Original Article

Methicillin-resistant *Staphylococcus aureus* among food handlers in Jordan: frequency, risk factors, toxigenicity and antibiotic resistance

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Objective: To evaluate the frequency, antibiotic resistance, toxigenicity of Methicillin-resistant Staphylococcus aureus (MRSA) in food chain workers at our institution.

Methodology: A total of 160 nasal swabs were collected from food chain workers in Al-karak district in the south Jordan. MRSA and its toxigenicity were detected using cultural and molecular methods. Antibiotic susceptibility was determined by the disc diffusion method.

Results: The frequency of MRSA was 18.7% (n=30). There was a significant difference for nasal carriage of MRSA by *recent* hospitalization workers (p=0.008) or having a family member who was a healthcare worker (p=0.001). Isolates were highly resistant to fusidic acid (33%) followed by rifampicin (17%) and gentamicin (7%). All MRSA isolates were resistant to Cefoxitin. Among *S. aureus* enterotoxins, *SEA* was the most commonly reported enterotoxin gene from the isolates, followed by *SEH*, *SED, SEC, SEE, and SEJ,* respectively.

Conclusion: Food handlers were a potential source for MRSA infection and food poisoning out breaks might be attributed to the carriage of the toxigenic MRSA strains. More hygienic restrictions and infection prevention plan must be applied in food industry to ensure the good food handling and storage. (Rawal Med J 202;45:295-299).

Keywords: Staphylococcus aureus, MRSA, antibiotic resistance, Staphylococcal Enterotoxins, Food poisoning.

INTRODUCTION

Staphylococcus aureus (*S. aureus*) is a Gram-positive pathogen, causing mild skin and soft tissue infection as well as life-threatening sepsis, pneumonia, and toxic shock syndrome.[1] The ability of the pathogen to grow in substrates with a low water activity, over a wide temperature range of 7 to 48°C, and at pH values ranging from 4.2 to 9.3,[2] renders them a potential source of food toxicosis that ultimately leads to life threatening diseases. Its pathogenicity is attributed to the production of a range of different enterotoxins, which cause vomiting with or without diarrhea and are responsible for *S. aureus* food poisoning (SFP).

To date, twenty-three serologically distinct superantigenic staphylococcal enterotoxins (SEs) and Staphylococcal enterotoxin-like (SEl) proteins have been identified.[3,4] These SEs share a common structure, function, sequence homology and are encoded by genes carried and disseminated by different mobile genetic elements, i.e., prophages, plasmids, pathogenicity islands (SaPIs), enterotoxin gene clusters (egc) and the staphylococcal cassette chromosome (SCC).[5] They are basic proteins with molecular weights of 25–30 kDa and made up of approximately 220–240 amino acids rich in lysine, aspartic acid, glutamic acid, and tyrosine residues.[6] SEs are heat-stable, resist many environmental conditions (low pH, freezing, drying) and proteolysis by digestive enzymes, which confer maintenance of their activity in the digestive tract after ingestion.[7] Unlike conventional antigens, SEs activate a large number of T-cells leading to proliferation and massive release of chemokines and proinflammatory cytokines.[8]

S. aureus colonizes asymptomatically the nasal passages and skin of approximately 50% of healthy individuals, persistently or intermittently.[9] Moreover, it was present on 88% of the hands and

48% of the aprons of food handlers working in the delicatessen sections of a retail store.[10] Therefore, food poisoning outbreaks (FBOs) may be, in part, attributed to food handlers who carry enterotoxigenic *S. aureus* during food processing.[8] In fact, *S. aureus* is considered as the third most important cause of foodborne illnesses reported worldwide[2] and its enterotoxins with other bacterial toxins were responsible for 17.7% of all FBOs in European Union in 2016, 86% of which were reported from France.[11]

The motivation behind this study was to investigate the possible cause of food poisoning by studying if the food-handlers play a role in such cases. Studying the molecular epidemiology and detecting the antibiotic resistance and toxiginicity of MRSA isolates from food chain workers will be useful for FBOs management and in setting the proper infection prevention plan and infection control measures of related food poisoning.[12]

METHODOLOGY

This descriptive cross-sectional study was conducted at AlKarak governorate from January to December 2018 including all restaurants in AlKarak city by simple random sampling technique. Food handlers who are resident of AlKarak governorate were included in the study, while those who had taken antibiotics and anthelminthic within one month prior to the study were excluded. A total of 160 nasal swabs collected from food handlers were included in this study. From the governorate's seven areas, samples were collected from five areas which represents 71.4% because the food services are concentrated in these areas. A signed informed consent and questionnaire with relevant data regarding risk factors for MRSA carriage were obtained from each participating person. The study was approved by the scientific research ethical committee in the Faculty of Medicine, Mutah University.

Nasal swabs from food handlers were obtained by rotating a cotton swab 3 times in the vestibules of the anterior nares. The swabs were immersed in nutrient broth (Oxoid, Cambridge, UK) containing 5% NaCl and incubated for 3h at 37°C. Each broth was subcultured on mannitol salt agar and incubated at 35°C for 24 hrs. Presumptive identifications of *S. aureus* were based on colony morphology, Gram staining, positive catalase, and tube coagulase test. Identification of MRSA isolates was confirmed by disk diffusion assay using 30µg cefoxitin discs and *mecA* gene detection by polymerase chain reaction (PCR) respectively.

The isolates' antibiotic susceptibility for the selected antibiotic battery was carried out in accordance with the Clinical and Laboratory Standards Institute guidelines (CLSI).[13] Antibiotics included fusidic acid 10µg, rifampicin 5µg, vancomycin 30µg, ciprofloxacin 5µg, linezolid 30µg, mupirocin 200µg, gentamicin and trimethoprim-sulfa-methoxazole 25µg.

Genomic DNA from MRSA was isolated using DNA extraction mini kit (OMEGA, Bio-TEK, USA). DNA amplification was carried out by PCR (XP Thermal cycler, Bioer Technology, China) using specific primer pairs for 23s rRNA, six SEs encoding genes (*SEA, SEC, SED, SEE, SEH, SEJ*) and *mecA* gene as described previously.[14,15]

Statistical Analysis: The data collection and data entry were checked for any error or missing data. Statistical analysis was performed by SPSS release 13. The study variables were described as frequency (number) and percentage (%). Categorical variables were tested by using chi-square test. A $p \leq 0.05$ was considered statistically significant.

RESULTS

Of the total 160 samples, 30(18.7%) were positive for MRSA. The antibiotic resistance testing revealed that 100% (n=30) of MRSA were resistant to cefoxitin, 33% (n=10) to fusidic acid, 17% (n=5) torifampicin, 7% (n=2) to gentamicin, and one isolate (4%) was resistant to ciprofloxacin and to trimethoprim-sulfamethoxazole. All MRSA isolates were sensitive to linezolid and mupirocin. Recent hospital admission and having a family member working in healthcare setting increased the risk of nasal MRSA carriage amongst food handlers (Table 1).

Table 1. Variables affecting nasal MRSA carriage amongst food handlers.

Variable	Nasal MRSA Carriage		*p* value*
	Positive n (%)	Negative n (%)	
Gender			
Male	27 (20.0)	108 (80.0)	0.346
Female	3 (12.0)	22 (88.0)	
Recent hospital admission			
Yes	5 (50.0)	5 (50.0)	0.008*
No	25 (16.6)	125 (83.4)	
Work experience in years			
Less than 10 years	16 (17.2)	77 (82.8)	0.555
More than 10 years	14 (21)	53 (79.0)	
Chronic disease			
Yes	3 (25.0)	9 (75.0)	0.564
No	27 (18.0)	121 (82.0)	
Having a family member who is a healthcare worker			0.001*
Yes	6 (60.0)	4 (40.0)	
No	24 (16.0)	126 (84.0)	

*Chi square test: Data were statistically significant at *p value* ≤0.05.

Table 2. Simple frequency distribution of SEs genes in isolated MRSA (N = 30).

Genotype	Positive isolates	(%)
SEA (Staphylococcal enterotoxin-A)	29	96.7
SEC (Staphylococcal enterotoxin-C)	6	20
SEH (Staphylococcal enterotoxin-H)	11	36.7
SED (Staphylococcal enterotoxin-D)	9	30
SEE (Staphylococcal enterotoxin-E)	6	20
SEJ (Staphylococcal enterotoxin-J)	5	16.7

MRSA: Methicillin-resistant *Staphylococcus aureus*; SEs: Staphylococcal enterotoxins.

As indicated in Tables 2, *SEA* was the most prevalent enterotoxin encoding gene in MRSA isolates representing 96.7% (n=29) of the total MRSA isolates followed by *SHE 36.7%* (n=11) and *SED 30%* (n=9), while *SEC, SEE* and *SEJ* were the least prevalent enterotoxin encoding genes representing 20% (n=6), 20% (n=6), and 16.7% (n=5), respectively.

DISCUSSION

S. aureus has been considered as a foodborne hazard for a long time and causing many outbreaks of food poisoning. Asymptomatic food handlers, as part of community population, may represent major threats and can act as significant vectors in the chain of infection.[12] Therefore, screening of this category of population is important for both, epidemiological and infection prevention purposes.

In the current study, the frequency of MRSA was 18.7% (n=30), which is slightly higher compared to previously reported frequency (4-15%) in several studies on Jordanian population.[16] This can be explained by different factors such as difference in the study population, geographical population, and exposure to different risk factors that might increase the nasal carriage.

Resistance pattern of MRSA to the commonly used antibiotics showed that the highest rate of resistance was for fusidic acid followed by rifampicin. Although resistance rate of isolated MRSA strains to fusidic acid comparable to previously reported rate, however, resistance to rifampicin was among the highest rates detected in Jordanian population in previous studies.[17] Therefore, such antibiotics should be prescribed with caution for infections caused by MRSA. Moreover, our findings on susceptibility of MRSA isolates to the remaining antibiotics, especially vancomycin, is in accordance to previously reported rates.[18]

Recent hospital admission and having family member working in healthcare setting were associated with an increased risk for MRSA nasal colonization. This is in agreement with what was previously suggested by other studies.[19] *S. aureus* produces many toxins and enzymes which may affect many human systems. Staphylococcal enterotoxins (SEs; SEA, SEE, SEG, SEI, SER, SET) are produced widely by *S. aureus* with demonstrated emetic activity. Each of these toxins is known to have strong effects on the cells of the immune system which inhibit primarily the host immune responses to *S. aureus in vivo*.[10]

SEs are the causative agents of staphylococcal food poisoning resulting from the ingestion of contaminated food.[20] SEs are stable and can withstand heat and low pH as a result cooking and stomach digestion will partially destroy them. Nausea, emesis, abdominal pain or cramping and diarrhea ensue after a short incubation period. The

disease is usually self-limiting.[8] Among the studied SEs, the current work showed that the SEA was the most commonly reported enterotoxin gene from the food samples, which is in consistent with a prior work,[21] other enterotoxin genes (SEH, SED, SEC, SEE and SEJ) were frequent detected in association with *SEA* at comparable percentages. Multiple toxins may increase the severity and duration of disease.[22] This association was observed in a previous study[21] and is justified by the fact that some enterotoxine either belong to one operon or carried by one or more plasmids at the same strain such as egc.

It was reported that majority (70.3%) of the *S. aureus* isolates are carrying one or more plasmids, and about 54.9% of these plasmid bearing isolates were resistant to multiple drugs.[23] SEA, SEE, and SED were detected in at least one of our samples, which considered the most common cause of food poisoning. In the US, more than 70% of SFP outbreaks are caused by SEA followed by SED (37.5%) and SEB enterotoxins (10%).[21]

Moreover, SEA was the most frequent detected staphylococcal enterotoxin coding gene where 79% of the S. aureus strains isolated from 359 SFP outbreaks in UK between 1969 and 1990 produces SEA enterotoxin coding gene, while it was detected in 69.7% of SFP outbreaks in France.[8] Most of these outbreaks were generated by improper food handling and food storage via contaminated hands and nose. Intriguingly, ability of S. aureus to cause food poisoning and producing various SEs is considered as an emergency condition especially in immune competent patients.

CONCLUSION

Food handlers were significantly considered as a potential source for MRSA infection. The food poisoning out breaks in the study area might be attributed to the carriage of the toxigenic MRSA strains. Our data indicated that enterotoxins are produced by MRSA isolated from our study samples are detected in the study area. Therefore, more hygienic restrictions and infection prevention plan must be applied in food industry to ensure the good food handling and storage.

Strict control measures in food handling practices, carriage surveillance of food handlers on a regular basis, and the introduction of periodic programs for food handlers covering health education and food safety and hygiene may help prevent serious outbreaks.

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