Enterotoxigenicity and antibiogram profile of *Staphylococcus aureus* isolated from food handlers in restaurants and cafeterias in Duhok city, Iraq

Ali Yahya Saeed

Department of Biology, Faculty of Science, University of Duhok, Duhok
Tel: 009647507957275; E-mail: ali.saeed@uod.ac

Abstract: *Staphylococcus aureus* is one of the main causes of food poisoning throughout the world. Food handlers serving in restaurants and cafeterias are the main source of *S. aureus* who carry it in their nasal cavities. Therefore, the purpose of the this study to find out the *S.aureus* carrier in the nasal cavities of food handlers serving in the restaurants and cafeterias in Duhok city as well as to detect their toxiginicity and antibiogram profile. A total of 106 nasal swabs were collected from food handlers served in restaurants and cafeterias in Duhok city from September 2013 to December 2014. *S. aureus* was isolated from 31 (29.24%) out of 106 samples in which 10 (16.6%) out 60 were from food handlers served in restaurants and 21 (45.6%) out 46 from those served in cafeterias. Staphylococcal Enterotoxin type E was only elaborated by 7 (58.6%) out of 12 isolates of *S. aureus*. All isolates were sensitive to vancomycin and 22.5% were methicillin resistant (MRSA). High percentages of resistant were found against ampicillin and augmentin which were 93.54% and 80.64% respectively, while lower percentage of resistant (3.22%) was found against both gentamycin and clindamycin. From the result of the present study nasal carrier of *S.aureus* is high among food workers served in the restaurants and cafeterias which required attention from local health authorities to minimize cases of food poisoning as well as to control both communities acquired and hospital acquired MRSA infections. Enterotoxigenicity test is important because not all strains of *S.aureus* are toxigenic.
[Ali Yahya Saeed. **Enterotoxigenicity and antibiogram profile of *Staphylococcus aureus* isolated from food handlers in restaurants and cafeterias in Duhok city, Iraq.** *J Am Sci* 2015;11(3s):21-24]. (ISSN: 1545-1003). http://www.jofamericanscience.org. 4

Key Words: *Staphylococcus aureus*, enterotoxins, food handlers

Introduction

One of the major health problems throughout the world is food borne illnesses. According to the data of World Health Organization (WHO) up to 30% of the population in developed countries suffer from food borne diseases each year (WHO,2007).

Staphylococcus aureus is one of the most important causes of food poisoning due to its ability to produce more than 22 different enterotoxins (Argudin *et al*., 2010). Among these Staphylococcus enterotoxins, five of them (A, B, C, D and E) are known to be responsible for 95% of Staphylococcal food poisoning cases (Jay *et al*., 2005). Food handlers particularly those working in cafeterias and restaurants are the main source of food contamination and spread food borne diseases (Zain and Naing, 2002;Scott, 2003). Nasal cavities are the preferred site for colonization by *Staph. aereus* in which 10-50% of people are carrier of both methicillin resistant and sensitive *S. aureus* (MRSA and MSSA) (Mainus *et al*., 2006; Yassin and Hassan, 2013; Habeeb *et al*., 2014) which can be source of food poisoning in cases of food handler carrier (Noor-Ariza *et al*., 2012; Bassyouni *et al*., 2012; Dagnew *et al*., 2012; Cepoglu *et al*., 2010) or as source of serious infections in cases carrier health care persons working in hospitals (Al-Dahbi and Al-Mathkhury, 2013; Al-Omer and Al-Yassiri, 2009). Not all strains of *S. aureus* are enterotoxigenic, therefore enterotoxin detections methods are requested for definitive diagnosis of Staphylococcal food poising. Moreover, some coagulase negative Staphylococcal species are also enterotoxigenic and can cause food poisoning (Ostyn *et al*., 2010). Several studies had been carried out on the nasal colonization of *S. aureus* including MRSA and the data are foggy due to the huge differences among their results concerning carrier state in community acquired and hospital acquired MRSA. Moreover, no data are available about the carrier state of *S. aureus* among food handlers working in restaurants and cafeterias and the most common type of Staphylococcal enterotoxin, accordingly this study tried to find out the percentage of *Staph. aureus* carrier in the nasal cavities of food handlers working in restaurants and cafeterias of Duhok center, to study their antibiogram profile as well as to detect the type of Staphylococcal enterotoxin.

Materials and Methods

Sample collection and bacterial identification

A total of 106 nasal swabs were collected from food handlers who work in different cafeterias and restaurants in Duhok center from September 2013 to December 2014 and visited health and prevention center for routine tests. Nasal swabs were taken from both anterior nares of each person. The swabs were initially moistened with sterile distilled water then

inserted gently into each nostril and rotated for few seconds. All samples were directly inoculated on Mannitol salt agar (Oxoid, UK) and incubated for 24hrs at 37 ºC. Diagnosis of *S. aureus* was dependent on mannitol fermentation, Gram stained morphology, catalase and coagulase tests.

Antibiotic susceptibility test

All *S. aureus* isolates were checked against eleven commercially available antimicrobial discs (Oxoid, UK) using modified Kirby-Bauer method according to Clinical Laboratory Standard Institute (CLSI) guidelines and Mueller-Hinton agar as standard media. The inhibition zone of each antimicrobial drug was compared to that provided by zone diameters of CLSI (2006). The following antimicrobial drugs were used: Ampicillin (Amp) 10 µg, Chloramphenicol (C) 30 µg, Clindamycin (DA) 2 µg, Erythromycin (E) 15 µg, Gentamycin (CN) 10 µg, Methicillin (Met) 5 µg, Penicillin (P) 10 µg, Streptomycin (S) 10 µg, Tetracycline (TE) 30 µg, and Vancomycin (VA) 30 µg.

Detection of Staphylococcal enterotoxins

For detecting Staphylococcal enterotoxins, all pure bacterial isolates were cultured overnight in brain heart infusion broth (Oxoid, UK) under aerobic condition at 37º C. Supernatants were collected by centrifugation at 4,000 x g for 10 minutes then sterilized by Millex syringe filters, pore size 0.22 µm (Sigma-Aldrich, Ireland), then tested for five different Staphylococcal enterotoxins A, B, C, D and E using an Enzyme Linked Immunosorbent Assay (ELISA) detection kit (RIDASCREEN® SET A, B, C, D. Art.No: R4101, R-Biophram AG, Germany).

Results

A total of 106 nasal swabs from food handlers were tested for *S. aureus* in which 31 (29.24%) were carrier. Among 106 nasal samples, 60 were from food handlers worked in restaurants in which 10 (16.6%) were carrier for *S. aureus* compared to 21 (45.6%) among 46 samples collected from food handlers worked in cafeterias (Figure 1).

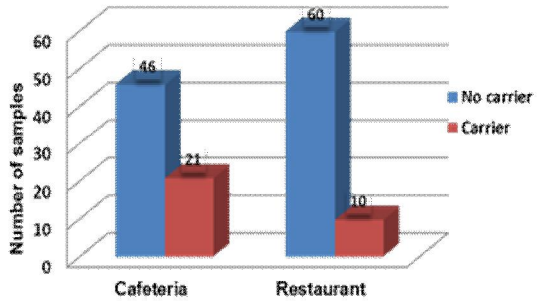

Figure 1. Nasal carriage of *S. aureus* carriers among food handlers

Due to the limited financial support only 12 *S. aureus* isolates were selected randomly and tested for enterotoxins by ELISA technique. The study found that 7(58.3%) from 12 isolates were positive for enterotoxins in which only enterotoxin type E (SEE) was produced by all tested isolates (Table1).

Table 1. Enterotoxins produced by *Staph.aureus*

Isolate	*Staphylococcus* enterotoxins				
	A	B	C	D	E
1	-	-	-	-	+
2	-	-	-	-	-
3	-	-	-	-	+
4	-	-	-	-	+
5	-	-	-	-	+
6	-	-	-	-	-
7	-	-	-	-	+
8	-	-	-	-	-
9	-	-	-	-	+
10	-	-	-	-	-
11	-	-	-	-	+
12	-	-	-	-	-

Regarding the results of antimicrobial susceptibility test, all isolates were sensitive to vancomycin, while showed variable resistant patterns against other drugs in which high percentages of resistance 93.54% and 80.64% were against ampicillin and augementin respectively. Lower percentages of resistance were recorded against both gentamicin and clindamycin which was 3.22%. Resistance to methicillin was 22.5% (Figure 2).

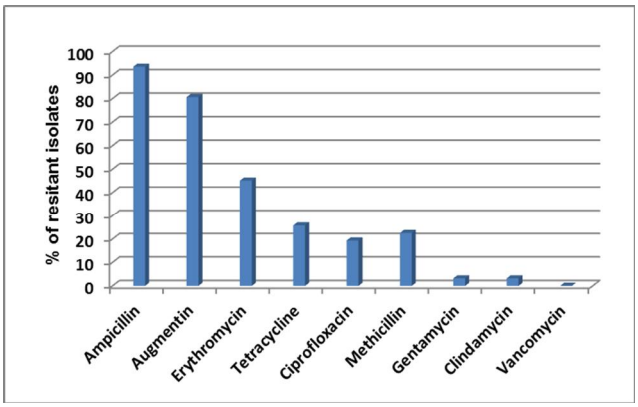

Figure 2. Antibiogram profile of *Staph. aureus* isolates.

Discussion

Food poisoning is a worldwide problem but occurs more frequently in developing countries due to the poor sanitary precautions. Food handlers especially those working in food services such as restaurants and cafeteria are the potential sources of

Staphylococcal food poisoning because they can carry *Saureus* in their nasal cavities and contaminate foods by their hands. Moreover, they are carrier of methicillin resistant *S. aureus* (MRSA) which can be serious problems in both community acquired and hospital acquired *S. aureus* infections.

In this study 31 (29.9%) out of 106 nasal swabs collected from healthy food handlers were positive for *S. aureus*. High percentage (45.6%) of carriers was found among those who worked in cafeterias compared to 16.6% who served in restaurants. The results of the current work were higher than the results of studies carried out in Malaysia (23.4%) (Noor-Ariza *et al*., 2012), USA (23%) (Mainous *et al*., 2006), UK (Wieneke *et al*., 1993), Ethiopia (20.5%) (Dagnew *et al*., 2012), Turkey (23.1%) (Simsek *et al*., 2009), Saudia Arabia (8.5%) (Dablool and Al-Ghamdi, 2011) and Kuwait (26.6%) (Al-Bustan *et al*., 1996). On the other hand, the results were less than those found among food handlers of a Chilean (65%) (Soto *et al*., 1996) and Botswana (57.5%) studies (Leoto *et al*., 2007), as well as, amongst healthy hospital staff working in an Iranian (35.7%), Indian (37.3%) and Nigerian (50%) hospitals (Saeed and Hamidi, 2010).

Although the results of the current study were higher and lesser than those recorded in different countries but were similar to those conducted in Sudan (30.1%) (Saeed and Hamidi, 2010) and Brazil (29.0%) (Carmo *et al*., 2006) and within the ranges reported for health personnel (20% - 32%) (Klaytmus *et al*., 1997). This variation in the percentages of nasal carriage of *S. aureus* is due to several factors such as countries, season of year, size and target studied population.

Because not all strains of *S. aureus* are toxigenic, therefore testing of enterotoxin production is important. In this study 58.3% of the *S. aureus* isolates from nasal cavities of healthy food handlers was toxigenic in which enterotoxin type E was the only enterotoxin produced by all isolates. The results were higher than those reported in Turkey (20%), Egypt (34.4%) and Saudi Arabia (35%) who detected more than one type of Staphylococcal enterotoxins. In most studies carried out throughout the world *Staphylococcal* enterotoxin type A (SEA) was the most dominant type except the study in France who found that enterotoxin type E was responsible for food poisoning outbreaks (Ostyn, 2009).

Another problem associated with nasal carriage of *S. aureus* is resistance to antibiotics particularly methicillin resistant *S. aureus* (MRSA) which can become a source of serious infections in both community and hospital acquired infections. The results of drug susceptibility test showed that all isolates were susceptible to vancomycin and 22.5% were methicillin resistant (MRSA). These results were lower than those carried out in the same city by Yassin *et al* (2013) who found that 23 out of 32 *S. aureus* isolated from the nasal cavities of physical education students were positive for MRSA. In another study conducted by Yassin and Hassan (2013) in the same city but among medical students found that 50% of the *S.. aureus* isolated from nasal cavities was MRSA. In another study conducted by Habeeb *et al*.(2014) in the same city but among secondary school students found 2.04% were MRSA. This discrepancy in the results is most likely due to the methodology adopted for interpretation of the results.

Conclusion

Nasal carriage of *S. aureus* among food handlers working in cafeteria and restaurants in Duhok city was high which required attention from health authorities. Test for enterotoxigenicity is important because not all strains of *S. aeureus* are toxigenic. Elimination of nasal carriage of MRSA is essential to prevent both community and hospital acquired Staphylococcal infections.

Acknowledgements

I would like to thank Mrs. Awaz Arshad Saadi and Hayfaa Mazin at Department of Biology/ Faculty of Science for their technical support.

Correspondence author:
Ali Yahya Saeed,
Tel: 009647507957275
E-mail: ali.saeed@uod.ac

References

1. Al-Bustan MA, Udo EE, Chugh TD. Nasal carriage of enterotoxin-producing *Staphylococcus aureus* among restaurant workers in Kuwait city. *Epidemiol. Infect*. 1996; *116*: 319-322.
2. Al-Dahbi AM, and Al-Mathkhury HJ. Distribution of Methicillin Resistant *Staphylococcus aureus* in Iraqi patients and Healthcare Workers. *Iraqi Journal of Science*. 2013; 54 (2): 293-300.
3. AL-Omer DK and Al-Yassiri HN. *Staphylococcus aureus* nasal carriage among medical staff in AL-Nasiriyah Teaching Hospital, Thi-Qar, Iraq. *Thi-Qar Medical Journal*. 2009; 5 (8):49-55.
4. Argudín MA, Mendoza MC,and Rodicio MR. Review: Food poisoning and *Staphylococcus aureus* Enterotoxins. *Toxins*.2010; 2:1751-1774.
5. Bassyouni RH, El-Sherbiny N, Hefzy EH, Wegdan AA. Perception of food safety and prevalence of *Staphylococcus aureus* and

Salmonella species carries among Fayoum University food handlers. *Life Science Journal*.2012; 9 (4):2934-2940.
6. Carmo LS, Dias RS, Linardi VR, Sena MJ and Dos Santos DA. An outbreak of staphylococcal food poisoning in the municipality of Passos, Minas Geralis, Brazil. *Braz. Arch. Biol. Technol*.2003; 46: 581-586.
7. Çepoglu H, Vatansever L, Oral NB. Isolation of *Staphylococci* from Food Handlers and Investigation of their Enterotoxigenicity and Susceptibility to Some Antibiotics. *Kafkas Univ Vet Fak Derg*.2010; 16 (Suppl-A): S1-S5.
8. Clinical and Laboratory Standard Institute. Performance standards for antimicrobial susceptibility testing.2006. M100-S16. Wayne, PA.
9. Dablool AS and Al-Ghamdi AA. Enterotoxigenicity of *Staphylococcal aureus* isolated from food handlers during Hajj season in Saudi Arabia. *Open Journal of safety Science and Technology*. 2011; 1: 75-78.
10. Dagnew M, Tiruneh M, Moges F and Tekeste Z. Survey of nasal carriage of *Staphylococcus aureus* and intestinal parasites among food handlers working at Gondar University, Northwest Ethiopia. *BMC public health*.2012; 12,837.
11. Habeeb A, Hussein NR, Assafi MS, Al-Dabbagh SA. Methicillin resistant *Staphylococcus aureus* nasal colonization among secondary school students at Duhok city-Iraq. *Journal of Microbiology and Infectious Diseases*.2014; 4 (2): 59-63.
12. Jay MJ, Loessner JM, Golden AD. *Staphylococcal* gastroenteritis. In: Modern Food Microbiology. 7th edition. Springer Science, New York. 2005. 545-560.
13. Kluytmans J, Van Belkum A and Verbrugh H. Nasal carriage of *Staphylococcus aureus*: epidemiology, underlying mechanisms and associated risks. *Clin. Microbiol. Rev*.1997; 13: 505-520.
14. Loeto D, Matsheka MI and Gashe BA. Enterotoxigenic and antibiotic resistance determination of *Staphylococcus aureus* strains isolated from food handlers in Gaborne, Botswana. *J. food Prot*.2007; 70: 2764-2768.
15. Mainous AG, Hueston WJ, Everett CJ and Diaz VA. Nasal carriage of *Staphylococcus aureus* and methicillin-resistant *S. aureus* in the United States. *Ann. Fam. Med*. 2006; 4,132–137.
16. Noor-Ariza AM, Mohammad-Faid AR, Shuhaimi M, Syafinaz AN, Hamat RA, Malina O. *Staphylococcus aureus* in food and nares of food handlers in Kuala Pilah, Malaysia. *Pertanika Journal of Tropical agricultural Science*. 2012; 35 (4): 853-862.
17. Ostyn A, De Buyser ML, Guillier F, Groult J, Felix B, Salah S, Delmas G, Hennekinne JA. First evidence of a food poisoning outbreak due to *Staphylococcus* enterotoxin type E. *Euro Surveill*. 2010; 15 (13). Available online: htt: // www. Eurosurviellance. org/ViewArticle.
18. Saeed HA and Hamid HH. Bacteriological and parasitological assessment of food handlers in the Omdurman Area of Sudan. *J. Microbiol. Immunol. Infect*. 2010; 43:70-73.
19. Scott E. Food safety and food borne diseases in 21st century homes. *Can J Infect Dis*.2003; 14:277–280.
20. Simsek Z., Koruk I, Kopur AC and Gurses G. Prevalence of *Staphylococcus aureus* and intestinal parasites among food handlers in Sanliurfa, Southeastern Anatolia. *J. Public Health Manag. Pract*.2009; 15: 518-523.
21. Soto A, Saldias ME, Oviedo P and Fernandez M. Prevalence of *Staphylococcus aureus* among food handlers from a metropolitan university in Chile. *Rev. Med. Chil*.1996; 124: 1142-1146.
22. Wieneke AA, Roberts D and Gilbertm RJ. Staphylococcal food poisoning in the United Kingdom, 1969-90. *J. Epidemiol. Infect. Dis*. 1993; 110:519-531.
23. World Health Organization: Food safety and food borne illness. Geneva: WHO; 2007.
24. World Health Organization: Food Safety – Food borne diseases and value chain management for food safety, "Forging links between Agriculture and Health" CGIAR on Agriculture and Health Meeting in WHO/HQ. 2007.
25. Yassin NA, Hassan AO. Nasal carriage of methicillin-resistant/sensitive *Staphylococcus aureus* among students in faculty of medical sciences, Duhok University. *Advance Tropical Medicine and Health International*. 2013; 3(2):65-72.
26. Zain M, Naing N. Sociodemographic characteristics of food handlers and their knowledge, attitude and practice towards food sanitation: a preliminary report. *Southeast Asian J Trop Med Public Health*.2002; 33:410–417.

8/23/2015