancomycin is considered one of the last options of treatment for *S. aureus* infections that are resistant to other antibiotics. Analysis of different studies showed the emergence of VRSA from different parts of the neighboring countries (Tiwari and Sen, 2006; Askari *et al.*, 2012). Fortunately, no VRSA isolates were found in this study. Similar results obtained in many studies done in Duhok province (Hussein *et al.*, 2010; Habeeb *et al.*, 2014; Saeed, 2015). This might be attributed to many reasons like vancomycin which had been used rarely in Iraq during the United Nation's sanction period from 1990 to 2003; the drug was expensive and was not available in many health facilities. Also, our result was in accordance with the results of neighboring countries (Udo *et al.*, 2009; Dangnew *et al.*, 2012; Shokouhi *et al.*, 2017).

In contrast, Alsamarai *et al.*, (2015) revealed that the 18.6% of studied food workers in Samara restaurants had vancomycin-resistant *S. aureus* and Loeto *et al.*, (2017) found that 27.3% of food handlers with MRSA also were resistant to vancomycin; this rate is near to that of the study done in Maharashtra among food handlers working in medical college restaurant (26.5%) (Kasturwar and Shafee, 2011). Moreover, Ferraria *et al.*, (2014) found a high percentage of VRSA 39.2% from the food handlers' hands and less percentage 23.5% of VRSA from their anterior nares.

5. CONCLUSION

The study revealed a high prevalence of MRSA among food handlers in Duhok city. Food handlers in public places are required to go through regular screening for both nasal and skin carriage of *S. aureus* for the early detection and treatment of carriers so as to protect the community from staphylococcal food poisoning and the spread of resistant *S. aureus* strains among the population.

COMPETING INTERESTS

The authors declare that they have no competing interests.

RECOMMENDATIONS

The current study recommends the demand for the regular screening of food handlers in all public places for both nasal and skin carriage of *S. aureus* in order to early detection and effective treatment of carriers so as to protect the community from staphylococcal food poisoning and the spread of resistant *S.*

aureus strains among the population. As proven, many staphylococcal foods borne disease is mostly caused by incorrect food handling practices. It is recommended to give proper training courses to food handlers so as to prevent the contamination of food.

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