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Nasal Carriage of Vancomycin- and Methicillin-Resistant *Staphylococcus aureus* among Intermediate Students of Urban and Rural Schools of Muthanna Province in Iraq

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Abstract

Staphylococcus aureus is one of the common causative agents of infections, from asymptomatic carriers to healthy individuals. It can colonize anterior nares of carriers with a high capability to resist different antibiotics. Students are susceptible to bacterial infection due to some factors, including poor health habits and surrounding school conditions. This study screened the rate of vancomycin- and methicillin- resistant Staphylococcus aureus nose carriers among secondary students in rural and urban schools and its association with some sociodemographic factors. The study sample included 300 male/female students aged 15-20 years from 12 schools of rural and urban areas during the period from November 2020 till May 2021. It was found that males are 2.3 times more of MRSA nose carriage and the rate of infections was higher in rural schools than urban whether among males or females. The prevalence of MRSA was 72/300 (24%) among students with 15/72 (21%) MDR-MRSA isolates with high resistance to Clindamycin and Erythromycin at rate 46% and 42% respectively, and a resistance ranging between (20-26) % for Gentamycin, Levofloxacin, Trimethoprim/Sulfamethoxazole, Rifampin, and Nitrofurantoin with high sensitivity to Vancomycin at 4% of resistance. There was no significant association between MRSA incidence with both medication and chronic diseases despite the 19% of students were self-medicating. Most schools were suffering from a shortage of potable water, disinfectants, and first aid materials. Students lack health awareness about transmissible diseases with unhealthy habits spread among students, as specialized health teams did not visit most schools.

Keywords: Methicillin, Vancomycin, MRSA nose carriage, Muthanna province

معدل الحمل الانفي بالمكورات العنقودية الذهبية المقاومة للفانكومايسين والمثيسيلين لطلاب المتوسطة في المدارس الحضرية والريفية لمحافظة المثنى في العراق

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الخلاصة

المكورات العنقودية الذهبية هي أحد العوامل الشائعة المسببة للعدوى من حامليها بدون أعراض إلى الأفراد الأصحاء. المكورات العنقودية ممكن ان تستعمر الفتحات الانفية الأمامية مع قدرة عالية على مقاومة المضادات الحيوية المختلفة. الطلاب عرضة للعدوى البكتيرية بسبب بعض العوامل، بما في ذلك العادات الصحية الخاطئة والظروف المدرسية المحيطة. فحصت هذه الدراسة معدل الحمل الأنفي للمكورات العنقودية الذهبية المقاومة للفانكومايسين والميثيسيلين بين طلاب المرحلة الثانوية في المدارس الريفية والحضرية ودراسة مدى ارتباطها ببعض العوامل الاجتماعية والديموغرافية. شملت الدراسة ٣٠٠ طالب وطالبة تراوحت أعمارهم بين ١٥ و٢٠ سنة من ١٢ مدرسة من المناطق الريفية والحضرية لمحافظة المثنى. وجد أن الذكور هم أكثر عرضة للإصابة من الاناث بمقدار ٢,٣ مرة وكان معدل الإصابة أعلى في المدارس الريفية منه في المناطق الحضرية سواء بين الذكور أو الإناث. كانت نسبة وجود بكتيريا المكورات العنقودية المقاومة للمضاد الحيوي المثيسيلين (المرسا) بين الطلاب هي ٧٢/٣٠٠ (٢٤٪) كان منها ١٥/٧٢ (٢١٪) متعددة المقاومة للمضادات الحياتية. كانت مقاومة العزلات البكتيرية عالية للكليندامايسين والإريثروميسين بمعدل ٤٦٪ و٤٢٪ على التوالي، بينما تراوحت المقاومة بين (٢٠- ٢٦) ٪ لجنتامايسين، ليفوفلوكساسين، وتريميثوبريم / سلفاميثوكسازول، ريفامبين، ونتروفورانتوين مع حساسية عالية للفانكومايسين بمقاومة ٤٪ فقط. لم يكن هناك ارتباط معنوي بين الاصابة بالمرسا مع كل من نسبة التداوي الذاتي والأمراض المزمنة بالرغم ان نسبة التداوي الذاتي كانت ١٩٪. معظم المدارس كانت تعاني من نقص بمياه الشرب، المطهرات، ومواد الاسعافات الاولية والطلبة كانوا يفتقرون الى الوعي الصحي حول الامراض الانتقالية بالإضافة الى بعض العادات الغير الصحية كما ان الفرق الصحية المتخصصة لم تزور معظم المدارس.

كلمات مفتاحية: الميثيسيلين، الفانكومايسين، معدل الحمل الانفي بالمرسا، محافظة المثنى

Introduction

Staphylococcus aureus is a leading source of infections ranging from superficial skin infections (SSTI) to invasive infections and death [(1)]. Different types of infections caused by *S. aureus* including skin infections, bacteremia, bone infections, endocarditis, food poisoning, pneumonia, and toxic shock syndrome [(2)]. One species of the staph germ, called methicillin-resistant *Staphylococcus aureus* (MRSA), is not easy to cure because MRSA is not eliminated by certain antibiotics used to treat other staph germs.

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For an extended period, methicillin-resistant *S. aureus* has been recognized as a pathogen associated with healthcare settings, but in the 1990s, community-associated infections of MRSA had emerged. MRSA infections cost millions of dollars and tens of thousands of patients around the world, with severe infections causing deaths between different ages [3]. Some healthy persons usually have staph germs on their body parts like skin and noses, and these persons are known as carriers, but they contagious to other people [4]. Carriers of staph germs are at risk to be colonized by staph that makes them sick. The most common transmission methods of staphs are skin-to-skin contact and personal items such as towel and clothing then enters damaged skins through scratches and cuts. The infections can diffuse deeper and affect the blood and other organs such as the heart, brain, lungs causing life-threatening infections [5].

Many community members are at a high level of risk of infection with MRSA, including athletes, prisoners, hospitalized patients, students, and military personnel [6]. Students are considered among the community groups at risk of MRSA infection, as most of the risk factors are existent in schools, especially in Iraq, such as overcrowding of students in classrooms and poor sanitation, as indicated by many previous Iraqi studies [7, 8]. In addition, the level of health education is low among students in Iraq, which is a direct cause of the spread of many transmissible diseases among them [9]. Therefore, there is an urgent need to focus more scientific research to discover some factors that may increase the spread of infection among students.

Subjects and Methods

Population and study design:

A cross-sectional survey study was conducted during November 2020 till May 2021 and worked on collecting nasal swabs with relevant medical information from high school students in rural and urban schools for both males and females aged 15-20 years. The 300 student that equally distributed as 150 participants with 75 males and 75 females for both of urban and rural schools, participants who abstained from giving the nasal swabs (n = 18) and who did not send the results of the questionnaire (n = 15) were excluded from the study. The questionnaire included a set of health information collected to investigate whether they are risk factors for infection with MRSA included medical counsel, chronic diseases, and medication in addition to investigating some unhealthy habits of students such as the rate of washing hands and exchanging personal items. Most of the answers were simplified in order not to include long and complex choices that may confuse the student's reliability and accuracy in the answer.

Detection of methicillin-resistance *S. aureus* and antibiotics susceptibility tests:

Both or one of the anterior nares were sampled with normal saline moistened sterile rayon-tipped swab and then placed in Amies transport medium (Oxoid, UK) and all specimens were cultured within a maximum of two days. Each swab was cultured on blood agar (HiMedia, India) and incubated for 24 hours at 37°C. Colonies with β-hemolysis were picked up and inoculated on both mannitol-salt agar (Oxoid, UK) and MRSA CHROMagar (HiMedia, India) with incubation for 24 hours at 37°C. Mannitol fermenter with positive green-bluish colonies on MRSA CHTOMagar were confirmed by slide/tube coagulase test [10]. The confirmed *S. aureus* isolates were stored for long preservation in 15% glycerol brain heart infusion broth at minus 30°C. The antibiotic susceptibility profile of methicillin-resistant *S.aureus* isolates was determined according to CLSI, (2017) [11] using Kirby-Bauer disc-diffusion method against eight antibiotics of different classes as following (Gentamycin 10 µg, Levofloxacin 5 µg, Erythromycin 15 µg, Clindamycin 2 µg, Nitrofurantoin 300 µg, Trimethoprim-sulfamethoxazole 1.25/23.75 µg and Rifampin 5 µg) with Etest for Vancomycin 0.016-256 µg.

Statistical analysis:

The electronic questionnaire data that included the students' answers were received, sorted individually, then entered into Excel and then into the SPSS version 23. The Chi-Square Test of Independence and Binomial Logistic Regression Test were performed to find out the degree of correlation between the incidence of MRSA nose carriage and other factors. The significant differences between male/female and rural/urban were analyzed using Mann–Whitney U Test. A p-value that ≤ 0.05 is statistically significant.

Results

The sociodemographic information showed that the proportion of participants that received medication was 33% with 14% of medical consultation which indicating about 19% of students underwent treatment without medical examination (Table 1). About 10% of the students were found to have arranged of chronic diseases including diabetes, asthma, physical urticarial, and psoriasis.

Table 1.Sociodemographic characteristics of 300 elementary school participants in Al-Muthanna province

Characteristics	Categories	Frequency n=300	Percentage
Residence	Urban	150	50
	Rural	150	50
Gender	Male	150	50
	Female	150	50
Chronic disease	Yes	31	10
	No	269	90
Medication Consultation	Yes	42	14
	No	258	86
Medication	Yes	100	33
	No	200	67
MRSA Carriage	Yes	72	24
	No	228	76
Characteristic	Urban (n= 150)	Rural (n=150)	Total (n=300)
Chronic Disease	15(10%)	16(11%)	31(10%)
Medical Consultation	19(13%)	23(15%)	42(14%)
Medication	51(34%)	49(33%)	100(33%)
MRSA Carrier	30(20%)	42(28%)	72(24%)

It was obvious that the health teams affiliated with the Ministry of Health or Education did not visit most schools, as 78% of students indicated that they were not visited or examined by any health teams. In addition, schools do not consistently have the simplest health requirements, such as water and soap, as all students who were asked about the availability of water and soap answered that they are not available at all times, which indicates that schools suffer from a shortage of water and detergents most of times. The lack of basic requirements for hygiene was not the only problem, as the questionnaire showed that schools lack first aid materials such as gauzes and wound disinfectants, as the students who were exposed to wounds and scratches (n = 93) during the school times only 27% of them received primary treatment for wounds, while the rest did not receive any type of treatment where their scratches and wounds remain susceptible to contamination. The sources of bacterial infection were not limited to poor school health facilities, as there was either deliberate negligence or ignorance on the part of the students. The results indicated that 61% of students borrow or exchange clothes among themselves, such as sports clothes. The biochemical and cultural properties of nose swabs revealed that 127/300 samples were β-hemolytic and only 78/127 samples were mannitol fermenter while 72/78 samples were coagulase-positive and grew on MRSA CHROMagar (figure 1).

Figure 1. Positive growth of MRSA/VRSA isolates on CHROMagar with greenish colonies due to the cleavage of chromogenic substrate

The factors of resident, medication, chronic diseases, and the medical consultation had no correlation with the MRSA nose carriage except the factor of gender, which has been proven to be associated with and contribute to increasing the prevalence of MRSA infections where males have a 2.3 probability higher for MRSA carriers than females (Table 2).

Table 2. Chi-square test of association between MRSA nose carriage and sociodemographic background of students.

Characteristics		MRSA Carrier		
		yes	no	Correlation
Residence	**Urban**	30	120	0.105
	Rural	42	108	
Gender	**Male**	47	103	0.003
	female	25	125	
Medical Consultation	**Yes**	10	32	0.975
	No	62	196	
Medication	**Yes**	22	78	0.57
	No	50	150	
Chronic Disease	**Yes**	7	24	0.845
	No	65	204	

No significant difference between male and female regarding treatment intake, medical consultation, and chronic diseases rate, wherefore these factors were excluded as possible reasons for high MRSA colonization among male (47/72, 65%) in comparison of female (25/72, 35%). Although the results of the statistical analysis did not indicate the existence of statistically significant differences between male and female infections in the rural and urban schools, it is still higher in rural than urban schools, if the infections increased by seven in the rural, the statistical difference would be 0.025 (Figure 2).

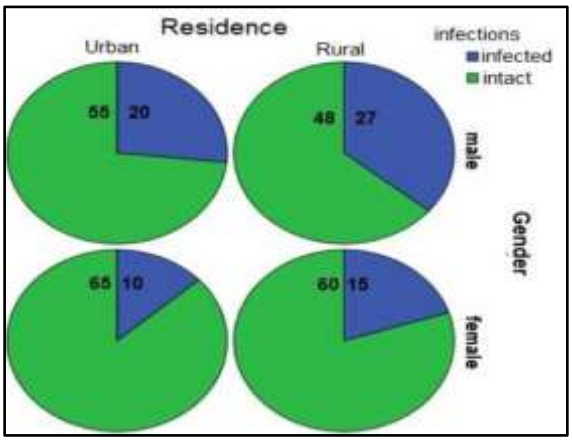

Figure 2. The prevalence of MRSA Nasal carriage among male and female in urban and rural schools

The antibiotic susceptibility profile of 72 MRSA isolates was interpreted according to the guideline of CLSI, (2017) (11), the isolates showed a varying resistance to the eighth antibiotics, where the highest resistance was recorded against Clindamycin and Erythromycin at rate above 40% in comparison to the resistance of other antibiotics of Gentamycin, Levofloxacin, Rifampin, Trimethoprim-sulfamethoxazole, and Nitrofurantoin which was ranging between 20% to 25% with high sensitivity to Vancomycin at rate of 4% of resistance as shown in figure (3). 15/72 MDR-MRSA isolates were detected at rate 21% (12 MRSA and 3 VRSA)

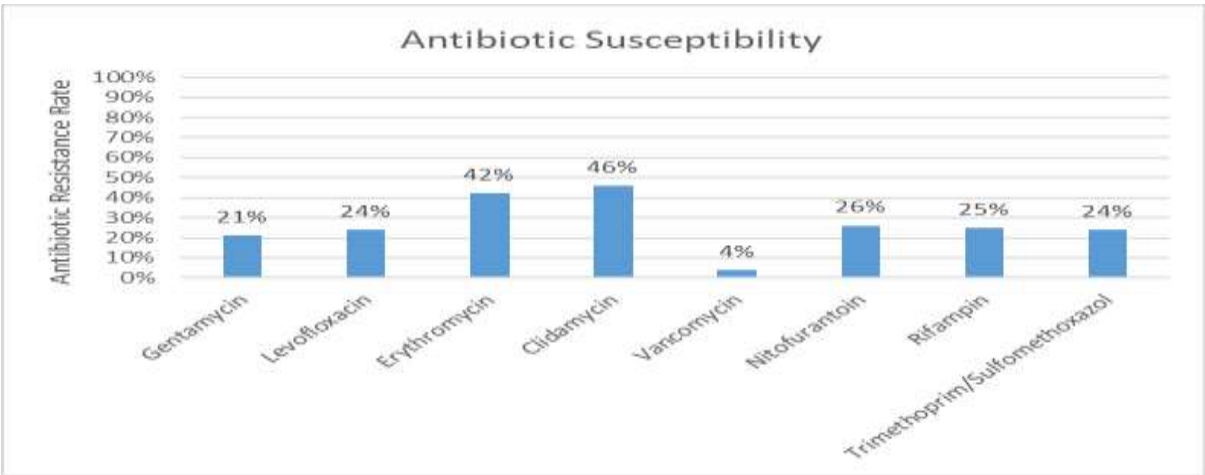

Figure 3.Antibiotic susceptibility profile of 72 MRSA isolates against eight antibiotics agents of different classes

Discussion

The results of questionnaire indicated that about 19% of students take treatments or what is known as self-medication without medical consultations which were more among the urban than the rural population as noted in Table (1).

Taking treatments without medical advice may not contribute to treating the disease, but rather causes an exacerbation of the health problem and the development of some side effects such as the development of drug allergy in the patient and an increase in antibiotic resistance and may even cause

death [12]. The cultural level has a role in increasing self-medication, as the higher the educational level of the family, the more self-medication will be resorted to, and this explains its rise in urban areas [13, 14]. About 10% of students suffer from chronic diseases mainly diabetes, asthma, and physical urticarial with no difference between urban and rural students. While some students suffer from respiratory and health problems, it was found that most of the schools in Iraq are destroyed and lack the most basic elements of a healthy environment, the most important of which are good ventilation and clean classrooms [15]. The present study also revealed that all students indicated that they had never been visited or checked up by any of specialist health teams.

There are many ways of transmitting bacterial infection among the members of society, including exchanging or borrowing personal items for infected persons, including clothes [16]. In the sense of the results, more than half of the students showed a lot of behavior, which is one of the main causes of bacterial transmission such as the exchange and borrowing of sports clothes between them because Iraqi schools lack educational health programs that teach students some healthy behaviors [17]. The failure was also evident by the health teams affiliated to the Ministry of Health and Ministry of Education, which either visited schools nominally or did not visit schools at all [18]. It is not surprising that schools suffer from a shortage of water; students do not find water for washing most of the time, in addition to the lack of safe drinking water [6, 8]. Among the most important causes of bacterial infection is the poor or neglectful treatment of wounds. Even superficial wounds need direct cleaning and sterilization, as open wounds are susceptible to bacterial infection [19]. About 73% of the students who were exposed to wounds during school time did not receive the initial treatment for wounds such as sterilization or putting on gauze, and this is completely consistent with a previous study that indicated the same problem where some students had to return to their homes to seek treatment which means that their wounds remain for several hours, exposed to bacterial contamination [20]. The issue goes beyond poor sanitation and school environment, as some schools lack first aid materials [21] and even if they are available, it has been found that educational personnel are not trained or lack primary medical information and have not been engaged in training courses on how to do first aid [22].

The results of the cultural properties showed that about a quarter of the students 24% of the students were MRSA carriers. The percentage of MRSA nose carriers is considered high, but it is expected in Iraq whereas in a similar study in Kurdistan, Iraq, the percentage was very close to the current study, as it was about 20% in general, whether in rural or urban students [23] but in another similar study, Hussein et al., (2019) [24] reported that the percentage of students carrying MRSA was about half. MRSA isolates found to have 100 percent of resistance to Cefoxitin and Oxacillin with about 40% of resistance to Clindamycin and Erythromycin especially among MDR-MRSA isolates that agree to a large extend with the results of Kistler et al., (2018) [25], Montravers and Eckmann (2020) [26], and Ullah et al., (2020) [27]. Over the past 10 years, there has been an increased resistance to Clindamycin where the resistance ratio is doubled 8 times [26]. Kistler et al., (2018) [25] reported that the resistance to Clindamycin and Levofloxacin increased consistently several times over the past years. Although vancomycin was the most effective agent with the lowest resistance reaching 4% in comparison to other antibiotics, there was a worrying number of increasing resistance to vancomycin reaching 14% by MRSA isolates recovered from clinical samples [28]. The multi-drug resistance isolates can be defined as that resist to one or more agents of three or more antimicrobial classes [29]. About 21% of MRSA isolates were multi-drug resistant isolates that were higher than results obtained by relatively similar study Pathak et al., (2010) [30] and close to Arali et al., (2016) [31] while no similar studies were found about Iraq.

The degree of correlation or influence of some factors that may contribute to an increase in MRSA nose carriage was studied. These factors did not prove their role in raising the infection, such as the self-medication and chronic diseases, but the factor of gender was very influential as the results showed that males are more susceptible to infection than females. The gender was found to significantly affect the increase in MRSA infections among males for reasons that have not been determined [32]. It was also indicated that the residence factor was not statistically significant to increase MRSA infections, but at the same time, it had a role in increasing the number of infections between males and females in rural students more than in urban, as shown in Figure 2.

Males were more affected by MRSA that may be due to multiple reasons, including levels of some vitamins such as vitamin D, as well as elevated smoking habits among males [33]. Among the other reasons that may increase the incidence of MRSA among males is the difference in some habits among them, for example, the level of sterilization and hand hygiene, in general, males are less sterilized than females that may be due to mingling between males or the practice of some sports [34]. Also, among the possible reasons that may increase the infection rate of MRSA among males are some habits such as going to public places such as football and sports halls and barber shops that may lead to sharing shaving tools and sports clothing [35].

It was noted that housing in rural areas contributes to an increase in the incidence of MRSA nose carriage. There are three main strains of MRSA which are livestock acquired MRSA LA-MRSA, community acquired CA-MRSA, and hospital acquired HA-MRSA [(36)]. The reason for the increase in MRSA nose carriage among students in rural areas may be due to the transmission of MRSA from animals to humans, either through direct contact with animals or indirect contact with animals' products such as milk, meat, and wool [(37)]. The coexistence between infected animals and humans, or vice versa leads to the transmission of infection between them. Where it was found that students, who visited animal farms even once, are more likely to be diagnosed with MRSA [(38)]. There is a clear difference between the level of hygiene between students in rural and urban schools. In general, the culture of hand hygiene and the availability of sterilizers, soap, and health facilities are to a lesser degree in rural schools [(39)] which may be the cause of an increase in the bacterial nose load and the spread of some other diseases more.

Conclusion

The results showed a high rate of MRSA nose carriage among students of intermediate schools, where male students are more prone to infections either in rural or urban schools and the countryside contributed to increase the prevalence of MRSA/VRSA nasal carriage in both genders.

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