Determine Nasal Carriage of Methicillin Resistant *Staphylococcus aureus* MRSA in Young Adult College Student

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Abstract

Present study was carried out to find prevalence of MRSA in healthy individual of second stage students, college of pharmacy/Baghdad University. A total of 74 student selected between age 18-23 years old were included in this study, nasal swabs collected and subjected to many diagnostic standard bacteriological identification methods. Culture, colonial morphology, Gram stain, mannitol fermentation, coagulase ,gelatinasetest, DNAase, MR/VP and antimicrobial susceptibility test was performed on tryptic soy agar by modified Kirby-Bauer muller hinton disc diffusion method and the result show that out of 74 nasal swabs,67(90.5%) were MRSA positive isolates, 21(31.4%) of them were mannitol ferment and 46(68.6%) non mannitol fermenter, among these isolates 33(44.6%)male and 41(55.4%) female, there was no significant sex difference in the prevalence of *Staph. aureus*, while show decrease in prevalence with age group,(54%),(27%),(9.5%) alternatively, MRSA positive isolates indicated relatively high rate of resistance to antibiotics, so to vancomycin 3(4.5%),ciprofloxacin 4(6%) , tetracyclin 9(13.5%) ,gentamycin 6(9%), erythromycin 15(22.5%) and keflex 20(30%). This study show a high prevalence of MRSA carriage in young adult college student (healthy people) that indicating the spread of MRSA in the community which consider high risk of spreading infections also we isolate non mannitol fermenter (MRSA) *Staph. aureus* that need further moleculer analysis to prove it.

Keywords: *Staphylococcus aureus* , MRSA, Modified Kirby-Baur methods.

تحديد الحاملين لبكتريا المكورات العنقودية الانفية المقاومة للمضاد الحيوي مثسلين في البالغين الاصحاء من طلاب الجامعة

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الخلاصة

اجريت هذه الدراسة لمعرفة معدل انتشار بكتريا المكورات العنقودية المقاومة للمضاد الحيوي المثسلين و المسماة (MRSA) في المجتمع بين الناس الاصحاء و ذلك باخذ عينات عشوائية لطلاب المرحلة الدراسية الثانيه في كلية الصيدله / جامعة بغداد بعدد ٧٤ مسحة بين الاعمار (١٨-٢٣) و قد تم عزل البكتريا و تشخيصها بالطرق البكتيرية المثالية من دراسة المزروع البكتيري و شكل المستعمرات و صبغة الكرام و الفعاليات البايوكيمياوية , وقد و جد انه من بين ٧٤ عزلة , ٦٧ (٩٠.٥%) تحوي على بكتريا المكورات العنقودية المقاومة للمثسلين (MRSA) وبانه ٢١(٣١.٤%)منها هي مخمرة لسكر المانتول بينما ٤٦ (٦٨.٦%) هي غير مخمرة لسكر المانتول و تعتبر هذه النسبة عالية بالمقارنة بمجتمعات اخرى , كما وجد بان الاعمار بين (١٨-١٩) سنة تحوي اعلى نسبة من العزلات (٥٤%) و بين (٢٠-٢١) سنة (٢٧%) ومن ثم الاعمار التي هي ضمن الفئة العمرية الاكبر من ٢١ سنة بنسبة (٩.٥%) مما يدل على تناقص معدل التواجد للبكتريا مع التقدم بالعمر كما تبين ان الجنس لا يؤثر في النسبة حيث لايوجد فرق بين الذكور و الاناث كما اظهرت الدراسة ان البكتريا المعزولة تظهر بعض المقاومة لمضادات حياتية اخرى مثل الفانكومايسين (٤.٥%) , البسبروفلوكساسين (٦%) الجنتامايسين (٩%) , التتراسايكلين (١٣.٥%) والارثرومايسين (٢٢.٥%) و اخيرا" الكفلكس (٣٠%) . تبين الدراسة خطورة ازدياد نسبة انتشار البكتريا في المجتمع و التي بدورها تزيد من نسبة الاصابات المكتسبة في المستشفيات Hospital acquired infection .و كما تبين الدراسة انتشار نوع من هذه البكتريا غير المخمرة لسكر المانتول والمقاومة للمثسلين و التي تعتبر عترة جديدة و تحتاج بدورها الى دراسة جزيئية لتثبيتها Molecular analysis.

الكلمات المفتاحية:المكورات العنقودية المقاومة للمضاد الحيوي مثسلين ، طريقة كيربي بور المعدلة .

Introduction

Staphylococcus aureus is a major pathogen responsible for nosocomial and community acquired infections [1, 2]. Methicillin resistant *Staph. aureus* (MRSA) has emerged as a nosocomial pathogen of a major worldwide importance and is an increasingly frequent cause significant morbidity and mortality [3], colonized person can serve as a reservoir for the nosocomial spread of (MRSA) . Active surveillance and timely identification of (MRSA) colonization of patients is an important infection control activity that help to prevent nosocomial spread and is cost effective [4,5]. Acquired infection with *Staph. aureus* have until very recently

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been reliably treated with B-Lactam antibiotics, B-lactam antibiotics normally bind to penicillin binding proteins (PBPS) in the cell wall, resulting in the disruption of synthesis of the peptidoglycan layer and death of bacterium. Since a lactam cannot bind to low affinity PBPS; synthesis of the peptidoglycan layer and cell wall synthesis are able to continue cited by [2] MRSA infection often require systematic antibiotic therapy and are an important health care burden since they increase treatment costs and patient morbidity ,MRSA carriage in many communities is largely un- known and it varies in different geographical regions ,so to control the spread of disease continuing study is needed to asses geographical distribution and epidemiology of infection and develop strategies to that [2,6]. The spread of MRSA can also be potentially minimized by prevention of risk factors such as previous antibiotic use, day care attendance , contact with a health care workers or nursing home resident, residence in a long - term care facility, diabetes mellitus ,hospitalization, admission to intensive care unit, intravenous drug use, invasive indwelling devices, hemodialysis or peritoneal dialysis, naso gastric tube, gastrostomy ,and external feeding ,mechanical ventilation, endotracheal tube, tracheostomy tube ,surgical procedures ,immune suppression ,chronic illness[2,7]. The anterior nares are the primary reservoir of *Staph. aureus* in both adult and children [8] Nasal carriage of *Staph* . *aureus* is important because most of *Staph .aureus* infection occur in person who are colonized with this organisms, this *Staph .aureus* carriage has been assumed to be one of the risk factors for subsequent infection [8],there for recognition of persons colonized orinfected with *Staph .aureus* is recommended for preventing the spread of the organisms within hospitalsor communities [4,8] .

Staphylococcus aureus —methicillin resistant mannitol fermenter negative strain were first isolated and reported as subtype of "antario epidemic" strain (MRSA-1) known In Canada lab.2003 by using un- enriched media such as Brain Heart Infusion (BHI) and incubate for 24-48 hours then sub cultured on blood agar, improve isolation and detection of these un usual strain which first reported by antario researchers need further molecular analysis [9, 10].

So the aim of this study is to estimate the frequency of MRSA *Staph .aureus* in community and control the spread of disease which need continuing study to asses geographical distribution and epidemiology of infection and develop strategies to that [3] .

Materials and Methods

Sample collection: nasal swabs of 74 student within age limit 21-23 years were collected for purpose of the study during a period from (October--Feb.2010), the specimens were collected with sterile cotton swabs available commercially The swabs was introduced 2-3 centimeter in the nasal cavity and rotated 4-5 times both clock wise and anti-clock wise before with draw. Each sample was labeled with code number and various other information including age, sex, location, etc. were recorded. The sample was transported to laboratory of microbiology in sidethe college of pharmacy and immediately inoculate on the special media (BHI, MSA, Blood Agar Media) for diagnosis [2, 11, 12].

Culture and Sensitivity

To be sure that none of the *Staph. aureus* were lost, Nasal swabs were inoculated into brain heart infusion broth, non selective media containing 0.5% salt,75 mg/Loztreonam, 5mg/L ceftizoxime (9,10) overnight incubation for 24hrs,37C°, then subcultured on blood agar and mannitol salt agar to indicate the type of hemolysin and fermentation of mannitol. Both hemolytic mannitol fermenter yellowish colonies and non hemolytic, non mannitol fermentor pink colonies, are subsequently identified by the gram stain, catalase test, slide and tube coagulase test, gelatinase test, MR/VP test[1,2,12,13]. Also, the colonies subcultured on tryptic soy agar (TSA) media for further examination.

Antibiotic Susceptibility test: All the identified *Staph. aureus* isolates from nasal swabs subjected to in vitro susceptibility test Modified Kirby-Bauer disc diffusion methods (2,14) antibiotics used in the study were Methicillin (10mcg), Tetracycline (30 mcg), Ciprofloxacin (30mcg), Erythromycin (15mcg), Vancomycin (30mcg), Gentamycin (10mcg) and Keflex (30 mcg), all the disc were obtained from (oxoid) . Quality control for the test: in this study the accuracy of the overall testingprocedure was monitored by using *Stap. aureus* ATCC 25923 as reference strain.

Statistical analysis

The computer programmer SSPS (Statistical Package for Social Sciences) version 17.0 was employed to manipulate the statistical analysis of present study results. The results were presented as percentage frequencies. Significant differences were assessed by Student T-Test in which $P<0.05$ was considered significant.

Result

Table 1 and 2 show that among 74 student under study, 33(44.5%) male and 41 (55.5%)

female, *Staph. aureus* could be isolated from isolates 29(43.3%) from male, while 38 (56.7%)from female. There was no significant sex difference in colonization of *Staph .aureus* between male and female, while the study showed that highest colonization of *Staph .aureus* in age group 18-19 years old (54%) 67 (90.5%) nasal swabs sample, among these follow by 20-21, age group (27%) and over 21 years old (9%), and only 7 which equalto (9.5%) show negative result means no *Staph. aureus* isolates, 3 (4.1%) 0f them male and 4 (5.4%) female .

Table(1) Number of Nasal swabs in correlation with age group and sex

Age group yrs.	Number of swabs No %	Male No %	Female No %
18-19	43(58)	22(29.7)	21(28.3)
20-21	24(32)	6(8.1)	18(24.3)
22>	7(10)	5(6.8)	2 (2.7)
Total No.	74(100)	33(44.7)	41(55.3)

Table (2) Frequency of *Staphylococcus aureus*(MRSA) isolates , Nasal carriage by age and sex group

Age group yrs.	Number of MRSAisolated No %	Male No %	Female No %
18-19	40(54.0)	20(27.0)	20(27.0)
20-21	20(27.0)	5(6.75)	15(20.2)
22>	7(9.5)	5(6.75)	2(2.70)
Total No.	67(90.5)	30(40.5)	37(50.0)

Total no. of sample 74 , no. of negative isolates 7(9.54) , male 3(4.1%) and female 4(5.4%)

Table 3-show the identification methods for *Staph. aureus*, and we can see all the mannitol fermenter *Staph. aureus* isolates show strong slide and tube coagulase test DNAase test, gelatinase test, MR/VP test as mention in reference lab. while mannitol fermenter negative 46 (68.6%) give weakly positive tube coagulase test andmostly negative for other test, which means that n.m.f. *Staph aureus* are different in some properties (new strain reported in 2003) [(10)].

Table 4- show the sensitivity pattern according to number of *Staph aureus* isolates, 67 (90.5%)were methicillin resistant MRSA which divided in to21(31%) mannitol fermenter and 46 (68.6%) non mannitol fermenter, indicated relatively some resistant to other antibiotics such as for Keflex (kf) 30%, Erylhromycin (E) 22.5%,Tetracyclin (Te) 13.5%,Vancomycine (V) 4.5% and for Ciprofloxacin (Cp) 6%.

Table (3) Identification methods used to diagnose *Staphylococcus aureus*

Test used	Type and no. result	
Tube coagulase	21 (+)	46(w+)*
Slide coagulase	67 (+)	-
Mannitolferment	21 (+)	46(-)
Gelatinase	21 (+)	46(-)
DNA ase	21 (+)	46(-)
Catalase	67 (+)	-
MR/VP	67 +/-	

*W=weakly positive non mannitol ferment 46

Table(4) Sensitivity pattern according to no. of isolated *Staphylococcus aureus* (MRSA)

Antibiotics used	Mannitolfermention total No. 21(31.4%)		Non Mannitolfermention 46(68.6%)	
	Sensitivity No %	Resistance No. %	Sensitivity No %	Resistance No. %
Vancomycin	19 28.4	2 2.9	45 67.2	1 1.49
Tetracyclin	19 28.4	2 2.9	39 58.2	7 10.4
Erythromycin	16 23.9	5 7.5	36 53.7	10 14.9
Keflex	14 20.9	7 10.4	33 49.2	13 19.4
Gentanycin	20 29.9	1 1.5	41 61.2	5 7.5
Cifrofloxacin	20 29.9	1 1.5	43 64.2	3 4.4

Discussion

Study on *Staphylococcus aureus* nasal carriage rates in various populations have been investigated in the developed countries with temperate climate [10], but nosuch study among healthy population had been reported, in India so far researchers reported that nasal carriage of *Staph. aureus* varied in different communities,[10] manyinvestigators have reported an increasing incidence of CA-MRSA infections in community settings [8,15] such increase without the usual risk factors related to MRSA infection or colonization have given the surveillance for *Staph. aureus* greater significance. So due to association between the carriages of *Staph.aureus* and subsequent infection [8] evolution of colonization prevalence may be useful for the estimation of potential *Staph.aureus* causing disease [8].

The result of present study showed that nasal carriage of Staphylococci was as high as (90.5%) among 74 nasal swabs which are under study table (1, 2), this result is slightly higher than what reported in previous studies on college student in Brazil (40.8%) [16],and in more recent studies in India reported that the rate of approximately (88.2 %)found to be positive Staphylococcus and (52.2%) of them MRSA were isolated [15], other found different ratio such as in two studies with pre-clinical medical student showed that (35. 2%) and (29%) were *Staph. aureus* nasal carriers [11,12]. The prevalence of MRSA in the apparently healthy community of Eask Sikkim was estimated to be (11.1%)a total of 129 (46.1%) among 280 healthy individual screened were nasal carriers of *Staph. aureus* ,similar findings were reported by Anwar *et al*, cited by Majuandar D *et al* [23] .

In their study in Lahore, Pakistan who screened 1024 and 636 apparently healthy persons from urban and rural area respectively for nasal carriage of *Staph. aureus* and MRSA and reported thaturban area prevalence of nasal carriers of *Staph. aureus* was estimated to be(16.99%) but rural areas was (11.32%) and the prevalence of MRSA in urban areas was found to be(22.98%) against (11.11%) in rural areas [8]. In a study by Lamikanra and colleagues [15] it was observed that (56.4%) of healthy Nigerian students were nasal carriers of *Staph. aureus*, Tanakaetal. While studying *Staph. aureus* in healthy individuals in Japan reported (24.3%) of them to be nasal carriers [11], another study conducted at university of Taxas, reported that 99 (58%) of 170 isolates are *Staphylococcus aureus* [22].

Also we found that there was no statistically significant difference in the prevalence of *Staph. aureus* between male and female subjected in the present study, this finding was contrary to that observed in the study done in Nigreian population where females harbored *Staph. aureus* more often than males [15], but most studies agree with our result that no significant sex differences [15, 19, 20, 21]and all of them agree with that increasing of colonization on healthy population in older adult between 18-40 years old which affected by many factors some of them depend on sampling, quality of culture media, using enriched media, population under study, geographical area,civilization of the country little knowledge about factors that make one person to be chronic carriers or transient carriers [2], so it need further investigation and epidemiological studies including genotyping to understand the dynamics of spread of MRSA in the community in more details. Table (3) shows that from the 67 (90.5%) MRSA isolates 21, (31.4%) were mannitol fermenter and 45 (68.6%) were non mannitol fermenter *Staph. aureus*, and this strain was first reported as subtype of epidemic strain C MRSA-1 in Canada research lab. [10], this strain of n.m.f. *Staph. aureus* which is community strain found to be different in there biochemical activity from m.f. MRSA *Staph. aureus* such as weakly positive tube coagulase test and gelatinase negativeand MR/VP variable this strain need further molecular analysis because it show high prevalence in community so further study is need. In table (4) the sensitivity pattern of MRSA isolates 67 (90.5%) to antibiotics show rate of resistance toward some antibiotics, Keflex(30%), Erythromycin(22.5%), Tetracycline (13.5%), Gentamycin (9%), Ciprofloxacin (6%) and Vancomycin (4.5%). PantandRai [17] reported rate of resistant for *Staph. aureus*, some antibiotics, Ampicillin (38.1), Erythromycin (33.3%1, Cloxacilin (14.3%), Gentamycin (9.5%)and Methicillin (9.5%) respectively. In comparison to these result we can see that our value nearly equal to some values, while other study like in Nepal medical study show high rate of resistant for MRSA isolates It show resistance toward cloxacilin (68.8%) , follow by ofloxacin (40.7%), Tetracycline (15.6%), Erythromycin (9.4%),ciprofloxacin (6.3%) , and Vancomycin (3.1%). The different in antibiotic resistant pattern may be due to different in " community strain, personal factors such as antibiotic therapy.

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