Molecular screening of *Staphylococcus aureus* enterotoxins associated with samples of meat / Iraq

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**ABSTRACT**

*Staphylococcus aureus* secreted many types of toxins accompanying Intestinal poisoning resulting from eating food contaminated with bacteria or their toxins. Five hundred meat samples were collected from local markets, including fresh, frozen, canned, sausage and hamburger to investigate their contamination with *S.aureus* and then determined their ability of these isolates to secrete enterotoxins by using polymerase chain reaction. The results showed that the ratio of isolated *S.aureus* is 30 (6%) and the percentage of encoding genes for toxins is 30(100%), 0(0%), 3(10), 0(0%), 0(0%), 3(10), 2(6.7), 1(3.3), 0(0%) and 3(10) to sea, seb, sec, sed, see, seg, seh, sei, sej, and sel respectively. The result shows the *S.aureus* isolated from contamination meat able to produce different type to enterotoxins sea, sec, seg, see, sei, and sel and present the sea toxin is the most prevalence type of staphylococcus enterotoxins.

**INTRODUCTION**

*Staphylococcus aureus* is coccus shape, with a diameter between 0.5 and 1.5 μm. It’s negative to oxidase, catalase-positive, coagulase-positive, non-sporing, non-motile bacteria. It is classified in the Staphylococcaceae family, facultatively anaerobic (Pal et al., 2020).

Foodborne diseases result from eating food contaminated with pathogenic bacteria, and *Staphylococcus* bacteria is considered one of the main pathogens causing foodborne diseases in hospitals (Touimi et al., 2020).

*S.aureus* produce various types of intestinal toxins including enterotoxin A, B, C, D, E, H, G and I which cause food poisoning as a result of food contamination with bacteria (Johler et al., 2016).

The molecular method, for example, Conventional PCR, multiplex PCR is recommended for the discovery of *S. aureus* enterotoxins genes instead of phenotypical strategies because the serological kits can not recognize all the serotypes (Saly et al., 2018).

The research was aimed to isolation and identification of *S.aureus* from meat and meat product and molecular study for the enterotoxin genes (sea, seb, sec, sed, see, seg, seh, sei, sej, sel) responsible of food intoxication produced by *S. aureus* isolated.

**MATERIALS AND METHODS**

Using traditional culture media to isolated *S.aureus* from the 500 meat samples isolated from different butcher shop and supermarket in Hilla city. Approximately 5 grams of shipped meat samples were inoculated into 10 ml of heart infusion broth, then streaking on Mannitol Salt Agar. The positive colony further confirmatory by cultivated on Staphylococcus chromogenic media (Sundararaj et al., 2019).

To validate the diagnosis of the bacteria, Coagulase assay was performe by using MAST plasma coagu-
lase kit (Subramanian et al., 2017).

DNA was extracted from the probable *S. aureus* isolates from broth cultures utilizing phenol /chloroform DNA extraction method (Akkou et al., 2018; Hassan et al., 2019).

There are 35 cycles of PCR performed to obtain large number copies of a gene, by using the primers listed in (Table 1), for detection the *S. aureus* toxins genes.

**RESULTS AND DISCUSSION**

*S. aureus* is part of the normal flora found in the mucous layer of the skin and mouth, But when the number of bacteria increases lead to the contamination the food and food poisoning as a result of eating this food (Silva et al., 2018; Pal et al., 2020).

The *S.aureus* isolated form yellow golden colony when growth on mannitol salt agar with mannitol fomentation, a purple colony on staphylococcus chromogenic media (Figure 1).

![Figure 1: Growth of *S.aureus* on A. mannitol salt agar B. Staphylococcus chromogenic media](image1)

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![Figure 2: Distribution of *S.aureus* isolated according to the type of meat](image2)

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![Figure 3: Distribution of *S.aureus* toxins gene according to the type of meat](image3)

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![Figure 4: Agarose gel electrophoresis for sea amplicon(102bp) sea encode for Enterotoxine A genes (n=30)](image4)

Figure 4: Agarose gel electrophoresis for sea amplicon(102bp) sea encode for Enterotoxine A genes (n=30)

![Figure 5: Agarose gel electrophoresis for sec amplicon (907bp) seg encode for Staphylococcus Enterotoxin c genes (n=30)](image5)

Figure 5: Agarose gel electrophoresis for sec amplicon (907bp) seg encode for Staphylococcus Enterotoxin c genes (n=30)
Table 1: PCR primers and conditions for the detection of staphylococcal enterotoxin genes

| Gene | Direction | Primer sequence (5-3) | Amplicon size (bp) | PCR condition | References |
|------|-----------|-----------------------|--------------------|---------------|------------|
| sea  | F         | CGGCACCTTTTTTCTCTCCGG | 102                | 94°C for 5 min | Chapa-     |
|      |           |                       |                    | 94°C for 30 sec| val et al. |
|      | R         | GTATATCATGTCCTCCGG    |                    | 57°C for 30 sec | (2006)     |
| seb  | F         | CCAATAAGCTGACGAGTTAGG | 164                | 94°C for 5 min |            |
|      |           |                       |                    | 95°C for 30 sec|            |
|      | R         | GTATGTTGAGTCAACTGAGC  |                    | 50°C for 45 sec|            |
| sed  | F         | CCAATAATAGGAGAAATAAAAAG| 278               | 94°C for 7 min | Chapa-     |
|      |           |                       |                    | 35 cycle      | val et al. |
|      | R         | ATGGGATTTTTTTTGTTCC  |                    | 72°C for 3 min | (2006)     |
| See  | F         | AGTTTTTTTCACAGGTCATCC | 209                | 94°C for 5 min |            |
|      |           |                       |                    | 94°C for 30 sec|            |
|      | R         | CTTTTTTTTCTTCCGTCATCC |                    | 57°C for 30 sec|            |
| Seg  | F         | AAGTAGACATTTTTTGCGTTCC| 287                | 94°C for 5 min | Omoe et al.|
|      |           |                       |                    | 94°C for 30 sec| (2002)     |
|      | R         | AGAACCATCAAACGTATAGC  |                    | 55°C for 30 sec|            |
| seh  | F         | GTCTATATGGAGGTACACGATC| 213                | 94°C for 5 min |            |
|      |           |                       |                    | 94°C for 30 sec|            |
| sei  | F         | GGATATGTGGTGAGTTAAC  | 454                | 94°C for 5 min | Chiang    |
|      |           |                       |                    | 94°C for 30 sec| et al. (2006) |
|      | R         | ATCCATTTCTTTGCTTACAG |                    | 58°C for 30 sec|            |
| sel  | F         | CATACAGCTCTATCTACGG  | 275                | 94°C for 5 min |            |
|      |           |                       |                    | 94°C for 30 sec|            |
|      | R         | TTTTCTGCTTTAGTAACTC   |                    | 72°C for 5 min |            |
|      |           |                       |                    | 35 cycle      |            |
|      |           |                       |                    | 72°C for 30 sec|            |
Table 2: Number of toxins gene in S. aureus isolated (n=30)

| No. of sample | Type of meat Sample | Toxin gene       | Number of toxins gene |
|---------------|---------------------|------------------|-----------------------|
| 1             | Fresh meat sea, sel | sea, sel         | 2                     |
| 2             | sea, seg            |                  |                       |
| 3             | sea, sel            |                  |                       |
| 4             | sea, sec            |                  |                       |
| 5             | sea, sec            |                  |                       |
| 6             | sea, seh            |                  |                       |
| 7             | sea, sec, seg       |                  | 3                     |
| 8             | sea                 |                  | 1                     |
| 9             | sea                 |                  |                       |
| 10            | sea                 |                  |                       |
| 11            | sea                 |                  |                       |
| 12            | sea                 |                  |                       |
| 13            | sea                 |                  |                       |
| 14            | sea                 |                  |                       |
| 15            | Frozen meat sea     |                  | 1                     |
| 16            | sea                 |                  |                       |
| 17            | sea                 |                  |                       |
| 18            | sea, seh            |                  | 2                     |
| 19            | sea                 |                  | 1                     |
| 20            | sea                 |                  |                       |
| 21            | sea                 |                  |                       |
| 22            | sea                 |                  |                       |
| 23            | sea, seg, sei       |                  | 3                     |
| 24            | sea                 |                  | 1                     |
| 25            | Hamburger sea       | sea              |                       |
| 26            | sea, sel            |                  | 2                     |
| 27            | sea                 |                  | 1                     |
| 28            | sea                 |                  |                       |
| 29            | Canned meat sea     | sea              | 1                     |
| 30            | Sausage sea         | Sea              | 1                     |

Figure 6: Agarose gel electrophoresis for seg amplicon (287bp) seg encodes for Staphylococcus Enterotoxin G genes (n=30)

Figure 7: Agarose gel electrophoresis for seh amplicon (213bp) seh encode for Staphylococcus Enterotoxin H genes (n=30)
The results indicated that 30(6%) isolates belong to the S. aureus bacteria out of 500 meat sample. The highest percentage was obtained from fresh meat was 14(2.8%). The lowest percentage from canned meat was 1(0.4%), while the percentage of S. aureus isolated from Frozen meat, Sausage and hamburger were 10(2%), 1(0.2%) and 4(0.8%) respectively (Figure 2).

When comparing the results of this study with other studies, it was found that the isolation rate is lower than the result of other researchers. In Duhok city Abdulrahman (2020) showed that 28 (28%) of 100 local chicken and 80 (80%) of 100 imported frozen chicken were found to be positive with S. aureus using conventional methods, Maestro et al. (2020) collected 48 sample from farm animals in Algeria present 41 (85.4%) belong to S. aureus. Dashen and Cirfat1 (2020) also found the percentage of S. aureus isolated from ready-to-eat meat samples higher than the percentage in this study. The difference in the isolation ratios of the bacteria may be attributed to the extent of application of the health conditions used everywhere, the nature of production, location of sample collection, and the technical development of the country or its backwardness and through this study, it observed the underdeveloped reality of slaughtering and our distance from the simplest health ingredients.

The result showed that all the S. aureus isolated contain sea gene 30(100%), a similar pattern of results was obtained by Saly et al. (2018) it was found sea gene are the most type of S. aureus causing Food Born Disease, Others have shown that over half of food contamination cases are brought about by staphylococcal enterotoxin A.

This result was also similar to the findings of other researchers Dehkordi et al. (2019) Who is found that sea were the most prevalent types in percentage (38.7%)(Figures 3 and 4).

But other researchers Saleh et al. (2016) and Babu et al. (2017) not detection the sea genes in S. aureus isolated.

In Karbala city, Mohammed and Alwan (2017) obtain 57 isolates of S. aureus from meats found the percentage of the sea was 20(35%) by using PCR assay, 12 (37.5%) from frozen and 8 (32%) from fresh meat samples. Adikwu et al. (2019) found the percentage of the sea was 17.5%.

Another researcher collected 100 MRSA and 100 MSSA present the prevalence of sea was (45.0%) among MRSA and the prevalence of sea 42.0% in MSSA (Arabestani et al., 2018).

The present result found the ratio of seb and sed toxin gene was 0% (Figure 3), but another researcher present the prevalence of sed gene was (75.7%) (Rehab et al., 2016). The prevalent seb gene was (12.9%) (Asgarpooor et al., 2018). Li et al. (2018) found The percentage of enterotoxin seb gene was (6.9%). The result shows the number of S. aureus contained seg toxin only 3 from 30, while Dehkordi et al. (2019) showed that seg was the most prevalent Staphylococcus enterotoxins types (Figures 3 and 6).

The result showed all S. aureus isolated not contain see and sej toxin gene (Figure 3), while the anther result show The prevalent see gene was (22.6%) isolated from cooked meat samples (Asgarpooor et al., 2018).
The prevalent see gene (16.1%) in S. aureus isolated from cooked meat samples (Asgarpoor et al., 2018). Li et al. (2018) found the percentage of enterotoxin see and sej gene was (2.4%) and (12.5%), respectively. The percentage of sec was 3(10%) Li et al. (2018) found the percentage of enterotoxin sec gene was (6.9%). Rehab et al. (2016) found the predominant of sei gene was (51.4%), But in this study, found only 3.3% of S. aureus isolated contain this gene (Figures 3 and 5). The percentage of she, sei and sel was 2(6.7%), 1(3.3%) and 3(10%) respectively (Figures 3, 7, 8 and 9).

The current results demonstrated that all the S. aureus isolated from meat sample contain one or more type of toxin gene (Table 2). When comparing the results of this study with other studies, it was found that the isolation rate is lower than what other researchers found, Adikwu et al. (2019) found only 8 (13.8%) from 54(22%) of S. aureus isolated containing enterotoxin genes, But close to the findings of other researchers who found that 95.7% of S. aureus carried the SE genes (Rehab et al., 2016).

The results of the study showed the type of toxin secreted by S. aureus isolated from fresh meat, was the sea, sec, seg, sel, she, frozen meat was sea, seg, seh, sei, the hamburger was sea, sel, sausage canned meat only sea type (Table 2).

If the workers in the restaurants do not follow the rules of hygiene, which lead to contamination of food in bacteria. It found 10 bacteria per gram of food sufficient to produce intestinal toxins by staph bacteria (Saly et al., 2018).

From the results, it is clear the widespread prevalence of detected SE genes may indicate a risk of food poisoning resulting from eating animal meat contaminated with bacteria and its toxins. The increased prevalence of this MRSA means increased resistance to antibiotics, which cause serious public health problems (Mashouf et al., 2015).

CONCLUSIONS

Staphylococcus aureus secreted many types of toxins contaminated by eating food. From 500 hundred meat samples were collected from local markets, including fresh, frozen, canned, sausage and hamburger obtain 30 S. aureus isolated able to produce different type to enterotoxins sea, sec, seg, see, sei and sel and present the sea toxin is the most prevalence type of staphylococcus enterotoxins.

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Conflict of Interest

The authors declare that they have no conflict of interest for this study.

REFERENCES

Abdulrahman, R. F. 2020. Detection of Staphylococcus aureus from local and imported chicken in duhok province -Kurdistan region of Iraq using conventional and molecular methods. Bas.J.Vet.Res, 19(1).

Adikwu, A. A., Okolocha, E. C., Luga, I. I., Ngbede, E. O. 2019. Microbial hazards associated with pig carcasses and molecular detection of enterotoxigenic Staphylococcus aureus at different stages of the slaughter process. Sokoto Journal of Veterinary Sciences, 17(1):27–27.

Akkou, M., Bentayeb, L., Ferdi, K., Medrouh, B., Bachtarzi, M.-A., Ziane, H., Kaidi, R., Tazir, M. 2018. Phenotypic characterization of Staphylococci causing mastitis in goats and microarray-based genotyping of Staphylococcus aureus isolates. Small Ruminant Research, 169:29–33.

Arabestani, M. R., Rastiyani, S., Alikhani, M. Y., Mousavi, S. F. 2018. The Relationship Between Prevalence of Antibiotics Resistance and Virulence Factors Genes of MRSA and MSSA Strains Isolated from Clinical Samples, West Iran. Oman Medical Journal, 33(2):134–140.

Asgarpoor, D., Haghi, F., Zeighami, H. 2018. Frequency of Enterotoxin Producing Staphylococcus aureus and Toxin Genes in Raw and Cooked Meat Samples. Infection Epidemiology and Microbiology, 4(2):53–58.

Babu, S. W. R. N., Senthilkumar, R. J. A. T., Rao, P. K. V. A., Porteen, K. 2017. Prevalence of Panton Valentine Leukocidin (pvl) Gene in Methicillin Resistant Staphylococcus aureus Isolated from Market Samples of Chicken Meat. International Journal of Current Microbiology and Applied Sciences, 6(4):2459–2466.

Chapaval, L., Moon, D. H., Gomes, J. E., Duarte, F. R., Tsai, S. M. 2006. Use of PCR to detect classical enterotoxins genes (ent) and Toxic Shock Syn-
drome toxin-1 gene (tst) in Staphylococcus aureus isolated from crude milk and determination of toxin productivities of S. aureus isolates harboring these genes. *Arq. Inst. Biol.*, 73(2):165–169.

Chiang, Y. C., Chang, L. T., Lin, C. W., Yang, C. Y., Tseng, H. Y. 2006. PCR Primers for the Detection of Staphylococcal Enterotoxins K, L, and M and Survey of Staphylococcal Enterotoxin Types in Staphylococcus aureus Isolates from Food Poisoning Cases in Taiwan. *Journal of Food Protection*, 69(5):1072–1079.

Dashen, M. M., Cirfat1, N. A. 2020. Microbiological Quality of Ready - To - Eat Dog Meat Sold In Some Parts of Plateau State. *Nanret Mark Jackden1, Lilian Umbulle Mashor1*, 15.

Dehkordi, M. K., Shamsabadi, M. G., Banimehdi, P. 2019. The occurrence of Staphylococcus aureus, enterotoxigenic and methicillin-resistant strains in Iranian food resources: a systematic review and meta-analysis. *Annali Di Igiene : Medicina Preventiva e Di Comunita*, 31(3):263–278.

Hassan, K. I., Ali, B. A. M., Mohammed, N. A. 2019. Detection of the origin of animal species in kebab meat using mitochondrial DNA based - Polymerase Chain Reaction (mtDNA-PCR). *Iraqi Journal of Veterinary Sciences*, 33(1):39–43.

Johler, S., Sihto, H.-M., Macori, G., Stephan, R. 2016. Sequence Variability in Staphylococcal Enterotoxin Genes seh, sec, and sed. *Toxins*, 8(6):169–169.

Li, S., Wang, P., Zhao, J., Zhou, L., Zhang, P., Fu, C. X., Wang 2018. Characterization of toxin genes and antimicrobial susceptibility of Staphylococcus aureus from retail raw chicken meat. *Journal of food protection*, 81(4):528–533.

Maestro, L., Maestro, D., Segalo, S. 2020. Antimicrobial Resistance Pattern of the Staphylococcus Strains Isolated from Farm Animals, Exposed and Non-Exposed Personnel. *United International Journal for Research & Technology*, 1(7):21–28.

Mashouf, R. Y., Hosseini, S. M., Mousavi, S. M., Arabestani, M. R. 2015. Prevalence of Enterotoxin Genes and Antibacterial Susceptibility Pattern of Staphylococcus aureus Strains Isolated from Animal Originated Foods in West of Iran. *Oman Medical Journal*, 30(4):283–290.

Mohammed, N. I. M. J., Alwan 2017. Isolation and identification of Staphylococcus aureus strains from fresh and frozen meat in Karbala province. *IJ.S.N*, 8(3):704–709.

Omoe, K., Ishikawa, M., Shimoda, Y., Hu, D. L., Ueda, S., Shinagawa, K. 2002. Detection of seg, seh, and sei genes in Staphylococcus aureus Isolates and Determination of the Enterotoxin Productivities of S. aureus Isolates Harboring seg, seh, or sei Genes. *Journal of Clinical Microbiology*, 40(3):857–862.

Pal, M., Kerorsa, G. B., Marami, M. L., Kandi, V. 2020. Epidemiology, Pathogenicity, Animal Infections, Antibiotic Resistance, Public Health Significance, and Economic Impact of Staphylococcus Aureus: A. Comprehensive Review *American Journal of Public Health Research*, 8(1):14–21.

Rehab, M. E., Dina, E. R., Shaymaa, H. A. R. 2016. Toxin gene profile and antibiotic resistance of Staphylococcus aureus isolated from clinical and food samples in Egypt. *African Journal of Microbiology Research*, 10(13):428–437.

Saleh, E., ElMohsen, R., Ibrahim, M. 2016. Molecular Identification of Staphylococcus Aureus in Imported Frozen and Locally Slaughtered Meat. *Alexandria Journal of Veterinary Sciences*, 51(1):162–162.

Saly, M. E., Tobar, A. A., Elbialy, M. M. M., El-Shafey, Z. A. S. 2018. Isolation of Staphylococcal Enterotoxins Causing Gastroenteritis. *AAStencil Journal of Health*, 5(2):34–38.

Silva, D., Nicolete, M., Lopes, G., Morita, B. D., Souza, C. H. D., Winkelstroter, J. M., K. L., Rodrigues, M. V. P. 2018. Evaluation of Antimicrobial Susceptibility by Staphylococcus aureus Isolated from the Nutrition Service of a Teaching Hospital. *Advances in Microbiology*, 8:270–285.

Subramanian, A., Chitalia, V. K., Bangera, K., Vaidya, S. P., Warke, R., Chowdhary, A. R. A., Deshmukh 2017. Evaluation of Hiaureus™ coagulase confirmation kit in identification of Staphylococcus aureus. *Journal of Clinical and Diagnostic Research*, 11(2):8–13.

Sundararaj, N., Kalagatur, N. K., Mudili, V., Krishna, K., Antonyssamy, M. 2019. Isolation and identification of enterotoxigenic Staphylococcus aureus isolates from Indian food samples: evaluation of in-house developed aptamer linked sandwich ELISA (ALISA) method. *Journal of Food Science and Technology*, 56(2):1016–1026.

Touimi, G. B., Bennani, L., Berrada, S., Moussa, B., Bennani, B. 2020. Prevalence and antibiotic resistance profiles of Staphylococcus sp. isolated from food, food contact surfaces and food handlers in a Moroccan hospital kitchen. *Letters in Applied Microbiology*, 70(4):241–251.