Staphylococcus aureus is implicated in the etiology of multiple sclerosis

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Abstract

Multiple sclerosis (MS) is an autoimmune-inflammatory disease that occurs in the central nervous system (CNS) may lead to demyelination., The most common form of the disease is the relapsing-remitting multiple sclerosis (RR-MS), including around 85% - 90% of all cases. It has been suggested that *Staphylococcus aureus* in the nasal carriage that related to several autoimmune diseases involving systemic lupus erythematous, multiple sclerosis & others. To investigate and measures the commonness of *Staphylococcus aureus* in the nasal carriage of recently diagnosed and aggravated multiple sclerosis patients and their correlation with multiple sclerosis etiology compared with healthy individuals. This study was conducted on 200 nasal swab specimens, (100 samples from multiple sclerosis patients, and 100 from apparently healthy volunteers). Preparation of culture media like blood agar, nutrient agar, mannitol salt agar and the bacterial culture identification to other media, biochemical tests then the Antimicrobial susceptibility were done.

The isolates of *Staphylococcus aureus* iin the MS patients was (81%) including 38.2% from newly diagnosed multiple sclerosis and 61.8% from relapsing-remitting" multiple sclerosis" patients. Females in multiple sclerosis patients were (52%) and (29%) were males, while isolates of *Staphylococcus aureus* in nasal of controls about (12%) only were (7%) females and (5%) males. All relapsing-remitting

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multiple sclerosis patients (61.8%) were colonized with *Staphylococcus aureus* in their nasal carriages and examined with an expanded disability status scale (EDSS) to determine the degree of disability along with staphylococcal colonization, *Staphylococcus aureus* isolates were tested for their susceptibility to 19 antimicrobial agents by standard disk diffusion method. In multiple sclerosis patients, all isolates were resistant to most types of antibiotics, 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 only (22.8%) as well as a high rate of sensitivity to most of the other antibiotics.High frequency of *Staphylococcus aureus* in the nasal carriage of multiple sclerosis patients mainly (R.R.MS) group compared with low frequency in healthy nasal carriers. Most of *the S.aureus* isolates in MS patients were MDR and sensitive only to the limited number of antibiotics whereas resistance was very limited in the control group.

Keywords*: Multiple sclerosis(MS), relapsing-remitting multiple sclerosis (RRMS),* Staphylococcus aureus.

المكورات العنقودية الذهبية المتورطة في تسبب مرض التصلب العصبي المتعدد

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الخلاصة

التصلب المتعدد (MS) هو مرض التهاب مناعي ذاتي يحدث في الجهاز العصبي المركزي (CNS) قد يؤدي إلى إزالة الميالين. ، الشكل الأكثر شيوعًا للمرض هو التصلب المتعدد الانتكاس والراكم (RR-MS) ، بما في ذلك حوالي 85٪ - 90٪ من جميع الحالات . تم اقتراح وجود بكتيريا Staphylococcus aureus في هيكل الأنف والتي لها علاقة بالعديد من أمراض المناعة الذاتية والتي تشمل الذئبة الحمامية الجهازية والتصلب المتعدد وغيرها. التحقيق في مدى شيوع المكورات العنقودية الذهبية في النقل الأنفي لمرضى التصلب المتعدد الذين تم تشخيصهم مؤخرًا وتفاقمهم وعلاقتهم بمسببات التصلب المتعدد مقارنة بالأفراد الأصحاء. تقيس هذه الدراسة تواتر المكورات العنقودية الذهبية في عربات الأنف لمرضى التصلب المتعدد الذين تم تشخيصهم حديثًا والذين يعانون من الانتكاس والهدوء والضوابط الصحية في هذه الدراسة. أجريت هذه الدراسة على 200 عينة من مسحة الأنف

، و 100 عينة من مرضى التصلب المتعدد ، و 100 عينة من متطوعين أصحاء على ما يبدو. تم تحضير وسائط الاستزراع مثل أجار الدم ، أجار المغذيات ، أجار ملح المانيتول مع الوسائط الأخرى المستخدمة لتحديد الثقافة البكتيرية وتكرارها في جميع العينات ، تم إجراء الاختبارات البيوكيميائية مع اختبارات الحساسية للمضادات الميكروبية.

بلغ تواتر عزلات المكورات العنقودية الذهبية في مرضى التصلب المتعدد (81٪) بما في ذلك 38.2٪ من التصلب المتعدد المشخص حديثاً و 61.8٪ من مرضى "التصلب المتعدد" الانتكاس والهاجر. الإناث في مرضى التصلب المتعدد كانت (52٪) و (29٪) ذكور. في حين أن تواتر استعمار المكورات العنقودية الذهبية في الضوابط الناقلة الصحية (12٪) كانت فقط (7٪) إناث و (5٪) ذكور. تم استعمار جميع مرضى التصلب المتعدد الناكس (61.8٪) بالمكورات العنقودية الذهبية في عرباتهم الأنفية وتم فحصهم بمقياس حالة الإعاقة الموسع (EDSS) لتحديد درجة الإعاقة جنبًا إلى جنب مع استعمار المكورات العنقودية ، وتم اختبار عزلات المكورات العنقودية الذهبية للتحقق من قابليتها للتأثر إلى 19 عاملًا مضادًا للميكروبات عن طريق طريقة الانتشار القرصية القياسية. في مرضى التصلب المتعدد ، كانت جميع العزلات مقاومة لمعظم أنواع المضادات الحيوية ، وكان متوسط معدل مقاومة المضادات الحيوية لجميع عزلات Staphylococcus aureus لهؤلاء المرضى (53.3٪) مع زيادة مستوى المقاومة في مجموعة الانتكاس المحولة إلى (60.6٪) أكثر مما تم تشخيصه حديثًا كان حوالي (46.3٪) ، بينما في المجموعة الضابطة كانت جميع العزلات مقاومة لـ 6 مضادات حيوية فقط بمتوسط نسبة مقاومة للمضادات الحيوية (22.8٪) بالإضافة إلى معدل حساسية مرتفع لمعظم المضادات الحيوية الأخرى. ارتفاع معدل الإصابة بالمكورات العنقودية الذهبية في النقل الأنفي لمرضى التصلب المتعدد بشكل رئيسي في مجموعة الانتكاس والهدوء (R.R.MS) مقارنة بالتردد المنخفض في حاملات الأنف السليمة. كانت معظم عزلات بكتريا المكورة العنقودية البرتقالية في مرضى التصلب المتعدد هي MDR وحساسة فقط للعدد المحدود من المضادات الحيوية بينما كانت المقاومة محدودة للغاية في المجموعة الضابطة.

الكلمات المفتاحية: *التصلب المتعدد (MS) , التصلب المتعدد الانتكاسي المتكرر (RRMS), المكورات العنقودية الذهبية.*

Introduction

Multiple sclerosis (MS) is a chronic inflammatory, autoimmune neurological disease of the central nervous system (CNS). MS attacks the myelinated axons within the CNS, [1].

Multiple sclerosis affects females more often than males in a ratio of 2:1 [2]. The clinical course of MS is around 87% of a remitting and relapsing (RR) in nature but may be secondary progressive (SP) or primary (PP) [3]. Cognitive impairment and alterations in vision, motor, and sensory disorders are common clinical features of MS [4]. Though the etiology of MS remains elusive, it is now well-known that several factors are believed to contribute to the progress of immune response involving; genetic, age and environmental agents, like bacteria, viruses, and other infectious pathogens are the main supposed environmental stimuli of autoimmunity [5].

The Expanded Disability Status Scale of MS is considered as an important scoring for measuring the degree of patients by physicians, disability that depends on the type of MS, clinical presentation, and duration of this disease, depending on neurologists physical examination this scale graduated from 0-10 points starting from no any obvious disability to death of patients affected by MS [3].

Staphylococcal Colonization can be either persistent or transient and maybe at multiple or single body sites. Most often, the site of colonization in humans is the anterior nares and they have been presented to be the main reservoir of *S.aureus* in both children and adults [6].The principle reservoirs, carriage of *S.aureus* in the nose, appear to play a key part in the pathogenesis and epidemiology of infection. It has been related to an increased risk of infection among patients undergoing renal dialysis and in patients after surgery due to those patients considered as an immunosuppressed. Moreover, nasal carriage of *S.aureus* was a risk for the increase of nosocomial bacteremia in intensive care units due to those admitted patients considered immunocompromised [7].

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Therefore *S.aureus* eradication from the nose has evidenced to be efficient in minimizing the occurrence of Staphylococcal infection. This shows that the anterior nasal area is a primary ecological reservoir of *S.aureus*, the prevalence of *S.aureus* nasal carriage changes, however, it is higher in white persons, men, young children, and hospitalized patients. Other colonization sites are skin and in certain regions in the body such as perineal region, umbilicus, axilla, vagina in 5% of females and mammary folds [8]. *Staphylococcus aureus* within nasal carrier individuals carry toxigenic genes, which can perform as superantigens [9]. Bacterial superantigens are potent activators of $CD4^+$ T cells that can have chronic or acute influences on the central nervous system causing proliferation of autoreactive $CD4^+$ T cells and massive cytokine activation in the brain, these superantigens have enterotoxigenic action such as exfoliative toxins, toxic shock syndrome toxine-1 (TSST-1) and enterotoxins (A, B, C) but mainly (A) that have been implicated in the etiology and/or exacerbation of patients with multiple sclerosis [10]. *Staphylococcus aureus* is a pathogen capable of bypassing the entire barriers of the host defense system because it owns a broad spectrum of virulence factors. In spite of the increasing knowledge on this topic it is still difficult to prevent or efficiently treat Staphylococcal infections in various cases [11].

Greater than 90% of *S.aureus* strains are resistant to penicillin G owing to most of such strains yield β-lactamase enzyme. These organisms may be treated with β -lactamase–resistant penicillins, e.g. oxacillin, nafcillin, cephalosporins, methicillin, or gentamicin is occasionally added [12]. Therapeutic problems are triggered by infections with strains which are resistant to various antibiotics and precisely to methicillin (MRSA, methicillin-resistant *S. aureus*) , the development of resistance to numerous antibiotics by *S.aureus* has included the acquisition of determinants either by mutations in chromosomal genes or by horizontal gene transmission of mobile genetic elements such as the Staphylococcal cassette chromosome, transposons, and plasmids,Methicillin-resistant *Staphylococcus aureus* (MRSA) has a gene named the

mecA gene that makes it resistant to methicillin and also to other antibiotics of beta-lactam, involving, beta-lactam/beta-lactamase inhibitor combinations, flucloxacillin, carbapenems, and cephalosporins , the most common position of colonization is the anterior nares, but MRSA may also be located in other regions such as the abnormal skin (e.g., wounds, eczema), axillae, urine, throat, and rectum. The colonized location may perform as a reservoir of MRSA [13-16].

Materials and methods

Collection of samples

Two hundred nasal samples were enrolled in this study. One hundred nasal swab samples from multiple sclerosis patients (50 relapsing-remitting and 50 newly diagnosed) and 100 from apparently healthy individuals were collected during a period from first of November 2021 to the end of January 2022 from Baghdad Teaching Hospital / Multiple Sclerosis Clinic In Medical City.

The Expanded Disability Status Scale (EDSS) of MS

To determine the degree of MS patient's disability, the Expanded Disability Status Scale (EDSS) was determined and estimated by MS physicians according to the course of the disease. The scale ranges between 0-10 points describing the patient's routine walk disability status graduated from 0 without any disability to 10 death of patients due to MS [17].

Preparation of culture media

All culture media were prepared in the laboratory as specified by the manufacturer instructions.

Isolation and identification of *Staphylococcus aureus*

From each patient and healthy controls two nasal swab samples were collected under appropriate conditions according to standard operating procedure (SOP), these samples were labeled with name and case number then transported to the laboratory for processing and investigations on the same day. Catalase test and coagulase test were done and resistance patterns of the *Staphylococcus aureus* isolate to 19 different

antibiotics were determined by disk diffusion test (DDT) according to Clinical and Laboratory Standards Institutes (CLSI) 2019 [8]. The data of the present study were analyzed by applying the SPSS software package version (22.0) to analyze and assess the results of the study. For measurement of association and correlation, the Chi-square test was used. T-test & ANOVA tests were used for parametric data while Mann-Whitney for non-parametric data. A P-value of less than 0.05 was considered statistically significant.

Results

Distribution of study groups

Among the overall MS patients, 36% were males and 64% were females in (1:1.7) ratio while in the case of control, 46% were males and 54% were females indicating a comparable gender distribution with no significant correlation ($P>0.05$) between them, table (1), Regarded to age groups the mean age of MS patients was 35.02 ± 11.18 years ranging between 17 and 59 years indicating no statistically significant differences at $P>0.05$ due to their similarity.

Concerning residency of patients and controls, results of studied groups showed 47% were from urban and 53% were from rural in MS patients while in controls 86% were from urban and 14% were from rural. Statistically, there is a significant differences at ($P=0.0$) concerning residency, table (1)

Table (1): Distribution of demographical characteristics regarding to gender, age groups and residency

SDCv.	Groups	MS		Control		*P*-value
		No.	%	No.	%	
Gender	Male	36	36	46	46	***P*=0.151 NS**
	Female	64	64	54	54	
	Total	100	100	100	100	
Age Groups	< 20	5	5	4	4	***P*=0.155**

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(Per yrs.)	20 _ 29	36	36	38	38	**NS**
	30 _ 39	25	25	36	36	
	40 _ 49	20	20	17	17	
	50 _ 59	14	14	5	5	
	Total	100	100	100	100	
	Mean ± SD	35.02 ± 11.18		32.03 ± 9.01		
Residency	Urban	47	47	86	86	***P*=0.0000 HS**
	Rural	53	53	14	14	
	Total	100	100	100	100	

(*) NS: Non Sig. at *P*>0.05; Testing based on a contingency coefficient test.

The Expanded Disability Status Scale EDSS of MS & *S.aureus*

All newly diagnosed MS patients were not subjected to EDSS score examination (no EDSS = 50%), while all the remaining patients were examined with EDSS score correlated with duration of disease (< 1 year to 19 years) duration in R.R. MS patients only. In this study, results of EDSS score in relapsing-remitting MS patients reported that the EDSS =0 with referred to ensure no disability and constituted 12 patients (12%) with statistically significant differences at *P*<0.01. Concerning the duration of disease with the EDSS score examination results revealed that 24 patients (24%) out of 50 R.R. patients had a duration of more than 5 years with an increased disability on EDSS scale that focused on minimal, moderate disability and assistance required to walk with EDSS score 2, 3 and 6 respectively, in addition, all those patients colonized with *S.aureus* in nasal carriages indicating a highly significant correlation between increase duration of MS disease, frequency of *S.aureus* and increase disability of patients in this scale at *P*<0.01, table (2).

Table (2): Correlation between EDSS score and duration of disease with *S.aureus* colonization

No. of patients with *S.aureus* positive	Duration of disease in R.R. MS				*P*- value
	< 1 year	1-5 years	> 5 years	Total	
	3	23	24	No.(%)	
NO EDSS	In newly diagnosed MS only			50(50%)	
EDSS score	In R.R. MS only				
0	2	9	1	12	
1	1	5	3	9	
2	0	3	6	9	
3	0	2	6	8	
4	0	2	1	3	***P*= 0.00 (HS) x^2 =49.64**
5	0	1	2	3	
6	0	1	5	6	
7	0	0	0	0	
8	0	0	0	0	
9	0	0	0	0	
10	0	0	0	0	
TOTAL	3(3%)	23(23 %)	24(24 %)	100	
***P*- value**	*P*= 0.00(HS) , x^2 =76.75			*P*= 0.00(HS) , x^2 =49.64	

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

***Staphylococcus aureus* colonization**

The results showed that multiple sclerosis patients (MS)colonization with *S.aureus* in the nasal carriage was 81% while the percentage of *S.aureus* in the control group was only 12% indicating a statistically significant difference in the frequency of colonization between them (P= 0.00), as shown in table (3).

Table (3): frequancy of *Staphylococcus aureus* in all study groups

studied groups	No. of collected samples	S.aureus No.	P-value
Control	100	12	*P*=0.0 (HS)
New diagnosed MS group	50	31	
MS exacerbated group	50	50	

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

Frequency of *S.aureus* regarding gender

The results revealed that the frequency of *S.aureus* growth in the MS group was 29(29%) male and 52(52%) female while in the control group was 5(5%) male and 7(7%) female with significant differences in the distribution of gender concerning the growth of *S.aureus* in all study groups (*P*= 0.00) as shown in table (4).

Table (4): Distribution of gender with *S.aureus* colonization

Gender		Overall study groups		*P*-value
		Multiple sclerosis group	**Non-MS control group**	
		***S.aureus* result**	***S.aureus* result**	
		(+ve)	(+ve)	
Male	**No(%)**	29(29%)	5(5%)	*P*=0.0 (HS)
Female	**No(%)**	52(52%)	7(7%)	*P*=0.0 (HS)
Total	**No(%)**	81(81%)	12(12%)	*P*=0.0 (HS)
		100(100%)	100(100%)	

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

The results also showed that the frequency of *S.aureus* colonization in newly diagnosed MS was 31(32.8%) including 9(18%) male and 22(44%) female while in relapsing remitting MS was 50(61.8%) including 20(40%) male and 30(60%) female with highly significant differences (*P*=0.00), table (5).

Table (5): Distribution of gender with *S.aureus* colonization in stages of MS

Gender		Overall study groups				*P*-value
		Newly diagnosed MS		R.R. MS		
		S.aureus result		*S.aureus* result		
		(+ve) No%	(-ve) No%	(+ve) No%	(-ve) No%	
Male	**No(%)**	9(18%)	7(14%)	20(40%)	0(0%)	*P*=0.0 (HS)
Female	**No(%)**	22(44%)	12(24%)	30(60%)	0(0%)	*P*=0.0 (HS)
Total	**No(%)**	31(32.8%)	19(19%)	50(61.8%)	0(0%)	*P*=0.0 (HS)
		81%+19%= 100%				

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

Frequency of *S.aureus* regarding age groups :

Regarding the frequency of *S.aureus* colonization according to age groups, this study involved five age groups ranging between (< 20 – 59) years. The highest rate of *S.aureus* colonization in multiple sclerosis patients was 37% in age group (20-29 years) as well as 26% in age group (30-39 years) while the lowest rate of colonization was 3.7% in the age group (< 20 years), compared with the highest rate of colonization in healthy controls which is 50% in age group (30-39 years) followed by 25% in age group (20-29 years) , while there is no *S.aureus* colonization (0%) in age group (< 20 years). Statistically, there is a highly significant correlation (*p*=0.00) between MS patients and controls in the frequency of *S.aureus* colonization according to the age group, table (6).

Table (6): Frequency of *S.aureus* regarding age

Age groups	Overall study groups				*P*-value
	Multiple sclerosis group		Non-MS control group		
	S.aureus		*S.aureus*		
	(+ve)	(-ve)	(+ve)	(-ve)	
	No.(%)	No.(%)	No.(%)	No.(%)	
<20	3(3.7)	2(10.5)	0(0)	4(4.6)	
20-29	30(37)	6(31.6)	3(25)	35(39.8)	
30-39	21(26)	4(21)	6(50)	30(34)	
40-49	15(18.5)	5(26.4)	2(16.7)	15(17)	
50-59	12(14.8)	2(10.5)	1(8.3)	4(4.6)	*P*= 0.0037 (HS)
Total	81(100)	19(100)	12(100)	88(100)	
	100(100)		100(100)		

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

Antibiotic sensitivity tests of *S.aureus*

The results of antibiotic sensitivity by disk diffusion test to all MS patients showed that there is a complete rate of methicillin resistance (100%), a high resistance to ceftriaxone (93.8%) , ampicillin (87.7%) , penicillin-G (84%) , erythromycin (82.7%) , sulphamethoxazole / trimethoprim (80.2%) and azithromycin (75.3%) , moderate resistance to tetracycline (72.8%) , amikacin (61.8%) and gentamycin (56.8%) , low resistance to cefotaxime (42%) , doxycycline (42%) chloramphenicol(40.8%) , clindamycin (40.8%) , rifampin (35.8%) , ciprofloxacin (33.4%) , vancomycin (10%) , meropenem (5%) and imipenem (3.7%) , while in control groups there is also complete resistance rate to methicillin (100%) in addition to penicillin-G (100%) , moderate resistance to ampicillin (66.6%) , ceftriaxone (66.6%) and erythromycin (66.6%) , low resistance to tetracycline (33.4%) , with complete sensitivity (100%) to azithromycin, amikacin, cefotaxime, chloramphenicol, ciprofloxacin, clindamycin, doxycycline, gentamycin, imipenem meropenem

rifampin, sulphamethoxazole / trimethoprim and vancomycin . Statistically there is a highly significant differences between MS and control groups in term of antibiotic resistance (P=0.00) except in meropenem with significant differences (P= 0.0235) and non-significant differences at (*P*>0.05) in imipenem and methicillin, as shown in table (7) .

Table (7): percentage of antibiotic resistance of *S.aureus* isolates in all study group.

ANTIBIOTICS	Multiple sclerosis patients	Non-MS
	Total	
	R No.(%)	R No.(%)
Methicillin[ME]	81(100%)	12(100%)
Vancomycin[VA]	8(10%)	0
Azithromycin[AZM]	61(75.3%)	0
Erythromycin[E]	67(82.7%)	8(66.6%)
Tetracycline[TE]	59(72.8%)	4(33.4%)
Amikacin[AK]	50(61.8%)	0
Rifampin[RA]	29(35.8%)	0
Penicillin-G[P]	68(84%)	12(100%)
Meropenem[MEM]	4(5%)	0
Sulpfa/tri[SXT]	65(80.2%)	0
Doxycycline[DOX]	34(42%)	0
Ciprofloxacin[CIP]	27(33.4%)	0
Clindamycin[DA]	33(40.8%)	0
Imipenem[IPM]	3(3.7%)	0
Cefotaxime[CTX]	34(42%)	0
Gentamicin[CN]	46(56.8%)	0
Ceftriaxone[CRO]	76(93.8%)	8(66.6%)
Ampicillin[AM]	71(87.7%)	8(66.6%)
Chloramphenicol[C]	33(40.8%)	0
TOTAL R%	53.3%	22.8%

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Discussion

In this study, the ratio of males to females in MS patients was (1:1.7) at a percentage of 36% and 64% for males and females respectively. The multiple sclerosis disease was higher in females than in males in the present study because of the effects of sex hormones, different environmental exposures, genetic differences, the presence of immunocompromised persons like pregnant women having MS, and modern lifestyle in men and women. This study coincided with a study conducted by Valadkeviciene *et al.,* 2018 [18], who reported that the females to males sex ratio in MS in Lithuania was increased from 1.5 to 2 times over the past 15 years in Lithuania.

The current results showed that the higher percentage of MS disease was 36% in the age group (20-29 years) and 25% in age group (30-39 years), this interprets the natural incidence of this disease affecting the patients around the world commonly in young adults. This study matched with Leray *et al*., 2015 [19] who said that the disease age of onset starts at (20-40 years) with a mean age of 33 years in 85% of MS diseases, whereas the present study disagreed with another studies conducted in Ireland by Connell *et al*., 2017 [20], who reported that 38% of MS patients were in the mean age of onset at (34 -50) year. In the control group, the study results revealed that 38% of healthy people were in the age group (20-29 years) and 36% were in the age group between (30-39 years). According to residency in this study the percentage rate of MS patients in rural areas was 53% and in urban was 47%, indicating a relatively increased incidence of MS disease in rural areas, and this may be due to there was no similarity in the selection of patients and controls, individuals who lived in rural areas tend to be poorer, less hygiene, less educated and acquire more disease and disability than peoples who are resident in urban areas. The study results agreed with the study on MS patients performed in Iran by Tolou-Ghamari, 2015 [21], who reported that the prevalence of MS in Isfahan and its rural provinces were 84% while in other cities was only 16%, while the current study disagreed with the study conducted in united states by Buchanan *et al.*, 2006 [22], who showed that the

percentage of relapsing-remitting patients in remote rural areas was 51.3% and in urban was 58.6%.

The current study showed that all relapsing-remitting MS patients examined with EDSS score were colonized with *S.aureus* in the nasal carriage since the EDSS score in patients with a duration of more than 5 years reached (6.0) indicating a strong relationship between increased disability of patients represented by increase EDSS score with persistent *S.aureus* in the nasal carriage of those patients. The explanation of this result gives more details about the important role of *S.aureus* enterotoxins mainly (*sea)* in the activation of autoreactive CD4+ T-cells in the brain that destroys the myelin protein surrounding the CNS which finally leads to non-specific polyclonal CD4+ T-cells activation and huge cytokine production that responsible for increasing severity of those MS patients.

This study demonstrated that the colonization of *S.aureus* in nasal carriage of healthy controls was only 12 % , while in MS patients, the frequency of *S.aureus* was 81% including (38.2%) of newly diagnosed and (61.8) of relapsing remitting MS patients, this result coordinated with study was performed by Mehrabi *et al.,* 2015 [23], who reported that the frequency of *S.aureus* colonization in MS patients was high (68.33%) in MS exacerbated group and (50%) in MS stable group compared with low in healthy individuals or non-MS group (23.75%), the interpretation of this result depends on the complex etiology of MS disease that includes environmental agents like bacterial superantigens frequency in *S.aureus* isolates accompanied with increase nasal colonization of those patients more than healthy carriers indicates that the colonization rate of *S.aureus* in nasal carriage of MS patients play a significant role in the development of infection and establishment of this autoimmune disease .

The result of the current study revealed that 100% of *S.aureus* isolates in MS patients were multidrug resistance (MDR) when determined by disk diffusion method that differ from *S.aureus* isolates in healthy controls which showed low resistant rate.

The high rate of resistance to several types of antibiotics was (53.3%) in MS groups whereas low resistance rate (22.8%) of *S.aureus* isolates was detected in healthy non-MS group .This result suggests that the nasal bacterial colonization with *S.aureus* in MS patients was pathogenic, virulent, more antibiotic-resistant (MDR), and can infect and cause disease which differs from nasal *S.aureus* in healthy carriers that appear as nonpathogenic due to having no virulence factors and low antibiotic resistance, the result of this study agreed with the study published in Taiwan; by Yang *et al*. 2018 [24],who reported that commensal *S.aureus* flora differs from pathogenic by many factors including the persistence of high-level antibodies to nasal *S.aureus* and circulating antibodies in human serum directed against *S.aureus* cell wall proteins that prevent Staphylococcal infection in healthy carriers.

While a study conducted in India by Karthik M *et al*., 2009[25] disagreed with the current study regarding *S.aureus* colonization, which reported that MRSA in the nasal carriage of healthy individuals plays a significant role in the epidemiology and pathogenesis of infection. Another explanation of this study is based on the fact that persistent Staphylococcal nasal colonization in healthy individuals is represented by the production of bacteriocins called staphylococcin which compete against staphylococcal infections [26].

In the current study, all *S.aureus* isolates in MS and non-MS groups were resistant to methicillin (100%) indicating complete methicillin-resistant *S.aureus* (MRSA) strains were distributed even in healthy carriers, this result coincides with a study performed in Iran by Emaneini M, *et al.*, 2017 [27], who showed the high percentage of nasal MRSA colonization (32.8%) among Iranian individuals, whereas this study is in disagreement with the study of Dağı *et al.*, 2015[28] was conducted in Turkey, and reported that low frequency of MRSA was detected in healthy carriers (2.9%).

In addition, to complete resistance to methicillin in study groups, S.aureus isolates in MS patients of this study demonstrated a very increased rate of resistance to ceftriaxone (93.8%), ampicillin (87.7%), penicillin (84%),erythromycin (82.7%) sulphamethoxazole/trimethoprim (80.2%) , and azithromycin (75.3%) , while in the healthy control group in addition to methicillin resistance, *S.aureus* isolates showed a high resistance rate to only penicillin-G (100%) , meaning that all strains of *S.aureus* isolates in MS patients were resistant strains and reliable for numerous drug resistance (MDR) which lead to increase the severity of multiple sclerosis (MS), while the *S.aureus* isolates were differed than in control group in term of lower antibiotic resistance results which occur as non-virulent. This result arranged with Mehrabi *et al.*, 2015 [23], who conveyed that resistant *S.aureus* strains in MS patients was considered as a risk factor for MS exacerbation with high level of resistance against tetracycline(80%),ampicillin(72.22%) , methicillin (66.66%), erythromycin (66.66%), oxacillin (63.33%) and sulphamethoxazole / trimethoprim (61.11%). As a result, *S.aureus* nasal carriage in MS patients is probably very important in the etiology, development, and pathogenesis of MS disease.

This result in match with the same study of Libbey *et al.*, 2015 [29], who reported that antibiotic resistance results in MS exacerbated group showed high rate of resistant against tetracycline (92.68%), erythromycin (87.80%), ampicillin (85.36%), methicillin (85.36%) and sulphamethoxazole/trimethoprim (75.60%), while in MS stable group of the same study there was moderate resistance to tetracycline (73.33%), ampicillin (66.66%), methicillin (56.66%) and erythromycin (53.33%). In the attending study involving MS groups it showed a moderate resistance against tetracycline (72.8%), amikacin (61.8%), and gentamicin (56.8%). furthermore, in the control group the percentage of resistance against tetracycline was (33.4%) and no resistance against amikacin and gentamicin (0%).

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This study also revealed a low percentage of antibiotic resistance in MS patients against cefotaxime (42%), doxycycline (42%), chloramphenicol (40.8%), clindamycin (40.8%), rifampin (35.8%), and ciprofloxacin (33.4%), whereas in the control group there was no any resistance (0%) against cefotaxime, doxycycline, chloramphenicol, clindamycin, rifampin, and ciprofloxacin respectively, this study was in agreement with a study conducted in Egypt by Foster *et al.*, 2017 [30] , who reported that *S.aureus* isolates from different clinical samples including nasal samples was low resistance to clindamycin(6%), ciprofloxacin(14%). A very low percentage of resistance to vancomycin (10%), meropenem (5%), and imipenem (3.7%) was detected in MS patients of the present study, whereas no level of resistance (0%) was determined in the control group against vancomycin, meropenem, and imipenem respectively.

Regarding MS patients this study coincides with a study was performed in India by Chakraborty *et al.*, 2011[31], who reported that the *S.aureus* isolated from postoperative wounds was (26.6%) resistant to vancomycin, regarding patients and control groups this study also agreed with study of ElSayed *et al.*, 2018 [32], who reported that 100% *S.aureus* isolated from different clinical samples including community individuals, patients, and healthcare workers were only (13.8%) resistant to vancomycin (VRSA). The explanation of these antibiotic results probably depend on the frequency of *S.aureus* colonization in nasal passages of MS patients with an increased level of resistance to several antibiotics accompanied the progression of multiple sclerosis disease particularly in those patients with relapsing-remitting stage compared with newly diagnosed MS stage and healthy non-MS group with low antibiotic resistance rate, all these results indicate that resistant *S.aureus* isolates were detected in MS patients including high virulent strains commonly in the relapsing-remitting group was responsible for etiology of MS, increased disability, development and pathogenesis of this disease.

Conclusions

High frequency of *S.aureus* isolates in MS patients occur at age group (20-29 yea while in control group occur at (30-39 year) and most common in female than in male . Most of *S.aureus* isolates in MS patients were MDR and sensitive only to limited number of antibiotics whereas resistance was very limited in control group. All *S.aureus* isolates in patients and controls were MRSA. All *S.aureus* isolates in patients and controls were highly sensitive imipenem , meropenem and vancomycin.

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