The role of staphylococcus aureus enterotoxin B (seb) in the exacerbation of multiple sclerosis disease

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Abstract

Staphylococcus aureus is a gram- positive bacteria that causes a number of clinical conditions. The toxic shock syndrome toxin (TSST) and the Staphylococcus aureus enterotoxins (A, B, C, D, and E) were the first superantigens to be broadly described as T cell activators, and it is these same enterotoxins (A, B, and C) that have been implicated in the severity or development of multiple sclerosis. This damage disrupts the ability of parts of the nervous system to transmit signals, resulting in a variety of signs and symptoms, in order to investigate the percentage of *Staphylococcus aureus* enterotoxin gene B (SEB) in MS patients compared with healthy participants to determine the role of Staphylococcus *aureus* in the exacerbation of multiple sclerosis disease. This study measures the occurrence of *Staphylococcus aureus* enterotoxin B in multiple sclerosis patients correlated with exacerbation of this disease 200 nasal swab samples categorized into multiple sclerosis group &control group in order to investigate the occurrence of staphylococcal and its enterotoxin B , all samples were cultured by ordinary laboratory media with antibiotic sensitivity , DNA extraction and PCR with gel electrophoresis to viewing the PCR products of enterotoxin genes (A,B&C). The age of patients and controls were ranged between (17- 59) year. The frequency of *Staphylococcus aureus* isolates in MS patients was (81%) including 38.2% from newly diagnosed multiple sclerosis and 61.8% from relapsing remitting multiple sclerosis patients. The frequency of *Staphylococcus aureus* isolates in multiple sclerosis patients was (52%) female and (29%) male, while the frequency of *Staphylococcus aureus* nasal colonization in healthy carriers controls was only (7%) female and (5%) male , the mean rate of antibiotic resistance to all antibiotics in *Staphylococcus aureus* isolates of those patients was (53.3%) , while in control groups all isolates were resistant to 6 antibiotics only with the mean percentage of antibiotic resistance was only (22.8%) as well as high rate of sensitivity to most of other antibiotics. High frequency of *S. aureus* isolates in MS patients occur at age group (20-29 year) while in control group occur at (30-39 year), Multiple sclerosis disease and frequency of *S. aureus* nasal colonization was most common in female than in male, there was a highly

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significant correlation between antibiotic resistance of MS patients and presence of enterotoxin genes *sea, seb and sec.*

***Keywords**: Multiple sclerosis(MS); Multi-drug resistant (MDR); Staphylococcus aureus. enterotoxins A, B, C; enterotoxin genes sea, seb & sec.*

دور السموم المعوية نوع ب لبكتريا المكورات العنقودية الذهبية في تفاقم مرض تصلب الأعصاب المتعدد

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الخلاصة

تعتبر بكتريا المكورات العنقودية الذهبية الموجبة لصبغة كرام المسبب الرئيسي لكثير من الحالات المرضية السريرية , حيث ان سم متلازمة الصدمة السمية والسموم المعوية نوع (A,B,C,D&E) من المستضدات الخارقة الاولى التي حفزت خلايا اللمفاوية نوع T-cellبصورة واسعة , وهي أيضا من نفس المستضدات التي تساهم في تطوير ومفاقمة مرض التصلب المناعي المتعدد وحسب الدراسات التي اجريت. للتحري عن نسبة تواجد جينات السموم المعوية نوع B الخاصة ببكتريا المكورات العنقودية الذهبية مقارنة مع نسبة تواجدها في الناس الأصحاء اللذين لا يعانون من مرض التصلب العصبي المتعدد كذلك لقياس تكرار تواجد هذه السموم ولمعرفة مدى خطورة ومفاقمة المرض وعلاقة افراز السموم البكتيريه بها.أجريت هذه الدراسة على 200 عينة من مسحات الأنف شملت 100 عينة من مرضى التصلب العصبي المتعدد و 100 عينة من متطوعين أصحاء تم جمعهم من مستشفى بغداد التعليمي / عيادة تصلب الاعصاب في مدينة الطب 2019 وذلك لمعرفة مدى تكرار حدوث المكورات العنقودية الذهبية المحمولة بالأنف لمرضى التصلب المتعدد وكذلك للتحقق من تواجد جينات السموم المعوية بالمقارنة مع الاشخاص الاصحاء وذلك من اجل تحديد دور المكورات العنقودية الذهبية في زيادة خطورة وتدهور مرض التصلب المتعدد. جميع العينات تمت زراعتها على اوساط تشخيصية مختلفة واجريت للبكتريا المعزولة فحص الحساسية لعدة انواع من المضادات الحيوية وكذلك استخلاص وتنقية DNA وفحص ال PCRوعملية الترحيل الكهربائي للجينات الخاصة بالسموم البكتيرية .كانت نسبة تكرار حدوث عزلات المكورات العنقودية الذهبية في مرضى تصلب الأعصاب (81 ٪) بما في ذلك 38.2 ٪ من المرضى المشخصين حديثاً و 61,8 ٪ من مرضى التصلب المتعدد الانتكاسي.

كان مجموع عزلات المكورات العنقودية الذهبية في مرضى التصلب المتعدد (52 ٪) في الإناث و (29 ٪) في الذكور، في حين أن نسبة تكرار حدوث المكورات العنقودية الذهبية في الاشخاص الاصحاء كان 12 ٪ فقط بما في ذلك الإناث (7 ٪) والذكور (5 ٪). اعتمادا على التجارب الخاصة بتفاعل البلمرة المتسلسل التقليدي ، فقد أظهرت النتائج الحالية أن التضخيم لتسلسل بادئات جينات السموم المعوية نوع *sea, seb , sec* في الحمض النووي الجيني الكلي لعزلات المكورات العنقودية الذهبية قد ادى الى انتاج مجاميع بحجم 564 زوج قاعدي ،599 زوج قاعدي و 257 زوج قاعدي على التوالي . كان توزيع الجينات السمية المعوية في المكورات العنقودية نوع *sea* A في مرضى التصلب المتعدد بمقدار 69,1 ٪ يليه 49,4 ٪ من الجينات المعوية نوع *seb* B وكانت النسبة المئوية الأقل شيوعا هي 8,6 ٪ للجينات المعوية نوع *sec* C. من الجدير بالذكر هنا بانه لم يتم اكتشاف كل هذه السموم المعوية في مجموعة الاشخاص الاصحاء. كشفت هذه الدراسة أيضًا عن وجود علاقة مهمة للغاية بين نسبة تكرار حدوث السموم المعوية للمكورات العنقودية نوع *sea, seb , sec* من العزلات المحمولة بالانف لمرضى التصلب المتعدد وبين نسبة مقاومة المضادات الحيوية المتمثلة بزيادة المقاومة لمعظم المضادات الحيوية لكل عزلة مصحوبة بوجود أكثر من سم معوي واحد. اثبتت الدراسة ان اكثر تكرار لعزلات المكورات العنقودية الذهبية في مرضى التصلب العصبي المتعدد كانت بين

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اعمار تتراوح من (20-29) سنة , بينما لدى الأصحاء كانت بين اعمار تتراوح بين (30-39) سنة , مرض تصلب الأعصاب المتعدد وتكرار تواجد المكورات العنقودية الذهبية المحمولة أنفيا في هذه الدراسة كانت اكثر شيوعا في النساء مقارنة بالرجال , وبالأضافة الى ذلك كانت هنالك علاقة وثيقة وذات اهمية احصائية بين نسبة مقاومة البكتريا العنقودية الذهبية لكثير من المضادات الحيوية لمرضى تصلب الأعصاب ونسبة تكرار الجينات السمية للبكتريا وخصوصا نوع B .

الكلمات المفتاحية *: تصلب الأعصاب المتعدد , مقاومة لأدوية متعددة , السموم المعوية لبكتريا المكورات العنقودية الذهبية نوع أ , ب, ج .*

Introduction

Multiple sclerosis (MS) is the most common demyelinating illness, characterized by the destruction of the insulating coverings of nerve cells in the brain and spinal cord. This damage affects the nervous system's ability to transmit messages, resulting in a wide range of signs and symptoms, including physical, mental, and psychiatric problems [1]. A high proportion of T cells can be activated by enterotoxigenic genes of the *Staphylococcus aureus* that have superantigenic property, owing to their capability of binding to both specific V-β regions, in the T cell receptor and molecules of the major histocompatibility complex (MHC) in antigen presenting cells, such activation leads to an increased production of pro-inflammatory cytokines and the polyclonal stimulation of T cells [2،3]. Superantigens are proteins produced commonly by some viruses and bacteria that potently activate autoreactive CD4+ T cells, inducing cytokine production and massive cell proliferation, of predominantly interferon (IFN)-γ and IL-2 [4]. The most substantial superantigens were toxic shock syndrome toxin and the *S. aureus* enterotoxins (A, B, C, D and E) that contribute to T cell activation [5]. Chronic or recurrent systemic exposure to a very small amount of superantigen in *S. aureus* carrier individuals can also lead to superantigen absorption. The absorption of the superantigen can cause the activation of the autoreactive B and T -lymphocytes in individuals that are genetically predisposed. The activation process of these autoreactive cells under proper conditions is able to participate in an autoimmune disease [4].

The relationship between bacterial superantigens and MS exacerbation has recently been studied in patients with multiple sclerosis (MS). As a result, nose colonization with enterotoxin A-producing *Staphylococcus aureus* could play a role in triggering MS exacerbations and activation of the autoreactive CD4+ T cell [5] Staphylococcal enterotoxins (SEs) are classified as superantigens because they can activate significant numbers of T lymphocytes (20–30%), resulting in the generation of a cytokine bolus and symptoms similar to S. aureus food poisoning, such as diarrhea and vomiting [6].The majority of the genes that code for Staphylococcal enterotoxins (SEs) are found on mobile genetic elements such bacteriophages, plasmids, and pathogenicity islands. As a result, horizontal transmission between strains is not uncommon [7].

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The role of S. aureus superantigens in MS etiology is currently unknown, but toxic shock syndrome toxin-1 (tsst-1), Staphylococcal enterotoxin A (sea), Staphylococcal enterotoxin B (seb), and Staphylococcal enterotoxin C (sec), all of which act as superantigens that contribute to the severity of MS patients and have been proven to be involved in [8]. Furthermore, a previous study found that *S. aureus* superantigens, when used at nanogram levels, were effective in eliciting T cell activation in a panel of 16 gamma delta T cell clones derived from MS patients and controls [9،10].

Materials and methods

Subjects

In this investigation, a total of 200 samples were enrolled. From the first of November 2021 to the end of January 2022, 100 nasal swab samples from multiple sclerosis patients. 50 relapsing remitting and 50 newly diagnosed) and 100 from apparently healthy persons were obtained from Baghdad Teaching Hospital / Multiple Sclerosis Clinic in Medical City. MS patients who were taking immunosuppressive medicines, antibiotics, or had viral or bacterial infections were excluded from the study.

1. Identification & investigation

All nasal swabs were cultured on blood agar, nutrient agar &broth, mannitol salt agar, brain heart infusion broth and agar, biochemical tests for catalase & coagulase. *Staphylococcus aureus* isolates tested to different antibiotics were determined by disk diffusion test (DDT) according to Clinical and Laboratory Standards Institutes (CLSI). Tris-Borate EDTA buffer was made from reagents with the highest purity and delivered in convenient ready to use for agarose gel electrophoresis according to the manufacturing company was relied.

2. Molecular Detection of enterotoxin genes of Staphylococcus aureus (sea, seb and sec)

Conventional PCR was done to detect (*sea, seb and sec*) genes of *Staphylococcus aureus* by amplification of *S. aureus* DNA strands according to Dağı HT *et al*.,2015 [11].

Determination of genomic DNA concentration and purity

The concentration and purity of DNA was quantified by use Nano-drop instrument according to the instruction of the manufacture. 3µl were aspirated by special tip (tips 10µl) and placed in socket in the machine, DNA was quantified by the refractive index using the wave length 260nm, 280nm. The purity was calculated with the OD_{260nm} /OD_{280nm} ratio while the concentration of DNA was

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calculated with the $OD_{260nm.}$ The ratio of ~1.8 was generally accepted as (pure) for DNA meaning a low degree of protein contamination.

Primers

Conventional PCR was used to detect the presence of staphylococcal enterotoxins (*sea, seb and sec)* genes in the DNA of *Staphylococcus aureus*. Primers sequences for (*sea, seb and sec)* genes were designed and synthesized in alpha DNA (Canada), as showed in tables (1,2, 3), the primers diluted by adding nuclease free water and prepared for PCR reaction according to manufacture instructions [12].

Table (1): Sequences and products of *sea* gene.

***sea* gene**	**Nucleotide sequences (5' ⟶ 3')**		**Products *bp***	**Reference**
sea	**F**	GGGAACAGCTTTAGGCAATC	**564**	*Pakbaz et al., 2017* [10]
	R	ATTTGAATACTGTCCTTGAGC		

Table (2): Sequences and products of *seb* gene.

***seb* gene**	**Nucleotide sequences (5' ⟶ 3')**		**Products *bp***	**Reference**
seb	**F**	CCAGATCCTAAACCAGATGAG	**599**	*Pakbaz et al., 2017* [10]
	R	TGCAGGCATCATGTCATACC		

Table (3): Sequences and products of *sec* gene.

***sec* gene**	**Nucleotide sequences (5' ⟶ 3')**		**Products *bp***	**Reference**
sec	**F**	GACATAAAAGCTAGGAATTT	**257**	*Mehrabi F et al., (2015)* [16]
	R	AAATCGGATTAACATTATCC		

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Accupower bioneer PCR premix kit

The accupower PCR premix contains DNA polymerase, dNTPs and reaction buffer in a premixed format that is freeze-dried into an individual packet. Patented chemical stabilizer maintains the activity of the premixture for over 2 years in the freezer (stored at -20°C). This kit was provided by bioneer (korea) corporation. In addition to non-template control(NTC) was used as a negative control which is prepared from all PCR master mix but without DNA template in order to ensure no contamination.

Conventional PCR screening (*sea, seb and sec*) genes

Conventional PCR was done to detect (*sea, seb and sec*) genes of *Staphylococcus aureus* by amplification of *S. aureus* DNA strands according to Dağı HT *et al*.,2015 [14]. To amplify these genes of *Staphylococcus aureus* isolates, DNA template was diluted to optimal concentrations. The mixture of conventional PCR working solution was prepared as described in table (4) and PCR master mix components according to the manufacturing company described in table (5).

Table (4): The mixture of conventional PCR working solution for detection of (*sea, seb and sec*) genes in *Staphylococcus aureus.*

Component	Concentration	Volume µl
Master mix	1X	5µl
Primer F.	20 picomole	2.0 µl
Primer R.	20 picomole	2.0 µl
DNA template	5 – 50 ng	5 µl
Deionized D.W.	-	6 µl
Total volume		20 µl

Table (5): PCR master mix components according to the manufacturing company.

Component	20 µl reaction
Each: dNTP (dATP, dCTP, dGTP, dTTP)	250 µM
KCl	30 mM
MgCl2	1.5 mM
Stabilizer and tracking dye	0
Taq DNA polymerase	1 U
Tris-HCl (pH 9.0)	10 mM

Statistical analysis

The data of this study were analyzed by using SPSS version (22.0) in order to analyze and assess the results of the study to determine the correlation between biofilm formation, antibiotic

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resistance and progression of multiple sclerosis course. P-value of less than 0.05 was considered statistically significant.

Results

Molecular results (DNA concentration)

After bacterial DNA extraction was made, a nano drop apparatus was used in the measurement of DNA concentration and purity of *S. aureus* isolates from collected samples. The final concentration of *S. aureus* DNA samples was in range between (19.2-195.5) and the DNA purity was ranged between (1.75-2.0) at OD 260/280.

Conventional PCR screening for *S. aureus* enterotoxin genes (A, B and C)

Conventional PCR technique was used in the amplification of specific sequences of the (*sea, seb and sec)* enterotoxin genes in the whole genomic DNA of *S. aureus* isolates. The enterotoxin genes amplicons were produced in size (564bp, 599bp and 257 bp) of (*sea, seb and sec*) respectively, as shown in figure (1), (2) and (3)ز

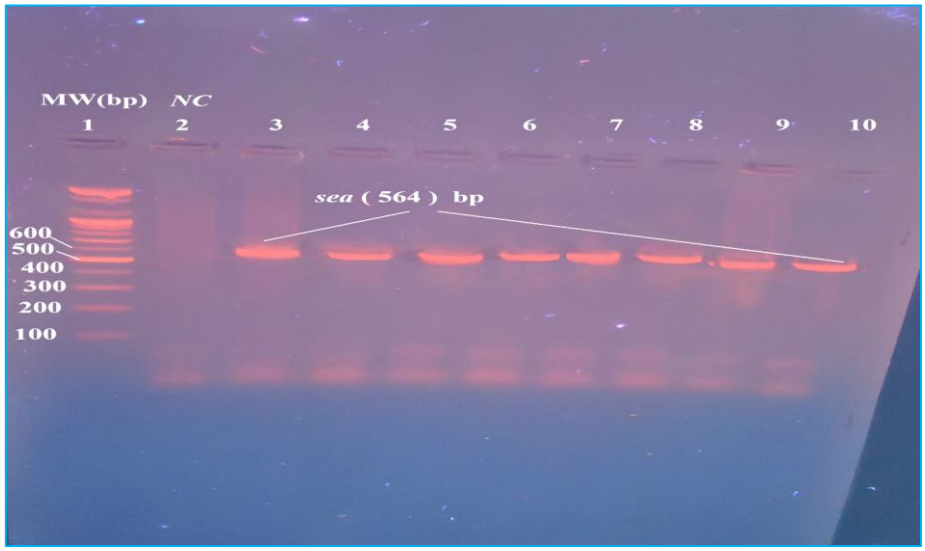

Fig. (1): Gene amplified PCR product (564 bp) gel electrophoresis. 100 bp ladder in lane one. Lane 2 is a negative control, while Lanes 3–10 are clinical isolates that have a positive result. (7 v/cm2, 1 hr, 1 percent agarose).

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Fig. (2): Gel electrophoresis of the seb gene amplified PCR product (599bp). 100 bp ladder in lane one. Clinical isolates in Lanes 2–9 reveal a favorable outcome.

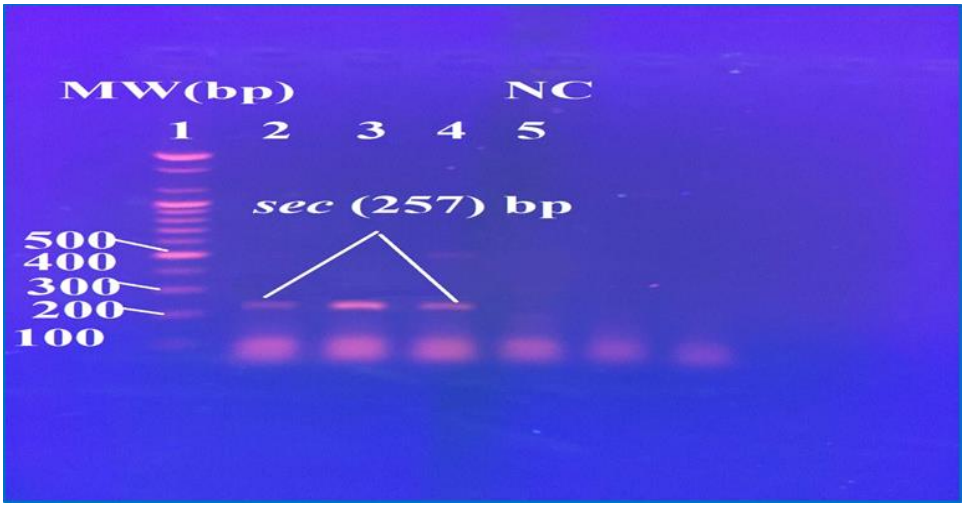

Fig. (3): Gel electrophoresis of the sec gene amplified PCR product (257 bp). 100 bp ladder in lane one. Clinical isolates in Lanes 2–4 reveal a favorable outcome. Lane 5 is a negative control (1% agarose, 7 v/cm2, 1 hour).

The total PCR results in the current study found that the frequency of all enterotoxins (*sea, seb and sec*) in MS patients were (42.4%) including (40.8%) were from newly diagnosed MS patients and (43.3%) were from relapsing remitting MS patients compared with no any enterotoxin gene was detected in control group indicating a highly significant differences (p<0.01) in the distribution of enterotoxins between study groups. The results also showed that *sea* gene was the most predominant one in all MS patients and the frequency of it was (69.1%) including (64.5%) in newly diagnosed MS and (72%) in relapsing remitting MS with highly significant differences (p<0.01) in the frequency compared with control group, table (6).

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Table (6): Distribution of *S. aureus* enterotoxin genes.

Study groups	*S.aureus* positive	Gene distribution				*P*-value
		sea	*seb*	*Sec*	Total	
	No.(%)	No.(%)	No.(%)	No.(%)	No.(%)	
Control	12(12)	0(0)	0(0)	0(0)	0(0)	
Newly MS	31(38.2)	20(64.5)	15(48.4)	3(9.7)	38(40.8)	
R.R. MS	50(61.8)	36(72)	25(50)	4(8)	65(43.3)	
Total	93	56(69.1)	40(49.4)	7(8.6)	103(42.4)	*P*=0.0000 (HS)
***P*-value**		*P*=0.0 (HS)	*P*=0.0 (HS)	*P*=0.138 (NS)	*P*=0.00002(HS)	

(*) HS: Highly Sig. at P<0.01; S: Sig. at *P*<0.05; Testing based on Contingency Coefficient test

Distribution of genes according to patient's gender

This study revealed that the percentage rate of *sea* and *seb genes* was higher in female than male in MS patients, in contrast to sec *genes* which is higher in male than in female of MS patients. Statistically there is no significant association between the presence of *sea and seb genes* in regard to gender while there is a highly significant correlation (*P*= 0.0) between *sec genes* and gender of MS patients, table (7).

Table (7): Distribution of enterotoxin genes with gender in all MS stages.

MS group	New Diagnosed MS Total (M=16)(F=34)			R.R. MS Total (M=20)(F=30)			*P*-value
Gender	**Male**	**Female**	**Total**	**Male**	**Female**	**Total**	
Genes	No.(%)	No.(%)	No.(%)	No.(%)	No.(%)	No.(%)	
SEA	6(30)	14(70)	20(100)	11(30.5)	25(69.5)	36(100)	*P*= 0.93 (NS
SEB	4(26.6)	11(73.4)	15(100)	8(32)	17(68)	25(100)	*P*=0.40 (NS)
SEC	2(66.6)	1(33.4)	3(100)	1(25)	3(75)	4(100)	*P*= 0.0 (HS)
Total	12(41)	26(59)	38(100)	20(29)	45(71)	65(100)	*P*= 0.07 (NS)
Total genes detected			38+65= 103 genes				

(*) HS: Highly Sig. at P<0.01; S: Sig. at *P*<0.05; Testing based on Contingency Coefficient test.

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Antibiotic resistance and presence of enterotoxins

In this study the results showed that there is a highly significant correlation (p = 0.00) between rate of antibiotic resistance and presence of enterotoxin genes (A, B and C) in both MS stage groups compared with healthy control group, table (8).

Table (8): Correlation between enterotoxin genes and antibiotic resistance**.**

Antibiotics		Enterotoxin genes						*P* value
		Newly Dx MS		R.R. MS		control		
		+ve	-ve	+ve	-ve	+ve	-ve	
ME	R	26	5	44	6	0	12	*P*= 0.00(HS)
	S	0	0	0	0	0	0	
VA	R	3	0	5	0	0	0	*P*= 0.0001(HS)
	S	23	5	20	6	0	12	
AZM	R	16	2	39	4	0	0	*P*= 0.00(HS)
	S	10	3	5	2	0	12	
E	R	38	3	41	4	0	8	*P*= 0.00(HS)
	S	7	2	3	2	0	4	
TE	R	16	2	38	3	0	4	*P*= 0.00(HS)
	S	29	3	6	3	0	8	
AK	R	16	1	32	1	0	0	*P*= 0.00(HS)
	S	29	4	12	5	0	12	
RA	R	7	2	18	2	0	0	*P*= 0.00(HS)
	S	19	3	26	4	0	12	
P	R	20	1	43	4	0	12	*P*= 0.00(HS)
	S	6	4	1	2	0	0	
MEM	R	1	0	3	0	0	0	*P*= 0.00(HS)
	S	25	5	41	6	0	12	
SXT	R	18	2	40	5	0	0	*P*= 0.00(HS)
	S	8	3	4	1	0	12	
DOX	R	8	0	25	1	0	0	*P*= 0.00(HS)
	S	18	5	19	5	0	12	
CIP	R	7	1	18	1	0	0	*P*= 0.00(HS)
	S	19	4	26	5	0	12	
DA	R	9	0	23	1	0	0	*P*= 0.00(HS)
	S	17	5	21	5	0	12	
IPM	R	1	0	2	0	0	0	*P*= 0.00(HS)
	S	25	5	42	6	0	12	
CTX	R	9	1	22	2	0	0	*P*= 0.00(HS)
	S	17	4	22	4	0	12	
CN	R	15	3	26	2	0	0	*P*= 0.00(HS)
	S	11	2	18	4	0	12	
CRO	R	23	4	43	6	0	8	*P*= 0.00(HS)
	S	3	1	1	0	0	4	
	R	20	3	43	5	0	8	*P*= 0.00(HS)

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AM	S	6	2	1	1	0	4	
	R	7	2	23	1	0	0	*P*= 0.00(HS)
C	S	19	3	21	5	0	12	

(*) **HS: Highly Sig. at P<0.01; S: Sig. at *P*<0.05; Testing based on Contingency Coefficient test.**

***Staphylococcus aureus* colonization, antibiotics resistance & enterotoxin genes**

In the present study, there is a highly significant association (P= 0.0) between S. *aureus* colonization, mean antibiotic resistance and the presence of staphylococcal enterotoxins (A, B and C) in MS groups compared with controls while there is no significant correlation (*p*=0.454) between two stages of MS disease, table (9).

Table (9): Correlation between *S. aureus* colonization, presence of enterotoxin genes and antibiotic resistance.

Different Tests	**Control non MS**	**Multiple sclerosis groups**		
		New MS	**R.R. MS**	**Total**
	No.(%)	No.(%)	No.(%)	No.(%)
***S.aureus* +ve**	12(12)	31(38.2)	50(61.8)	81(100)
Mean antibiotic[R] resistance No.= 19 types	(22.8)	(46.3)	(60.6)	(53.3)
Sea +ve	0	20(64.5)	36(72)	56(68.2)
Seb +ve	0	15(48.4)	25(50)	40(49.2)
Sec +ve	0	3(9.7)	4(8)	7(8.8)
***P*-value**		P= 0.454 (NS)		-
	P= 0.000 (HS)			

(*) HS: Highly Sig. at P<0.01; S: Sig. at *P*<0.05; Testing based on Contingency Coefficient test.

Discussion

Multiple sclerosis patients considered immunocompromised due to they have impaired T-cell function and disregulated immunity , in the present study all relapsing remitting patients 50(61.8%) received immune modulatory drugs as a course directed by clinician for several months such as betaferon , avonex and re-bif, whereas any patients obtained immunosuppressive drugs like gilenya and tysabri were excluded , in order to prevent unexpected growth of *S.aureus* bacterial colonization in nasal passages of those patients either by hand contamination with other carriers or via air born

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transmission. In this study three enterotoxigenic genes having superantigenic activity were identified in *S. aureus* isolates of MS patients (*sea, seb and sec*) whereas none of any one was detected in controls. The result of this study revealed that the frequency of *S. aureus* isolates in nasal carriage of MS patients was (81%) harboring a total of (42.4%) enterotoxin genes of which (40.8%) in newly diagnosed MS patients and (43.3%) in relapsing remitting MS patients, while there is no any enterotoxin gene was demonstrated in healthy nasal carriers control.

This study coincides with Libbey *et al.,* 2015 (13), who reported the total percentage of superantigen genes was (46.8%) in MS patients including (35.3%) in MS stable group and (58.3%) in MS exacerbated group. Concerning healthy nasal carriers in current study no any enterotoxin genes was detected in *S.aureus* isolates of control group that disagreed with study of of Dağı *et al*. 2015 (14) who reported that the frequency of *S.aureus* colonization in healthy nasal carriers was (17.3%) harboring (13.5%) *sea* , (8.7%) *seb* , (52.9%) *sec* and other enterotoxin genes the result of turkey study about low frequency of *S.aureus* isolates in healthy nasal carriers and high frequency of enterotoxin genes indicate that *S.aureus* in nasal carriers have many virulence factors that play a role in the establishment and pathogenesis of infections like food poisoning , skin infections , septic shock but not responsible for activation of CD4+ auto reactive T-cells and induction of autoimmunity , whereas the explanation of the current study results in Iraqi participants as healthy carriers regarding the absence of these genes completely from all *S.aureus* isolates in healthy nasal carriers ,also the *S.aureus* in those volunteers was non (MDR) and weak biofilm producers , all these results indicate that apparently healthy individuals were carrying nonpathogenic isolates of *S.aureus* in nasal passages that most reported as a normal commensal flora protect against infections by pathogens according to this study explanations , in contrast , *S.aureus* isolates in MS patients of the current study were have many virulence factors like enterotoxin genes , more MDR and strong biofilm producers particularly in a stage of relapsing remitting or exacerbated MS that responsible for causing increase sequel and severity of this autoimmune disease.

The result of the present study also showed that the most common enterotoxin distributed in *S.aureus* isolates of MS patients was *sea* (69.1%) participate in (64.5%) of newly diagnosed MS and (72%) in relapsing remitting stage , the second most common distributed gene was *seb* about (49.4%) including (48.4%) in newly diagnosed group and (50%) in relapsing remitting group while this study revealed that the least common frequent enterotoxin among *S.aureus* isolates of MS patients was *sec* (8.6%) included (9.7%) in newly diagnosed stage and (8%) in relapsing remitting stage , this result coincides with Sadeghi *et al*., 2019 [15], who reported that the frequency of *sea* was (40%) , *seb*

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(27%) and *sec* (15%) in MS patients , whereas this result in disagreement with Pakbaz *et al.*, [10] , who reported that the most common enterotoxin distributed in MS patients was *seb* (28.6%) then *sea* (25.4%) and *sec* (9.5%) , in addition to these results the current study determined that the frequency of enterotoxins (*sea , seb and sec*) was more distributed in relapsing remitting MS patients than newly diagnosed stage that agreed with Mehrabi *et al* ., 2015 [16], who reported that the frequency of *sea* in relapsing remitting stage was (41.7%) while in MS stable group was (29.4%) and the frequency of *seb* in relapsing remitting stage was (25%) while in MS stable group was (5.9%). The explanation of the current study results regarding distribution of enterotoxin genes in *S.aureus* isolates of MS patients indicate the important role and significant correlation of increase frequency of these enterotoxins in relapsing remitting stage of MS more than newly diagnosed MS group particularly (*sea and seb*) which is the reason for increase virulence of these bacteria and finely superantigenic property of these enterotoxins that induce activation of autoreactive CD4+ T-cells considered as a very essential factor in determining relapse and severity of MS disease and participate in the autoimmune diseases severity .

The reason for more frequent enterotoxin genes in females than in males especially (*sea and seb*) due to the occurrence of MS in this study was higher in female than in male in a ratio (1:1.7) that corresponding the incidence rate of MS in the world, while the frequency of *sec* was more in males than in females naturally due to very low frequency of this gene was detected in MS patients of the present study. In addition to these results the current study showed a highly significant correlation between *S. aureus* nasal colonization , mean antibiotic resistance of these isolates against several types of antibiotics with distribution of enterotoxin genes (*sea , seb and sec*) of *S.aureus* in MS patients stages compared with healthy control carriers explain that the enterotoxin genes of *S.aureus* isolates in MS patients participate in increasing antibiotic resistance also may be due to high frequency of these genes mainly in relapsing remitting MS stage contribute in increasing bacterial virulence and multiple sclerosis pathogenesis.

Finally the current study approved that there is a highly significant correlation between the presence of enterotoxins (*sea ,seb and sec*) and increase percentage of antibiotic resistance in MS patients particularly in those with exacerbation or relapsing remitting group than newly diagnosed MS group depending on the fact that high frequency of enterotoxin genes mainly (*sea and seb*) correlated with high percentage of resistance to different types of antibiotics that more frequent in relapsing remitting group, play a primary role in the development of MS exacerbation and other consequences .The current study is in accordance with study was conducted in Egypt by Mohammed *et al*., 2016 [17], who reported that there is a highly significant correlation between elevated *sea*

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(66%) expression and multidrug resistance , while current study not identical with the result of other study performed by Tokajian *et al*., 2011 [18] , who reported that out of 130 *S.aureus* isolates including 93 MRSA and 37 MSSA harboring more than one enterotoxin tested for 18 types of antibiotics, only one was multidrug resistance (MDR) with highest resistance to oxacillin (32%) , tetracycline (42%) and other antibiotics was intermediate in resistance.

Conclusions

1- There was a highly significant correlation between antibiotic resistance of MS patients and presence of enterotoxin genes *sea, seb and sec*.

2- Staphylococcal enterotoxin B was the second most common enterotoxins after sea in MS patients that play a major role in the antibiotic resistance & exacerbation of MS disease this disease in the future.

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