PREVALENCE OF ENTEROTOXIN TYPE A IN *STAPHYLOCOCCUS AUREUS* ISOLATED FROM DIFFERENT SOURCES

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ABSTRACT

This research was aimed to investigate the prevalence of enterotoxin type A in (82) *Staphylococcus aureus* isolates from different sources(Pathogenic, Soil, Food, Tape water& Nasal swabs from Normal source), the results had shown the capacity of (58) isolates (70.73%) to produce the enterotoxin by using culture method (in solid and liquid medium), concerning the liquid medium, the results conducted that there was a significant differences at P≤ 0.05, among bacterial isolates in the amount of produced enterotoxin in terms of optical density. By using serological ELISA specific kit, the results had confirmed the ability of (47) bacterial isolates(57.32%) to produce enterotoxin type A with concentrations were ranged between(3.7 - 15.7) ng/ml, which represents (81%) of all isolates producing enterotoxins, also the results indicated that there were isolates from different isolation sources can produce this toxin , and the prevalence of enterotoxin type A producing isolates was in pathogenic isolates(43.9%), soil (66.7%), food(92.3%), Tape water(55%) & Nasal swabs(80%), This prevalence of enterotoxin type A which is the most dangerous of Staphylococcal enterotoxins in studied isolates reflects the risk and healthy importance of this bacteria, regardless of the source of its isolation.

Key words:drinking water, ELISA, optical density, food, soil .

انتشار السم المعوي نوع A في بكتريا *Staphylococcus aureus* المعزولة من مصادر مختلفة

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المستخلص

يهدف البحث التحري عن انتشار السم المعوي من النوع A في (82) عزلة من المكورات العنقودية الذهبية معزولة من مصادر مختلفة (مرضية، تربة، غذاء، ماء الشرب ومسحات الأنف كفلورا طبيعية)، وأظهرت النتائج قدرة (58) عزلة (70.73) % على إنتاج السم المعوي باستخدام طريقة الزرع (في الوسط الصلب والسائل)، فيما يتعلق بالوسط السائل، أظهرت النتائج وجود فروق معنوية (عند مستوى احتمال 0.05 ≥p) بين العزلات البكتيرية في كمية السم المعوي المنتج بدلالة الكثافة الضوئية . باستخدام عدة فحص الاليزا ELISA المصلي المتخصص، أكدت النتائج قدرة (47) عزلة بكتيرية (57.32%) على إنتاج السم معوي من النوع A بتراكيز تراوحت بين (3.7 – 15.7) نانوغرام / مل ،وهي تمثل (81%) من مجمل العزلات المنتجة للسموم المعوية، كما أشارت النتائج إلى وجود عزلات من مصادر عزل مختلفة يمكنها أن تنتج هذا السم، وكانت نسبة انتشار السم المعوي من النوع A في العزلات الممرضة (43.9%)، التربة (66.7%)، الغذاء (92.3%)، ماء الشرب (55%) ومسحات الأنف (80 %)، إن انتشار الذيفان المعوي من النوع A الذي يعد أخطر السموم المعوية العنقودية في العزلات المدروسة يعكس خطورة هذه البكتيريا وأهميتها الصحية ، بغض النظر عن مصدر عزلها.

الكلمات المفتاحية: ماء الشرب، الاليزا، الكثافة الضوئية، غذاء، تربة.

Received:1/4/2021, Accepted:9/6/2021

INTRODUCTION

Staphylococcus aureus was considered as one of the gram positive bacteria that well known by its ability to occurrence of broad range of clinical diseases beginning with superficial skin infections and soft tissues infections ending with life threatning septicemia (32), besides that it had considered as important etiological agents for nosocomial infection(14).This bacteria had possessed a variety numbers of virulence factors such as producing different toxins and enzymes, biofilms and their capacity to resist a broad spectrum of antibiotics (16). One of the important toxins that secreting by this bacteria were enterotoxins(24), toxic shock syndrom toxin-1 and haemolysin (19). Because of the ability of this bacteria to produce enterotoxins , so it considered as an important etiological agent for food poisoning and it comes with third order after *Vibrio* and *Salmonella* (21), where it had secreted these toxins in foods during preparation and treatment, the food poisoning occurs after ingestion the contaminated foods with these toxins (11). These staphylococcal enterotoxins had characterized by their capacity for stability and heat resistance which subjected to it during cooking of foods, as well as , these enterotoxins had known as their high capability to resist the proteins digestive enzymes and the lower PH of stomach and all these factors assists the bacteria to causing food poisoning and effects the function of gasterointestinal tract (18), the experiments had conducted that when treating mushroom with 121^{o}C for 28 minutes or with 127^{o}C for 15 minutes, these enterotoxins remain saving their biological toxic activity and persists to cause food poisoning (1). In general these enterotoxins had counted as superantigens through their high stimulation of T cell proliferation (6). There were about more than 23 types of these enterotoxin which marked from SEA to SEIY (29), among these enterotoxins there were five classical types which were SEA to SEE which had responsible of 95% of food poisoning states , while the SEA and SED were the more occurrence and common for poisoning ,while other types of enterotoxins such as ESG , SHE, SEI, SER, SES and SET which counted as non classical and discovered after the five classical types also had the strength to occure the symptoms of food poisoning such as vomiting (13). Besides that there were other types of enterotoxins had produced by this bacteria but couldn't occure the symptoms of vomiting and diarrhea , these enterotoxins had put under staphylococcal enterotoxins like enterotoxin (25). The enterotoxin type A was important type of enterotoxin causing food poisoning with 50% of these states (9), So the aim of this research to detect the prevalence of enterotoxin type A in *Staphylococcus aureus* isolated from different sources by using culture and immunological methods

MATERIALS AND METHODS

Bacterial Isolates:

Eighty two isolates belongs to *Staphylococcus aureus* had used in this research , these isolates had isolated and diagnosed in Department of Biology/ College of Science/ University of Mosul, and the number of isolates as followings (Pathogenic 41 , Soil 3, Food 13, Tape water 20 & Nasal swabs from Normal source 5) .

Bacterial growth standardization:

Bacterial isolates was activated by streaking Brain heart infusion agar (Thermofisher scientific), and the plates incubated with 37^{o}C for 24 hr.(8). One colony of bacterial isolates had picked and suspended in tube with 5ml normal saline and mixed well, then the bacterial growth turbidity had compared with McFarland first tube which contains 1.5 X 10^{8} cell per ml (7).

Screening on enterotoxin using culture method:

We had screened about this enterotoxin by two methods according to (5, 22) as the following:-

A- In solid medium:- the bacterial isolates from standardized tubes had cultured on plates containing brain heart infusion Agar supplemented with phenol red indictor 0.2 gm per liter of medium at PH 5.4, then these cultured plates had incubated with 37^{o}C for 24, then the results were recorded.

B- In liquid medium:- this method was modified from previous solid medium method, 1ml of bacterial isolates from standardized tubes which positive in previous method had cultured on tubes containing 5ml of brain heart infusion broth (Himedia) Supplemented with

phenol red indicator 0.2gm per liter of medium at PH 5.4, then these cultured tubes were incubated in shaker with 150 rpm/ min. at 37^{o}C for 22 hr., after the incubation period these tubes had centrifugated with 3000 rpm/ min. for 5 minutes , then we taken 1ml of supernatant and put in new sterile test tube, then the optical density for each sample had measured at wave length 650 nm by using spectrophotometer (12) and the equipment had initialized by the blank solution which contains the brain heart infusion broth with phenol red at PH 5.4, the results had been recording.

Screening on Enterotoxin A using ELISA method:

The bacterial isolates under study were prepared by culturing them in tubes with 5ml of tryptic soy broth medium (salucea) and incubated with shaker 150 rpm/ min. at 37^{o}C for 22 hr (this period of incubation was detected in previous experiment), after ending the incubation time , the tubes centrifugated and the supernatants put in another sterile clean tubes for using them in screening about enterotoxin type A by (Staphylococcal enterotoxin A ELISA kit-My biosource) according to the instructions of the company supplied it and the optical density for for microplate was measured at wave length 450 nm, then results were recorded, as well as, the standard curve of enterotoxin type A was prepared by using standard solution of enterotoxin A supplemented with the kit to calculate the concentrations of enterotoxin A in samples.

Statistical analysis

The statistical analysis of the results was performed using chi- square test, SAS program and LSD at P≤ 0.05 and P≤ 0.01 (26).

RESULTS AND DISCUSSION

In this research, the culture method was used to investigate the ability of (82) isolates belongs to *S.aureus* isolated from different sources, to produce enterotoxins in general, as it is a non-specialized method, the results showed the ability of (58) isolates with percentage (70.73%) to produce the enterotoxins by this method whether in solid or liquid medium ,(Table 1). Where the ability of bacteria to produce toxin is detected through the change in color of test medium from yellow to red as a result of rising the PH of medium and transforming from acidic (yellow) to alkaline (red) . The results of other researchers did not agree with the percentage of toxin-producing isolates in our study by culture method (22) .This may be due to the differentiations in isolates and isolation sources and the different geographical areas for studies.

Table 1.Enterotoxin producing isolates by culture methods

Isolates	No.	%
Enterotoxin producing	**58**	**70.73**
Non Enterotoxin producing	**24**	**29.27**
Total	**82**	**100**

As for concering of detection of enterotoxins by liquid culture method, the results conducted that the measurments of optical density at wave length 650 nm, there is a significant differences among isolates in amount of producing enterotoxin at propability level(P ≤ 0.05), although this method nonspecific but it gives clear indicator for amount of producing enterotoxin , so when increasing of produced enterotoxin, the density of red color had increased and the optical density of solution also increased at this wave length (27,28) as shows in results at (Table 2) optical density ranged between (0.134 - 2.1) Which reflects the different amounts of enterotoxin produced from different isolates.

Table 2. Results of optical densities (at 650 nm) for liquid culture method as indicator of enterotoxin production .

N0.of isolates	Optical density (650 nm)	N0.of isolates	Optical density (650 nm)	N0.of isolates	Optical density (650 nm)
1	0.68	28	0.66	58	1.3
3	0.42	29	1.632	60	0.92
4	0.78	31	0.74	62	0.88
5	0.67	33	1.75	63	1.12
6	0.44	35	0.63	64	1.01
8	1.33	36	0.67	65	0.49
11	0.68	38	0.54	66	2.1
12	0.77	40	0.61	68	1.305
13	0.89	41	0.56	69	1.52
15	0.77	42	0.91	70	0.94
16	0.73	43	1.6	71	0.725
17	0.96	44	0.8	72	0.781
18	0.77	45	0.75	73	0.642
19	1.1	46	1.34	75	0.843
20	0.59	47	0.134	77	1.59
22	1.55	48	0.71	80	2.01
23	0.83	52	0.59	81	0.835
24	0.92	53	0.91	82	0.75
26	0.84	55	0.58	LSD	0.3661 *
27	0.26	56	0.93	* (P≤0.05)	

***means there were significant differences**

Results of screening the enterotoxin type A by immunological specific method using ELISA kit had confirmed that (47) isolates with percentage (57.32%) producing enterotoxin type A with concentrations was ranged from (3.74 - 15.7) ng/ml, (Table 3) . These results confirm the results observed in the liquid culture method in terms of the presence of variation in the quantities of enterotoxin produced by different isolates, also our results had nearly committed with (4) where the percentage of enterotoxin A production was (51.6%), as well as our results was not identical for the result of (3,5) where the percentage of enterotoxin A producing isolates were (25.5%) and (20.8%) respectively, as we explained earlier these differences may belongs to the differentiations in isolates and isolation sources and the different geographical areas for studies, as well as the type of kit used in the method technique in addition to that , our results indicated that the prevalence of enterotoxin type A among most the isolates under study, and it was distributed to all isolation sources without exception and in different percentages ranged between (43.9% to 92.3%), and enterotoxin type A producing isolates formed (57.3%) of all studied isolates, (Table 4), also formed (81%) of all enterotoxin producing studied isolates. The highest rate of enterotoxin type A production was in food isolates (92.3%) followed by Normal source isolates(80%) and soil, tape water, pathogenic isolates with(66.7%),(55%) and (43.9%) respectively as showed in (Table 5). The high percentages of enterotoxin-producing *S. aureus* isolates from various isolation sources, whether environmental or pathogenic, and even normal flora reflects their seriousness and health importance, especially as it is a major cause of bacterial food poisoning in which enterotoxins play a essential role, especially type A (1),in addition the prevalence of enterotoxin type A among the studied isolates which is a rate that warrants attention to it especially with regard to currency, workers in restaurants, cafes, bakeries, and ovens who present the food and drink services to consumers (2) . We conclude from our results that enterotoxin-producing especially type A *S. aureus* isolates, are exist in most environments around us, which calls for caution about the danger that these bacteria pose to our public health.

Table 3. Results of detection enterotoxin A in studied isolates by ELISA method .

No. of isolates	O.D at 450 nm	Enterotoxin A con. ng/ml	No. of isolates	O.D at 450 nm	Enterotoxin A con. ng/ml	No. of isolates	O.D at 450 nm	Enterotoxin A con. ng/ml
1	0.827	6.1	26	1.030	7.5	56	1.145	8.3
3	0.514	3.74	29	2.159	15.7	58	1.655	11.64
4	0.953	6.93	31	0.911	6.63	60	1.126	8.19
5	0.820	5.96	33	2.145	15.6	62	1.085	7.89
6	0.535	3.9	35	0.775	5.64	63	1.369	9.95
8	1.633	11.9	36	0.817	5.94	64	1.235	8.98
11	0.834	6.1	38	0.661	4.81	66	1.217	8.9
12	0.939	6.83	40	0.743	4.4	68	1.600	11.64
13	1.084	7.9	41	0.685	4.98	69	1.862	13.54
15	0.944	6.9	43	1.935	14.1	70	1.156	8.41
18	0.945	6.87	45	0.916	6.7	71	0.710	5.2
19	1.291	9.39	46	1.648	11.98	72	0.881	6.41
20	0.720	5.24	48	0.857	6.23	73	0.671	4.88
22	1.898	13.8	52	0.718	5.22	75	0.943	6.9
23	1.021	7.43	53	1.114	8.1	77	1.829	13.3
24	1.131	8.23	55	0.717	5.21	LSD	2.073 *	* (P≤0.0)

***means there were significant differences**

Table 4. Enterotoxin producing isolates by ELISA methods

isolates	No.	%
Enterotoxin A producing (from all sources)	47	57.32
Non Enterotoxin A producing (from all sources)	35	42.68
total	82	100

Table 5. Percentage of *S.aureus* producing enterotoxin type A according to their isolation sources

Isolation source	No. of isolates from each source	No. of isolates producing enterotoxin type A	%
Pathogenic	41	18	43.9
Soil	3	2	66.7
Food	13	12	92.3
Tape water	20	11	55
Normal source (nasal swabs)	5	4	80

REFERENCES

1. Abril, A. G.; T. G., Villa; J. B. Velazquez; B., Canas, A. S., Perez; P. C. Mata and M. Carrera .2020. *Staphylococcus aureus* exotoxins and their detection in the dairy industry and mastitis .Toxin. 12:537- 554..

2. Balaban, N. and A. Rassoly . 2000. Staphylococcal enterotoxins. Int. J. Food. Microbiol.61(1-10).

3. Boynukara, P.B.; T. Gulhan; M.A.Sarli; K. Gurturk, and H ., Solmaz. 2008. Classical enterotoxigenic characteristics of *S.aureus* strain isolated from bovine subclinical Mastitis in Van, Turkey .Int.J.Food. Microbiol. 125(2):209-211

4. Carfora, V.; A.Caprioli,; N.Marri; D.Sagrafoli ; C., Boselli and G., Giacinti. 2015. Enterotoxin, genes, enterotoxin production and methicillin resistance in *S.aureus* isolated from milk and dairy products in central Italy. Int. Dairy J. 42:12-15

5. Cencigoga, B.T.; M. Karama; P.V., Rossitto ; R.A., Morgante and J.S., Cullor. 2003. Eenterotoxin production by *S.aureus* Isolated from mastitis cow. J.Food. Prot. 66(9): 1693-1696.

6. Cheng, M. H.; S., Zhang; R. A, Porritt; M. V., Rivas; L. Paschold; E. Willescher,; M., Binder; M. Arditi, and I., Bahar. 2020. Superantiginic character of an insert unique to SARS-COV-2 spike supported by skewed TCR repertoire in patients with hyperinflamation .PNAS.117(41):25254-25262

7. Clinical and Laboratory Standards Institute. 2010. Methods for antimicrobial dilution and disk susceptibility testing of infrequeutly isolatated or fastidious bacteria ; Approved guideline, 2nd. ed. M45-A2. CLSI, Wayne, PA.pp:78-82

8. Cowan, M. K. and K. P., Talaro. 2006. Micorbiology A System Approach, 1st. ed. McGraw Hill Company. pp : 21.

9. Daracova, M.; J., Klimentova; A.Fucikova and J., Dresler. 2018 . Proteomic methods of detection and quantification of protein toxin .Toxin. 10:99-128

10. Doyle, M. E.; F. A., Hartmann and A. C., Lee Wong. 2012. Methicillin- resistant *Staphylococci*: Implications for our food

supply . Anim. Health Res. Rev.,13(2):157-180

11. Fisher,E.L.; M., Otto. and Y.C., Cheung. 2018. Basis of virulence in enterotoxin mediated staphylococcal food poisoning. Front. Microbiol. 9:436(1-18).

12. General Organization for Technical Education and Vocational Training .2009. Spectroscopy Methods. KSA . pp:16.

13. Hait, T.; S .,Talent; D.,Melka; C., Keys and R. , Bennett. 2014. Prevalence of enterotoxins and toxin gene profile of *S. aureus* isolates recovers from a bakery involved in a second staphylococcal food poisoning occurrence. J.Appl. Mccrobiol.117:866-875.

14. Hassan, K.I and S.K. ,Hamasaleh .2016. A rapid methods for PCR based on detection of *Salmonella spp*. and *S. aureus* in Spiked and naturally contaminated food . Iraqi J. Agric . Sci. 47(7): 66-73

15. Hen, M. M. S.; W. S. J., Boardman; I., Smith; A. E., Goodman and M. H.,Brown. 2014. Nasal colonization of *Staphylococcus spp*. among captive and free ranging Wallabies in South Australia . J. Vet. Sci. Med. Diag.. 3(2):136- 144.

16. Ibrahim, R. N.; M. S., Al- Salmani and T.H. Zedan. 2019. Study the antibacterial activity of aqueous extraction of onion (*Allium cepalae L.*) against *Staphylococuus aureus* isolated from otitis media. Iraqi J. Agric. Sci. 50(4): 1186-1192.

17. Jassim, S. A. and N. J. Kandala. 2021. Molecular detection of enterotoxin genes of multi resistant *Staphylococcus aureus* isolated from different source of food. Iraqi Journal of Science .62(1): 61-74

18. Johler, S.; P., Giannini; M., Jemini ; J., Hummerjohann ; A. , Baumgartner and R. Stephan. 2015. Further evidence for Staphylococcal food poisoning out breaks Caused by egc- encoded enterotoxins. Toxins, 7:997-1004

19. Joo, H. S.; S. S., Chatterjee; A. E., Villaruz; S. W., Dickey; V. Y., Tan; Y., Chen; D. E., Sturdevant ; S. M. ,Ricklefs and M., Otto. 2016. Mechanism of gene regulation by a *Staphylococcus aureus* toxin .Ameri. Socie. Microbiol.J.7(5):16-25

20. Jusko, M.; T., Potempa; T., Kantyka; E., Bielecka; H.K., Miller; M., Kalinska; G., Dubin; P., Garred; L.N. ,Shaw and A.M. , Blom, 2014. Staphylococcal proteases aid in evasion of the human complement system. J. Innate Immun., 6(1):31-46.

21. Liu, J.; L.W., Bai ; H., Han; P., Fu; X., Ma; Z., Bi; X.,Yong ; X. , Zhang and S.,Zhen . 2018. Trends of food borne disease in China: Lessons from laboratory based surveillance since 2011. Front. Med.12:48-57

22. Mohammed, S.A.; M.I.J., Al- Ani; D.L., Mohammed ; L.R. , Salar and B.M., Rasul. 2019. Inhibition of *S. aureus* enterotoxin genes by using plant extracts. Innovaciencia.6(2): 1-8.

23. Nadhom, B.N. 2018 . Study of molecular composition of Virulence bacteria isolated from bovine mastitis with biofilm production. Iraqi J. Agric . Sci. 49(5): 840-846.

24. Norri, H.A and G.M., Aziz. 2020. Purification, Characterization, and evaluation of fibrinolytic activity of staphylokinase from locally isolated *Staphylococcus aureus* GH38. Iraq.J.Agric.Sci. 51(4):1195-1203

25. Omoe, K.; D.L., Hu; H.K., One ; S., Shimizu; O.H.,Takahashi ; A.,Nakane ; T., Uchiyama ; K. , Shinagawa, and K., Imanishi. 2013. Emetic potentials of newly staphylococcal enterotoxin like toxins. Infect. Immun. 81:3627-3631

26. SAS.2012.Sraristical Analysis System, User's Guide.Statistical. version. 9.1th ed.SAS. Inst.Inc.Cary.N.C.USA.pp:23-25.

27. Starr, C.; C.A. , Evers and L ., Starr. 2006. Biology Concepts and Applications. Thomson, Books 1 cole, Brojstranica.pp :54

28. Viennot, L. and C.D. , Hosson. 2012. Colour Phenomena and partial Absorption. MUSE group .European Physical Society. pp :6

29. Zhao, Y.; A., Zhu; C.,Tang ; J., Chen and J., Liu. 2016. Identification and measurement of staphylococcal enterotoxin like protein I(SE/I) secretion from *Staphylococcus aureus* clinical isolates. J. Appl.Microbiol.121:539-546.