RESEARCH ARTICLE

Molecular Detection of *Sea* Gene and Antimicrobial Susceptibility Pattern of *Staphylococcus aureus* Strains Isolated from Food Handlers in Kirkuk City, Iraq

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ABSTRACT

Asymptomatic carriers of enterotoxigenic *Staphylococcus aureus* are potential source of food poisoning. This study aimed to determine the prevalence and risk factors associated with *S. aureus* nasal carriage among food handlers in Kirkuk city, Iraq. A total of 500 nasal swab samples were collected and analyzed using standard conventional methods of microbial analysis in isolation and identification of *S. aureus* including, culturing on selective media (mannitol salt agar); also blood agar medium was used to detect β hemolysin toxin, catalase, coagulase, and DNase tests were used. Staphaurex Plus test was used to detect protein A and clumping factor. Also RapID ^{TM} STAPH PLUS System was used to complete the diagnosis of *S. aureus* isolates. Penicillin-Binding Protein (PBP2a) Latex Agglutination test was also used to investigate the presence of penicillin-binding protein (PBP2a). Twelve antibiotics were used to detect the antimicrobial susceptibility of the isolates. The results showed that the prevalence of *S. aureus* nasal carriage among food handlers was 87 (17.4%) and 82% of them were harbored *sea* gene which encodes enterotoxin type A that detected by Polymerase Chain Reaction (PCR) using specific primer. Virulence factors (protein A, coagulase, DNase, β hemolysin rates were (100%, 100%, 100%, 65.5%) respectively. Free coagulase production by using the tube method was measured in different periods (2, 4, 6, and 24 hours) to indicate the degree of severity of *S. aureus* strains and the results were (50%, 34%, 0% and 16%). 100% of the isolates had PBP2a. The isolates showed high resistance to Oxacillin (98.8%) used in this study as a preliminary test to detect methicillin-resistant *S. aureus* (MRSA). Double disc diffusion test was performed to detect (MS), inducible clindamycin resistance (iMLSB) and constitutive MLSB (cMLSB) and the results were (16%), (14.9%) and (9.1%), respectively.

Keywords: Food poisoning, Methicillin resistant Staphylococcus aureus (MRSA), Sea gene, *Staphylococcus aureus*.

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INTRODUCTION

Staphylococcus aureus is a principal etiological agent of food poisoning worldwide.[1] Food handlers have been implicated in a plethora of foodborne diseases, and it has been reported that one of important pathogens often transmitted via food contaminated by infected food handlers is *S. aureus*.[2] *S. aureus* strains produce a range of different exotoxins like enterotoxins (SEs), which causes vomiting with or without diarrhea and are responsible for food poisoning.[3] Staphylococcal enterotoxins (SEs) have been divided into five serological types (SEA through SEE) based on their antigenicity. In recent years, the existence of new types of SEs (SEG-SEY). Enterotoxin type A is the most potent for inducing the disease. Staphylococcal enterotoxins are resistant to inactivation by gastrointestinal proteolytic enzymes such as pepsin. In addition, they are heat stable.[4] *S. aureus* strains also produce additional virulence factors such as protein A, ß hemolysin toxin, DNase, coagulase and beta-lactam antibiotic resistance factors.[5]

MATERIALS AND METHODS

Isolation and Identification

500 nasal swabs were collected from food handlers in Kirkuk city, and each swab was inoculated onto mannitol salt agar. Then the plates were incubated at 37ºC for 18–24 hours. Mannitol fermenting yellow colonies were selected, and further identification was carried out with Gram stain, catalase,

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Coagulase (Tube method), DNase. The Staphaurex Plus test was used to detect protein A and clumping factors. Also Penicillin-Binding Protein (PBP2´) Latex Agglutination test was used to detect the presence of penicillin-binding protein (PBP2a). RapID^{TM} STAPH PLUS System tests were used to complete the diagnosis of *S. aureus* isolates. The isolates were subcultured on blood agar and incubated at 37ºC to detect β hemolysin toxin.

Antimicrobial Susceptibility Test

All strains were analyzed for antimicrobial susceptibility by using Kirby-Bauer disk diffusion method according to Clinical Laboratory Standard Institute (CLSI) guidelines and Mueller-Hinton agar as standard medium. Twelve antibiotics were used, including (Penicillin, Oxacillin, Gentamycin, Erythromycin, Ciprofloxacin, Levofloxacin, Rifampicin, Chloramphenicol, Tetracycline, Trimethoprim, Trimethoprim/Sulfamethoxazole and Clindamycin. The results were recorded as resistant, intermediate, and susceptible by measuring inhibition zone diameter in a milliliter. A double-disc diffusion test was used to detect MS, inducible clindamycin resistance(iMLSB) and constitutive (cMLSB) phenotypes.

Molecular Detection of Sea Gene

Genomic DNA was extracted from (50) *S. aureus* isolates according to the protocol of Wizard Genomic DNA Purification Kit, (Promega USA) according to the manufactures instructions. After the extraction of DNA Quantus Florometer was used to detect the concentration of extracted DNA in order to detect the goodness of samples for downstream applications.

Polymerase Chain Reaction (PCR) and Gel Electrophoresis were done according to the protocol of the manufacturers. The following primer was used in this study for gene.[6]

PCR was performed under initial denaturation at 95ºC for 5minutes, followed by 30 cycles of 95ºC for 30 seconds, 72ºC for 30 seconds, and a final extension of 7 minutes at 72ºC. PCR products were analyzed using Gel electrophoresis, which was performed at 100 v/mAmp for 75 minutes. Gels were viewed by UV trans-illumination and photographed.

RESULTS AND DISCUSSION

A total of 500 specimens 87 (17.4%) were identified as *S. aureus,* and 57 (65.5%) of the isolates were produced β hemolysin, which was absent at 37ºC but produced at 4ºC this is called "hot-cold phenomenon" the hemolysis being initiated at 37ºC, but become evident only after chilling[7,8] was revealed (66%), while other studies[9,10] were reported (84.35%), (96.6%), respectively. All isolates were positive with Staphaurex Plus test, and this result indicated the presence of protein A and clumping factor for *S. aureus.* Protein A, synthesized by almost all strains of *S. aureus.*[11] In the current study, all isolates (100%) were positive for tube coagulase test, and the results were obtained by the formation of a clotted plasma in the tube after four different periods of incubation 2, 4, 6, and 24 hours (Table 1). The correlation between coagulase production and the time indicates the degree of severity of *S. aureus* strains, so if the isolate produces coagulase and clot the plasma in a short time, that means it is more virulent and vice versa.

Also the isolates (100%) were positive for DNase test, and the result was similar to the study by[12] that revealed (100%), while other studies by[13] and by[14] reported a lower percentage (72.2%) and (65.7%), respectively. Detection of this enzyme is a useful diagnostic marker as other *Staphylococci* small amounts of this enzyme or none at all.[15]

Table 1: Production of coagulase in tube method on different periods

	Total	*Time of incubation*							
Test	*No.*	*2h*	*%*	*4h*	*%*	*6h*	*%*	*24h*	*%*
Coagulase	50	25	50	17	34	0	0	8	16

***S. aureus* Carriers and Gender**

In this study, the results (Table 2) indicate that the number of males who work as food handlers and carry *S. aureus* was high (86.2%) compared with the low number of females (13.7%). This result disagreed with other studies which had revealed slightly differences between males and females for example, the study by[16] that reported a high prevalence of *S. aureus* among females (58.6%) while in male (44%) another study[16] reported (52.6%) in females and (47.4%) in males. This may be due to our country's tradition, which prevents females from working in restaurants and public places.

***S. aureus* Carriers and Age Group**

The age was categorized from less than 20 to more than 40 years in Table 3. The results were reported in Table 3 the higher number of isolates was between (20–29) years with 58.6% followed by the age group (30–39) years with 17.2% then the age group more than (40) years with 16% and the less number was in age group less than 20 with 8% the results were close to a study[17] that revealed (61.5%, 7.7%, 7.7%, and 15.4%), respectively.

Results of Antibiotic Susceptibility Test

Antibiotic susceptibility testing is not usually carried out for *S. aureus* associated with food poisoning because the illness is self-limiting and antibiotics are not usually required for treatment. However, antibiotic-resistant *S. aureus* strains can be involved in cases of food intoxication. In our study the antibiotic resistance profile of the *S. aureus* isolates were presented in Table 4.

Oxacillin was used in this study as a preliminary test to detect MRSA. The prevalence of nasal carrier of MRSA

Table 2: Distribution of *S. aureus* carriers acorrding to gender

Total No.	*Positive No.*	*Negative No.*	*Female No.*	*Positive No.*	*Male No.*	*Positive No.*
500	87(17.4%)	413(82.6%)	48(9.6%)	12(13.7%)	452(90.4%)	75(86.2%)

among food handlers was found to be 86 (98.8%) out of 87 that corresponded with local study conducted in Samara City, Iraq by[18] that revealed (100%). While a study in Duhok City, Iraq by[19] reported (73%). In Jordan, a study report[20] were reported (57.3%). In contrary to our study, no cases of MRSA were detected by other studies.[8,21] Many different factors contributed to *S. aureus* and MRSA distribution, including crowded housing, bad hygienic practices, sharing of personal utensils, recurrent antibiotic usage, hospital admissions, and intravenous drug addicts.[22]

Table 3: Distribution of *S. aureus* carriers according to age

Age group	*Total No.*	*Positive No.*	*%*
<20	67	7	8
20-29	229	51	58.6
30-39	129	15	17.2
>40	75	14	16

In our study the prevalence of MS, iMLSB and cMLSB phenotypes (Table 5) among MRSA isolate*s* were (16%), (14.9%) and (9.1%), respectively (Table 5 and Figure 1 are in agreed with other previous reports).[23] which is in agreement with that reported by[23] (20%), (18.6%), and (12%) consistent with the study[24] reported (17.6%), (11.75), and (8.8%) and with

Table 4: Antibiotic susceptibility pattern of *S. aureus* isolates

	Resistant		*Intermediate*		*Sensitive*	
Antibiotic	*No.*	*%*	*No.*	*%*	*No.*	*%*
Penicillin 10 µg	87	100	0	0	0	0
Oxacillin 1 µg	86	98.8	0	0	1	1.1
Gentamicin 10 µg	1	1.1	3	3.4	83	95.4
Clindamycin 2 µg	21	24.1	3	3.4	63	72.4
Erythromycin 15 µg	33	37.9	6	6.8	48	55.1
Tetracycline 30µg	16	18.3	4	4.5	57	65.5
Ciprofloxacin 5 µg	15	17.2	9	10.3	63	72.4
Levofloxacin 5 µg	15	17.2	2	2.2	70	80.4
Trimethoprim 5 µg	1	1.1	0	0	86	98.8
Trimethoprim/Sulfamethoxazole 1.25/23.75 µg	1	1.1	0	0	86	98.8
Rifampicin 5 µg	0	0	0	0	87	100
Chloramphenicol 30 µg	7	8	0	0	80	91.9

Table 5: Susceptibility patterns of isolated MRSA against erythromycin and clindamycin.

Erythromycin sensitive Clindamycin sensitive		*Erythromycin resistant Clindamycin sensitive (D zone negative)*		*Erythromycin resistant Clindamycin sensitive (D zone positive)*		*Erythromycin resistant Clindamycin resistant*	
No resistance		*MS*		*iMLSB*		*cMLSB*	
No.	%	No.	%	No.	%	No.	%
46	52.8%	14	16%	13	14.9%	8	9.1%

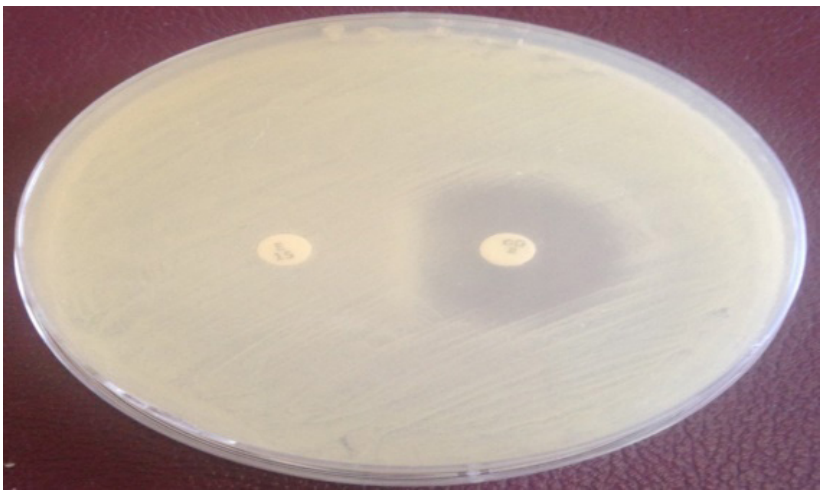

Figure 1: D-test positive. Halo formed as letter D form due to erythromycin-induced resistance

Table 6: The Primer sequence

Primer Name	*Sequence*	*Annealing temp.ºC*	*Product Size*
SeA-1	5`-CATTGCCCTAACGTGGACAACAAG-3`	57	587bp
SeA -2	5`-ATCCCCTCTGAACCTTCCCATC-3`		

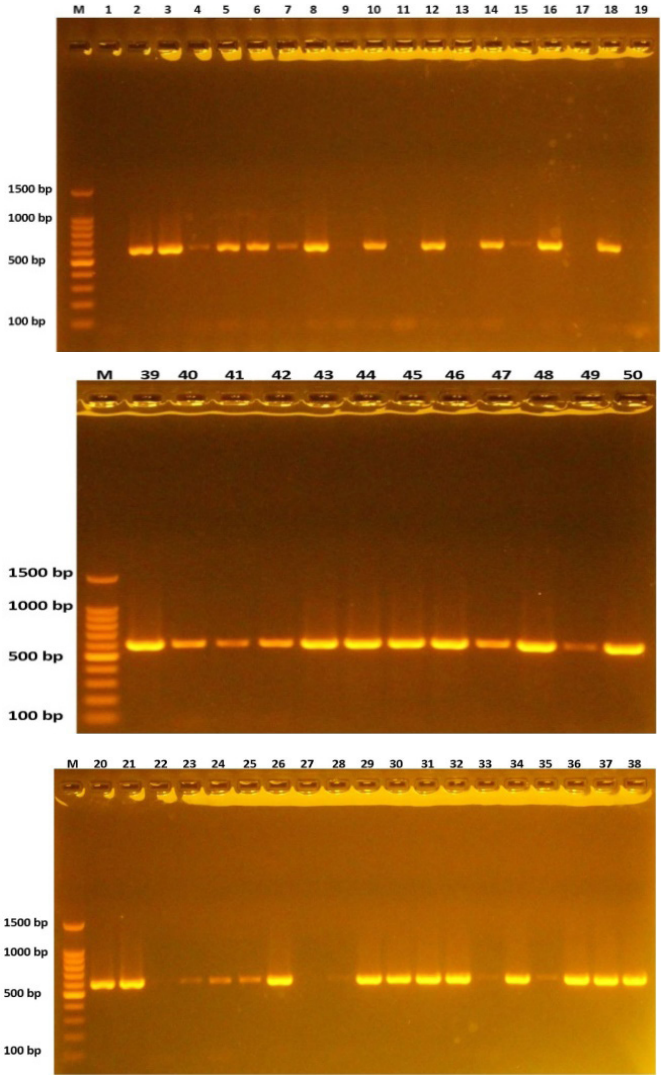

Figure 2: Results of the presence of *sea* gene of *Staphylococcus aureus* samples were fractionated on 1% agarose gel electrophoresis stained with Eth.Br. Lane1:100bp DNA marker

the study[25] that revealed (25.5%), (23.2%) and (9.3%). 52.8% of *S. aureus* isolates were sensitive to both clindamycin and erythromycin, which corresponded with another[26] reported (50%).

Molecular Detection of *Sea* Gene

DNA was extracted from (50) *S. aureus* isolates. The concentrations of extracted DNA samples were determined; the results showed that concentrations of DNA samples ranged from (18-30) ng/µL. The result of PCR assay was investigated a high prevalence of *sea* gene, 41(82%) were harbored *sea* gene (Figure 2). Our result was in agreement with the study carried out in China[27] that reported (81%). In Japan[28] also was reported a high prevalence (71%) of this gene.

While other studies were investigated a lower prevalence of *sea* gene among *S. aureus* strains isolated from food handlers, such as a study in Karbala City,[2] in Khartoum State[29] and (16%), (18.7%) and (22%),[30] respectively. SEA predominance in staphylococcal food poisoning outbreaks were aso documented in Italy,[31] Belgium,[2] and Germany.[32] SEA is produced in foods under a wider range of pH, redox potential and water activity (a_w) than are the other SEs, which explain why SEA is principal toxin involved in staphylococcal food poisoning.[33] The Primer sequence is mentioned at Table 6.

CONCLUSIONS

The prevalence of enterotoxigenic *S. aureus* carriers among food handlers is high.

High number of the enterotoxigenic *S. aureus* produce coagulase within 2–4 hours, so they are considered more virulent strains.

Most isolates have (PBP2a) a product of mec A gene, so they are MRSA, which means they are resistant to all beta-lactam antibiotics. The carrier state of MRSA among food handlers indicated must be considered as a problem with health impact.

The prevalence of inducible clindamycin resistance phenotype is not high.

Isolated *S. aureus* strains produce β-hemolysin toxin at 4ºC in other wards this toxin is less effective in the human body.

REFERENCES

1. Loeto D, Matsheka M L, Gashe B A. Enterotoxigenic and antibiotic resistance determination of *Staphylococcus aureus* strains isolated from food handlers in Gaborone. Botswana. Journal of food protection. 2007; 70(12): 2764.
2. Alhashimi H M M, Ahmed M M, Mustafa J M. Nasal carriage of enterotoxigenic *Staphylococcus aureus* among food handlers in Karbala City. Karbala International Journal of Modern Science. 2017; 3(2): 4.
3. Denayer S, Delbrassinne L, Nia Y, Bottdoom N. Food Outbreak and Molecular Typing: High diversity of *Staphylococcus aureus* strains and importance of toxin detection. Journal/ toxin. 2017; 9(12): 407.
4. Nazari R, Godarzi H, Baghi F, Moeinrad M. Enterotoxin gene profiles among *Staphylococcus aureus* isolated from raw milk. Iranian Journal of Veterinary Research. 2014; 15(4): 409.
5. Ou Q, Peng Y, Lin D, Bal C, Zhang T, Lin J, Ye X, Yao Z A. Meta-Analysis of the Global Prevalence Rates of *Staphylococcus aureus* and Methicillin-Resistance *S. aureus* Contamination of different Raw Meat Products. Journal of Food Protection. 2017; 80(5): 736.
6. Sezer C, O¨zgur Ç, Aksem A, Leyla L. Food handlers: a bridge in the journey of enterotoxigenic MRSA in food. J. Verbr. Lebensm. 2015; 10: 125.
7. Kumer, S. Text book of microbiology. 1thed. Jaypee Brothers Medical Publishers (P) Ltd. Aegean Offset Printers. Greater Noida; 2012. P.232.

8. Castro A, Santos C, Meireles H, Silva J, Teixeira P. Food handlers as Potential Sources of Dissemination of Virulent Strains of *Staphylococcus aureus* in the Community. Journal of Infection and Public Health. 2015; 9(2): 156.
9. AL Bahry S, AL Mushrufi S K, Mahmood I Y, Nallusamy S. *Staphylococcus aureus* contamination during food preparation, processing and handling. international Journal of chemical engineering and applications. 2014; 5(5): 388.
10. Islam M J, Uddin M S, Nasrin M S, Nazir K H, Rhman M T, Alm M M. Prevalence of Enterotoxigenic and Toxic Shock Syndrome Toxin-1 Producing Coagulase Positive *Staphylococcus aureus* in Human and their Characterization. Bangl. J. Vet. Med. 2007; 5 (1 & 2): 117.
11. Samarnayake L. Essential Microbiology For Dentistry. 4thed. CHURCHIL LIVINGSTONE Elsevier. China; 2012. P.125.
12. Samira A W, Ahmed S, Meundi M D. *Staphylococcus aureus*-A versatile Biochemical Characterization and Antibiogram. International Journal of Current Microbiology and Applied Sciences. 2018; 7(2): 2935.
13. Kulkarni S, Anuradha K, Venkatesha D. Demonstration of Virulence Markers and Methicillin Susceptibility of *Staphylococci* in Various Clinical Isolates. International Journal of Current Microbiology and Applied Sciences. 2014; 3(4): 52.
14. Javid F, Taku A, Bhat M A, Badroo G A, Mudasir M, Soft T A. Molecular typing of *Staphylococcus aureus* based on coagulase gene. Vet World. 2018; 11(4): 426.
15. Ford M. (2014). Medical Microbiology. 2thed. Oxford University Press. Great Britain by Bell & Bain Ltd. Glasgow. P.56.
16. Emeakaroha, Chioma M, Nkwocha, Gertrude I, Adieze, Chibuzo N, Adieze, Ifechukwu E. Antimicrobial susceptibility pattern of *Staphylococcus aureus* and their nasal and throat carriage among food handlers at the Federal University of Technology, Owerri Nigeria. International Journal of Biomolecules and Biomedicine. 2017; 6(3): 4.
17. Omololu-Aso J, Oluwatoyin O, Olutobi O, Otusanya O, Ochada H C, Shesha A. Nasal Colonization of Methicillin Resistance Staphylococcus aureus among food Handlers in the Eateries Obafemi Awolowo University Ile Ife, Nigeria. Journal of Clinical Nutrition & Dietetics. 2017; 3(1:9): 2.
18. Alsamarai A M, Abbas H M, Atia Q M. Nasal carriage of methicillin resistant *Staph aureus* in food provider in restaurant Samara City. World journal of pharmacy and pharmaceutical sciences. 2015; 4 (6): 54.
19. Abdulrahman M A, Taher I A. Prevalence of methicillin resistant *Staphylococcus aureus* among food handlers in Duhok City. Science Journal of University of Zakho. 2018; 4(4): 142.
20. Al-Zubi M S, Al-Tayyar I A, Hussin E, Al-Jabali A, Khudairat S. Antimicrobial Susceptibility Pattern of *Staphylococcus aureus* isolated from clinical specimens in Northern of Jordan. Iranian Journal of Microbiology. 2015; 7(5): 269.
21. Vatansever L, Sezer C, Bilge N. Carriage rate and methicillin resistance of *Staphylococcus aureus* in food handlers in Kars City, Turkey Springer Plus. 2016; 5(608):
22. Lehne, R. A. Pharmacology for Nursing Care. 8thed. Elsevier. United States of America; 2013. P.1066.
23. Supriyarajvi, Gupta A, Tina G, Sharma B P. Detection of inducible clindamycin resistance among Staphylococcal isolates from various clinical specimens in a Tertiary care Institute in north west region of Rajasthan, India. International Journal of Current Microbiology and Applied Sciences. 2015; 4(1): 746.
24. Koppad M, Parameshwar S, Haleesh LH, Siddesh KC. Detection of inducible clindamycin resistance in *Staphylococcus aureus* and CoNS at tertiary care hospital. Indian J. Microbiol Red. 2015; 2(4): 193.
25. Hanci H, Ayyidiz A, İgan, H. Investigation of inducible clindamycin resistance in methicillin resistant *Staphylococcus aureus* strains. Mamara phrmma Ceutical Journal. 2017; 21(2): 408.
26. Prabhu K, Roa S, Roa V. Inducible Clindamycin Resistance *Staphylococcus aureus* Isolated from Clinical Samples. Journal of Laboratory Physicians. 2011; 3(1): 25-26.
27. Chen Q, Xie S. Genotypes Gene Profiles, and Antimicrobial Resistance of *Staphylococcus aureus* Associated with Food handlers Outbreaks in Hangzhou, China. Toxins. 2019; 11(307): 1.
28. Sato'o Y, Omoe K, Ono H K, Nakane A, Hu D. A novel comprehensive analysis method for *Staphylococcus aureus* pathogenicity islands. ORIGINAL ARTICLE. Microbiol Immunol. 2013; 57: 91.
29. Hamza A O, Seed H A. Enterotoxigenic *Staphylococcus aureus* amongst Food handlers in Khartoum State, Sudan. EUROPEAN ACADEMIC RESEARCH. 2016; IV(4): 3459.
30. Aung M H, San T, Aye M M, Mya S, Maw W W, Nyein K, Htut, W H W, Kawaguchiya M, Urushibara N, Kobayashi N. Prevalence and Genetic Characteristics of *Staphylococcus aureus* and *Staphylococcus argenteus* Isolates Harboring Panton-Valentine Leukocidin, Enterotoxins, and TSST-1 Genes from Food Handlers in Myanmar. Toxins. 2017; 9(241): 2.
31. Ercol L, Gallina S, Nia Y, Auvray F, Primavilla S, Guidi F, Pierucci B, Graziotti C, Decastelli L, Scuota, S. Investigation of Staphylococcal Food Poisoning outbreak from a Chantilly Cream Dessert, in Umbria (Italy). Food borne Pathog Dis. 2017; 14(7): 411.
32. Johler S, Petra S, Tichaczek-Dischinger, Rau J, Sihto H M, Lehner A, Adamand M, Stephan R. Outbreak of Staphylococcal Food Poisoning Due to SEA-Producing *Staphylococcus aureus.* Food borne Pathog Dis. 2013; 10(0): 4.
33. Medvedova A, Valik L, Studenicova A. The Effect of Temperature and Water Activity on the Growth of *Staphylococcus aureus*. Czech J. Food Sci. 2009; 27(2): 28-29.