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Keywords: Antibacterial; Food handlers; Nasal carriage; Staphylococcus aureus; Sana'a restaurants; Yemen

Article

Nasal Carriage of *Staphylococcus aureus* and Its Antibacterial Susceptibility Profiles among Food Handlers in Sana'a Restaurants, Yemen

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Abstract: Background: *Staphylococcus aureus* is a ubiquitous organism that normally colonizes the human body and causes food poisoning. **Aim:** This study aimed to determine the rate of nasal carriage of *S. aureus* among food handlers in Sana'a restaurants in, Yemen and performed an antibacterial susceptibility test. **Methods:** This cross-sectional study was carried out among healthy food handlers from November 2022 to March 2023. Approximately 420 nasal swabs were collected and *S. aureus* was isolated and identified according to standard bacteriological procedures. Moreover, the antibacterial susceptibility pattern was determined using the disc diffusion method. Additionally, the required data were obtained by using the pretested questionnaire. **Results:** Of 420 nasal swabs, 78 (18.6%) food handlers were *S. aureus* nasal carriers. The prevalence rate of *S. aureus* was significantly higher among subjects aged >30 years (23.1%), males (18.8%), and uneducated subjects (25%). Additionally, a higher rate of *S. aureus* nasal carriers was observed among food handlers who worked as cooks (19.4%), had less than 2 years of experience (20.5%), and had low hygiene (29%). The *S. aureus* isolates were sensitive to ciprofloxacin (71.8%), vancomycin (76.9%), and amoxicillin-clavulanic acid (61%). Conversely, the isolated *S. aureus* was resistant to oxacillin (69.2%) and methicillin (66.7%). **Conclusion:** These data stress that food handlers with a high *S. aureus* nasal carrier rate may pose significant risks to consumers. Therefore, creating restaurant policies and rules is necessary to provide safe and healthy food to consumers.

Keywords: antibacterial; food handlers; nasal carriage; *Staphylococcus aureus*; Sana'a restaurants; Yemen

Introduction

Staphylococcus aureus is found in normal human flora and causes serious diseasesassociated with high morbidity and mortality worldwide (Balasubramanian *et al.*, 2017). This type of bacterium is a ubiquitous microorganism that commonly colonizes the skin of the human body and mucous membranes, mainly in the nasal cavity of healthy individuals (Sakr *et al.*, 2018).

Transmission of *S. aureus* between individuals occurs through direct skin-to-skin contact or indirect contact with contaminated inanimate objects (Brown *et al.*, 2013). Frequently, carriers of *S. aureus* can be branched into persistent and intermittent nasal carriers. Globally, about 20% of the

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general population is *S. aureus* persistent, while 30–50% are intermittent carriers of *S. aureus* (Ritchie *et al*., 2016; Sakr *et al*., 2018).

S. aureus can be transferred to food through manual contact by hands as well as respiratory secretions during the food preparation and handling process, resulting from poor hygiene practices of food handlers. Certainly, during food handling, nasal carriers' hands constitute the major vector for *S. aureus* dissemination, and unhygienic people are the leading cause of contamination. Asymptomatic food handlers are the main vector for contaminating food with *S. aureus* during food handling (Ghasemzadeh-Moghaddam *et al*., 2015; Sakr *et al*., 2018). In addition, food can also be contaminated through manual contact or via respiratory secretions resulting from asymptomatic food handlers, which eventually become the source of staphylococcal food poisoning (Leibler *et al*., 2016; Bencardino and Vitali, 2019).

Foodborne diseases are major global health difficulties, particularly in low-income nations that use insecure water for food cleaning and processing, and have poor food production and handling (WHO, 2015). Based on the World Health Organization estimation, nearly 600 million people globally become infected with foodborne illnesses, and at least 420 thousand people die each year (WHO, 2022).

In an investigation on foodborne illness outbreaks conducted among food services, a global team of food safety investigators recorded 816 outbreaks associated with the practices of food handlers, resulting in 80,682 cases of foodborne illness (Greig *et al*., 2007). Food handlers with poor practices appear to be the most important source of contaminating food, particularly food prepared to eat, such as that served in restaurants (McIntyre *et al*., 2013).

Globally, the overall rate of *S. aureus* nasal carriage was reported among food handlers to be 26.6% in Kuwait, (Al-Bustan *et al*., 1996), 21.6% in Sudan (Saeed and Hamid, 2010), 38–60% in Nigeria (Eke *et al*., 2015; Okareh and Erhahon, 2015), 23.8% in Lebanon (Osman *et al*., 2019), 7.1% in Bosnia and Herzegovina (Šegalo *et al*., 2020), and 50% in Malaysia (Seow *et al*., 2021).

Yemen is a developing country where endemic diseases are commonly prevalent among the population. This can be due to factors such as overcrowding, lack of safe water, poor disposal of waste, poor hygienic practices and attitudes, and inadequate environmental sanitation (Edrees and Al-Awar, 2020; Edrees and Anbar, 2021). In addition, the absence of rules regulating restaurants and their employees, unqualified restaurant workers, and unhygienic cooking and storing of food can lead to increased risks of staphylococcal food poisoning.

Data on nasal carriers of *S. aureus* and its antibacterial susceptibility tests among food handlers employed in Yemen are inadequate. Therefore, this study is the first to determine the prevalence of nasal carriage of *S. aureus* and its antibacterial susceptibility test among food handlers working in restaurants in Sana'a City, Yemen.

Material and Methods

Study area and period

This cross-sectional study was carried out at restaurants located in districts in Sana'a city which includes ten official districts. This study was carried out for a period of five months, from November 2022 to March 2023.

Study population

A total of four hundred and twenty (420) specimens were collected from restaurant workers aged between 17 and 44 years old who are working in different districts belonging to Sana'a capital. The districts subjected to this study were Al-Sabain, Al-Wahdah, Assafi'ya, Az'zal, Ma'ain, Ath'thaorah, and Shu'aub districts. The participating food handlers include cooks, waiters, and cleaning workers.

Sample size

The *Epi Info* 7 program (version 7.2.5.0) was used to calculate the sample size as follows: The population size was 7500. The expected mean of *S. aureus* prevalence was 17.5%, according to a previous report (Osman *et al*., 2019; Šegalo *et al*., 2020; Vicar *et al*., 2023). Additionally, the acceptable

 doi:10.20944/preprints202311.0495.v1

margin of error was set at 5%, and the confidence level was set at 99%. Therefore, a sample size of 365 was determined by the program. Then, the sample size was increased to 420 to obtain more precise results.

Data collection

A structured questionnaire was used to collect the necessary data from participants. The items in the questionnaire were designed based on a past study, and few changes were made (Seow *et al*., 2021). The needed data such as age, gender (male/female), district name, educational level (illiterate/primary/repertory/secondary/graduate), job (cook/waiter/cleaning), experience years (< 2 years/between 2–5 years/ > 5 years), and level of personal hygiene (high/moderate/ ow) were gathered via face-to-face interviews and recorded in the questionnaire sheet which was initially constructed in Arabic and then translated into English after data collection. The age group was clustered into three categories: < 20 years, 20–30 years, and > 30 years.

Inclusion and exclusion criteria

All participants who worked in selected restaurants and who signed the informed consent, agreed to bring specimens, and did not take antimicrobial agents before sampling were included in this project. Restaurant workers who refused sample collection, signed the informed consent form, or used antibiotics within seven days before sampling were excluded from this study.

Specimen collection

Under aseptic conditions, the nasal swabs were sampled using a sterile swab moistened with a sterile saline solution. The tip of the swab was inserted into the nasal cavity (approximately 2–3 cm), rotated six times before swab withdrawal, and returned to its sterile container. Swab specimens were labeled with participating information and immediately transported to the microbiology lab at Queen Arwa University (QAU) for further processing and examination within 1–2 hours (Mahon *et al*., 2015; Luis *et al*., 2020).

Identification of *S. aureus*

Nasal swabs were inoculated on a mannitol salt agar plate and incubated at 37°C for 48 h. After incubation, the yellow colonies (mannitol fermentation) on mannitol salt agar were subcultured on nutrient agar. Additionally, further Gram staining and biochemical tests were used for *S. aureus* isolation and identification according to standard microbiological methods (Mahon *et al*., 2015; Luis *et al*., 2020).

Antimicrobial susceptibility test

The Kirby–Bauer disc diffusion method was used to determine the antibacterial tests of *S. aureus* isolates on Mueller–Hinton agar according to guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2021). The antibiotic discs used included amoxicillin-clavulanic acid (AMC, 25 μg), ciprofloxacin (CIP, 5 μg), methicillin (MET, 5 μg), oxacillin (OX, 1 μg), and vancomycin (VA, 30 μg) (HiMedia Comp., India). The inhibition zone was measured, and the results were interpreted as resistant or sensitive according to CLSI (2021) guidelines.

Ethics considerations

The project proposal was primarily submitted to the Medical Sciences College of Queen Arwa University and ethical approval for this study was obtained from the Ethics Research Committee of Queen Arwa University on 15 October 2022. Additionally, QAU was formally communicated with restaurants in the selected districts by an official letter that explained the aims and purpose of the study delivered to the Health and Public Office in Sana'a Capital. Before the start of data and sample collection, the goals of this study were well explained to the study subjects for their agreement for inclusion in this study. Written informed consent was obtained from all the study subjects. Food handlers with positive results for *S. aureus* were advised for proper treatment at the nearest health center.

 doi:10.20944/preprints202311.0495.v1

Statistical analysis

The obtained data are presented in the tables. The variables of values are described as frequencies and percentages in tables and figures. The SPSS Statistic program (26 version, IBM Co., Armonk, New York) was used for statistical analysis of the observed data. Chi-square (χ^2) and confidence interval (CI 95%) tests were used to verify the existence of associations. Statistical significance (*P*-value) was considered at < 0.05.

Results

Approximately 420 food handlers were enrolled in this study. The majority of nasal swabs were collected from the age group of 20–30 years (51.7%), male (98.8%), those who had secondary school certificates (51.0%), and equally from each district (14.3%) (Table 1).

Table 1. Sociodemographic characteristics of study subjects.

Variables	Category	Frequency	Rate (%)
Age group (in years)	< 20	129	30.7
	20–30	213	50.7
	> 30	78	18.6
Gender	Male	415	98.8
	Female	5	1.2
Educational level	Illiterate	4	1.0
	Primary	34	8.1
	Preparatory	134	31.9
	Secondary	214	51.0
	Graduate	34	8.1
Districts names	Al-Wahdah	60	14.3
	As Sabain	60	14.3
	Ma'ain	60	14.3
	Assafi'ya	60	14.3
	Ath'thaorah	60	14.3
	Shu'aub	60	14.3
	Az'zal	60	14.3

Frequency of *S. aureus* nasal carriage

The present study revealed that approximately 78 (18.6%) food handlers were *S. aureus* nasal carriers while 342 (81.4%) food handlers were reported as noncarriers, as shown in Figure 1.

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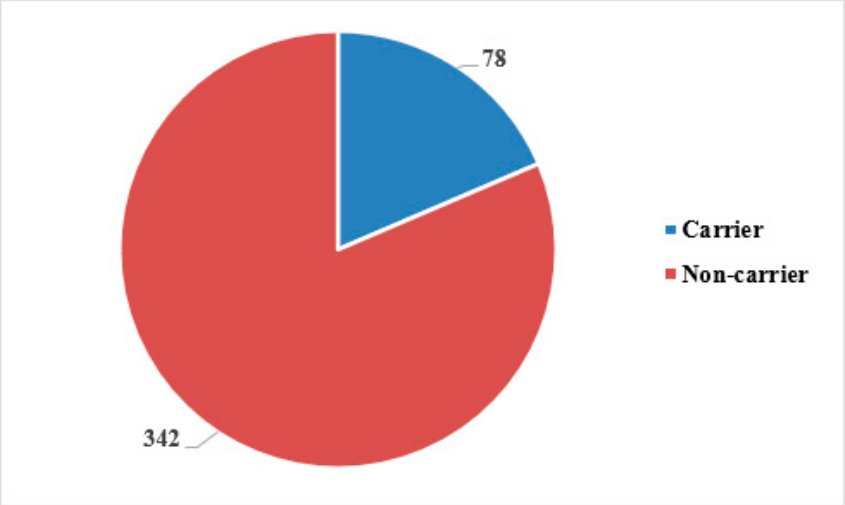

Figure 1. Frequency of *S. aureus* carriage among food-handlers.

Distribution of *S. aureus* concerning sociodemographic characteristics

The frequency of nasal carriage of *S. aureus* was higher among food handlers aged >30 years (23.1%), male food handlers (18.8%), and uneducated study subjects (25.0%) with a nonsignificant difference (*P* >0.05) as revealed in Table 2.

Table 2. Distribution of nasal carriage of *S. aureus* according to sociodemographic characterizatics.

Variables	Category	Carrier	Noncarrier	χ^2	95% CI	*P* value
Age group (in years)	< 20	23(17.8)	106(82.2)	1.297	1.81-1.94	0.525
	20–30	37(17.4)	176(82.6)			
	> 30	18(23.1)	60(76.9)			
Gender	Male	78(18.8)	239(81.2)	0.007	1.00-1.02	0.934
	Female	0(0)	5(100)			
Educational level	Illiterate	1(25.0)	3(75.0)	2.704	3.50-3.65	0.612
	Primary	7(20.6)	27(79.4)			
	Repertory	24(17.9)	110(82.1)			
	Secondary	43(20.1)	17(79.9)			
	Graduate	3(8.8)	31(91.2)			

Significant statistics at *P* value <0.05. χ^2 = Chi-square test. CI 95% =confidence interval,.

Regarding district names, it was noticed that the highest rate of *S. aureus* nasal carriage was isolated from food handlers working in Ma'ain (31.7%), while the lowest rate was found among food handlers working in Al-Wahdah (8.3%) with significant differences (*P* =0.019) as shown in Table 3.

Table 3. Distribution of nasal carriage *S. aureus* among participants according to district names.

District	F. (%)	Carrier	Noncarrier	χ^2	95% CI	*P* value
Al-Wahdah	60(14.3)	5(8.3)	55(91.7)	15.083	3.81-4.19	0.019
As Sabain	60(14.3)	8(13.3)	52(86.7)			
Ma'ain	60(14.3)	19(31.7)	41(68.3)			

 doi:10.20944/preprints202311.0495.v1

District	F. (%)	Carrier	Noncarrier	χ^2	95% CI	*P* value
Assafi'ya	60(14.3)	16(26.7)	44(73.3)			
Ath'thaorah	60(14.3)	10(16.7)	50(83.3)			
Shu'aub	60(14.3)	10(16.7)	50(83.3)			
Az'zal	60(14.3)	10(16.7)	50(83.3)			

Significant statistics at *P* value <0.05. χ^2 = Chi-square test. CI 95% =confidence interval,.

Risk factors associated with nasal carriers of *S. aureus*

Table 4 reveals that a higher rate of *S. aureus* nasal carriers was observed among food handlers who worked as cooks (19.4%), had less than 2 years of experience (20.5%), and had low hygiene (29.0%; *P* = 0.008).

Table 4. Frequency of nasal carriage *S. aureus* among food handlers according to some risk factors.

Variables	Category	F. (%)	Carrier	Noncarrier	χ^2	95% CI	*P* value
Job	Cook	217(51.7)	42(19.4)	175(80.6)	0.183	1.49-1.61	0.913
	Waiter	175(41.7)	31(17.7)	144(82.3)			
	Cleaning	28 (6.7)	5(17.9)	23(82.1)			
Experience years	< 2	161(38.3)	33(20.5)	128(79.5)	1.627	1.84-2.00	0.445
	2–5	133(31.7)	20(15.0)	113(85.0)			
	> 5	126(30.0)	25(19.5)	101(80.0)			
Personal hygiene	High	106(25.2)	18(17.0)	88(83.0)	9.731	1.92-2.05	0.008
	Moderate	214(51.0)	31(14.5)	183(85.5)			
	Low	100(23.8)	29(29.0)	71(71.0)			

Significant statistics at *P* value <0.05. χ^2 = Chi-square test. CI 95% =confidence interval,.

Antibacterial susceptibility profile of *S. aureus* isolates

The *S. aureus* isolates showed variable susceptibility patterns for the different antibiotics tested. It was found that *S. aureus* isolates were highly sensitive to ciprofloxacin (71.8%), vancomycin (76.9%), and amoxicillin-clavulanic acid (61%). Conversely, a high resistance rate of *S. aureus* isolates was only observed for oxacillin at 69.2% and methicillin at 66.7% (Table 5).

Table 5. Antibacterial susceptibility pattern of *S. aureus* isolates.

Antibiotic name	Symbol	S No. (%)	I No. (%)	R No. (%)
Amoxicillin-clavulanic acid	AMC	48 (61.5)	18(23.1)	12 (15.4)
Ciprofloxacin	CIP	56 (71.8)	13(16.7)	9 (11.5)
Methicillin	MET	26 (33.3)	0 (0)	52 (66.7)
Oxacillin	OX	24 (30.7)	0 (0)	54 (69.2)
Vancomycin	VA	60 (76.9)	0 (0)	18 (23.1)

S=sensitive, I=intermediate, R=resistant.

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Discussion

To the best of our knowledge, this study is the first to determine the nasal carriage of *S. aureus* among food handlers in Yemen. The overall rate of *S. aureus* nasal carriage in this study was 18.6% among food handlers. This finding is significantly higher than that reported at 7.1% in Bosnia and Herzegovina by Šegalo *et al.* (2020). Additionally, this result is lower than previous results reported at 26.6% in Kuwait, (Al-Bustan *et al*., 1996), 21.6% in Sudan (Saeed and Hamid, 2010), 20.5% in Ethiopia (Dagnew *et al*., 2012), 60% in Ekpoma (Eke *et al*., 2015), 38% in Benin City-Nigeria (Okareh and Erhahon, 2015), 20% in Iran (Nasrolahei *et al*., 2017), 53% in Brazilian (da Silva *et al*., 2018), 23.8% in Tripoli, Lebanon (Osman *et al*., 2019),15.5% in Nigeria (Anowai *et al*., 2019), 50% in Malaysia (Seow *et al*., 2021), and 21.7 % in Ghana (Vicar *et al*., 2023).

The variation in results could be attributed to numerous factors such as demographic location, sample size, collection and diagnostic methods, personal hygiene habits, education level, hygiene of the environment, and rules employed in each country.

In this result, the nasal carrier of *S. aureus* was significantly higher among participants aged >30 years (23.1%) compared with the other age groups. This study is in agreement with the report by Beyene *et al*. (2019), which revealed that the age group of 30–39 years had a higher rate of *S. aureus* (30.7%). A different report by Eke *et al*. (2015) noted that the age range of 26–30 years had the highest prevalence of 67%. Conversely, several studies revealed that nasal carriage of *S. aureus* was associated with younger age (Skramm *et al*., 2011; Holtfreter *et al*., 2016; Akinnola *et al.*, 2022).

The current results showed that *S. aureus* carriage was detected only in males handling food at 18.8%. A similar study by Eke *et al*. (2015) noticed that males had a higher prevalence rate than females. Additionally, in numerous reports, nasal carriage of *S. aureus* was correlated with males (Holtfreter *et al*., 2016). In dissimilar findings, a high carriage rate of *S. aureus* was detected among females (30.5%) compared to males (25.2%) by Beyene *et al*. (2019) and 63.49% for females and 56.75% for males by Akinnola *et al.* (2022).

The traditions and culture of the Yemeni community restrict the employment of women in public places such as restaurants (Edrees and Anbar, 2021). Yemeni women rarely work in restaurants. This explains why only a few samples were collected from females in this study.

These data showed that the highest carriage rate of *S. aureus* was recovered from uneducated subjects (25%), while the lower rate was recovered from study subjects holding a bachelor's certificate (8.8%). In contrast, there was a high carriage rate of *S. aureus* (35.7%) among workers who had college certificates (Beyene *et al*., 2019) and high school-leavers (44%) (Akinnola *et al.*, 2022).

The participants in this study who worked as cooks were found to have the highest rate of *S. aureus* nasal carriers at 19.4% compared with the others, and there was a significant difference (P = 0.008). This result is consistent with the study by Beyene *et al*. (2019) which revealed that the high carriage rate of *S. aureus* was 36.3% among cook participants. Additionally, approximately 50% of cooked food was reported as a nasal carrier of *S. aureus* (Seow *et al*., 2021).

Regarding years of experience, it was found that the highest rate of *S. aureus* nasal carriage was isolated from food handlers who had less than 2 years of experience at 20.5%. This is in line with reports performed in Ethiopia showing that *S. aureus* prevalence was higher among food handlers with work experience (Assefa, *et al.*, 2015; Beyene *et al*., 2019). In addition, Seow *et al*. (2021) showed a high rate among food handlers working between 1 and 5 years (44%). The dissimilar report by Akinnola *et al.*, 2022) documented that the highest rate was among food handlers with service experience of 1 –10 years (59%).

Furthermore, it demonstrated that food handlers who had many years of work experience were less exposed to bacterial contamination by hand (Assefa, *et al.*, 2015). The prevalence of *S. aureus* was found to be significantly correlated with years of working in food service. This anticipated outcome could be elucidated by the statement that the food handlers who practiced in the restaurant for a long time had improved their awareness of food safety knowledge about personal hygiene, prompting them to accomplish better food hygiene practices (Ismail *et al*., 2016). An investigation conducted in some settings among food handlers documented that many years of work are remarkably positively correlated with their practices and attitudes ($P < 0.05$) in the workplace (Bou-Mitri *et al*., 2018).

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Therefore, it can be implied that food handlers with many years of experience have better personal hygiene practices than food handlers without experience.

This finding showed that food handlers with low hygiene had a higher rate of *S. aureus* nasal carriage at 28.2%. It has been demonstrated that inadequate personal hygiene habits on the part of handlers can have major consequences for food safety, encouraging the spread of S. aureus strains during manufacturing, processing, and distribution (Al-Bahry *et al*., 2014).

In this result, it was found that *S. aureus* isolates were sensitive to ciprofloxacin (71.8%), vancomycin (76.9%), and amoxicillin-clavulanic acid (61%). A report by Eke *et al*. (2015) documented that the isolated *S. aureus* was completely sensitive to ciprofloxacin (100%). Additionally, it was recorded that *S. aureus* isolates were sensitive to vancomycin (100%), and ciprofloxacin (70%) (Dagnew *et al*. 2012). A study by Beyene *et al*. (2019) detected the sensitivity of *S. aureus* isolates to ciprofloxacin (96.5%), cefoxitin (95.3%), amoxicillin-clavulanic acid (94.2%), oxacillin (93%), vancomycin (93%), methicillin (93%), and vancomycin (93%) Recently, it was observed that 100% of isolated *S. aureus* strains were methicillin-susceptible *S. aureus* (Seow *et al*., 2021).

In this result, a high resistance rate of *S. aureus* isolates was observed only for oxacillin at 69.2% and methicillin at 66.7%. In a similar investigation, it was reported that the isolates of *S. aureus* were resistant to penicillin (51.2%), ampicillin (46.3%), amoxicillin (39%), cotrimoxazole (31.7%), and methicillin and ciprofloxacin (9.8% in each) (Dagnew *et al*., 2012). Another study by Eke *et al*. (2015) revealed that all *S. aureus* isolates were resistant to ampicillin and 75% to Ampiclox. Moreover, a report by Beyene *et al*. (2019) indicated that 90.7% of the isolates were resistant to ampicillin and penicillin. Isolated strains of *S. aureus* were found to be resistant to penicillin (99.5%), ampicillin (97.9%), and amoxicillin (94.9%) (Šegalo *et al*., 2020). Additionally, approximately 93.75% of *S. aureus* isolates were resistant to penicillin and 50% to oxacillin (Akinnola *et al*., 2022). Moreover, Al-Khawlany *et al*. (2021) revealed that vancomycin was the most effective antibacterial against all isolated Methicillin-Resistant *Staphylococcus* aureus (MRSA) strains. Additionally, *S. aureus* isolates presented high resistance to broad-spectrum antibiotics such as ampicillin (40%-75%), tetracycline (40%-80%), amoxiclav (20%-80%) and chloramphenicol (7.7%-50%) (Vicar *et al*., 2023).

Currently, the increasing resistance of pathogenic bacteria to commonly used antimicrobial agents, particularly in Yemen, could result from the availability of antibiotics as over-the-counter drugs. The widespread use and misuse of antibiotics, the absence of antimicrobial susceptibility tests, the empirical use of broad-spectrum antibacterial drugs, and the quality of antibiotics are also factors contributing to antibiotic resistance increasing (Al-Haik *et al*., 2017; Alhlale *et al*., 2019; Edrees *et al*., 2020; Alhlale *et al*., 2020; Elmanama *et al*., 2023).

Limitations of the study

The limitation of this investigation is representing on the study was a cross-sectional study conducted over a short period of time and in a one Yemeni governorate. Furthermore, the majority of individuals subjected to sample collection have difficulties getting consent mainly because of their fear that the results will be given to official authorities. In addition, there is a lack of modern technology that are used in the genetically identifying of bacteria.

Conclusion

The prevalence rate of *S. aureus* nasal carriers among food handlers, remains a health problem for restaurant consumers. The prevalence of *S. aureus* among study subjects could be attributed to factors such as poor personal hygiene, inadequate education, the absence of regular monitoring and control of food handlers' hygiene and restaurants' environmental sanitation. In addition, there was a lack of routine diagnosis and treatment for food handlers. Therefore, food handlers must adhere strictly to restaurant policies and rules, and training programs are needed to improve food handlers' food safety knowledge, attitudes, and practices through periodic medical checkups, and regular monitoring of food handlers' hygiene.

Author's Contributions: Nasser Al-Aomary, and Bashir Al-Ofairi conceived and designed the experimental study. Jameela Thabit and Nasser Al-Aomary shared with team workers for specimen collection and analysis.

Wadhah Edrees and Bashir Al-Ofairi statistically analyzed and presented the data in tables and figures. Additionally, Wadhah Edrees and Nasser Al-Aomary prepared and wrote the manuscript. All authors have reviewed, revised, and accepted the manuscript for submission.

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Date Availability: The data used to support the findings of this study are available from the corresponding author upon request.

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