

THE PREVALENCE OF *S. AUREUS* NASAL COLONISATION AND ITS ANTIBIOTIC SENSITIVITY PATTERN AMONGST PRIMARY SCHOOL PUPILS

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ABSTRACT:

Staphylococcus aureus is one of the most adaptable human pathogens. Nasal *Staphylococcus aureus* is the main cause of community associated staphylococcal infections. This project aimed to study the prevalence of nasal carriage of *Staphylococcus aureus* and community-associated methicillin resistant *Staphylococcus aureus* (CA-MRSA) and evaluate their antibiotic susceptibility tests among primary school children at Zakho city, Kurdistan region, Iraq. Nasal swabs were taken from a total of 300 primary school pupils aged 8-12 years. Collected nasal swabs were processed according to the standard bacteriological culture and isolates were identified using mannitol fermentation, gram stain, catalase test and coagulase test. Antimicrobial susceptibility test was carried out on Muller-Hinton agar (MHA) to determine the susceptibility of *S. aureus* and CA-MRSA towards antibiotics. 30% (90/300) of the primary school children carried *S. aureus*. The nasal carriage of MRSA was 4% (12/300) among participants. All MRSA isolates were sensitive to vancomycin, doxycycline, amikacin and ciprofloxacin. This study showed that the incidence of *S. aureus* and CA-MRSA is comparable with reports from elsewhere. Measures are needed to keep the emergence and transmission of these pathogens to a lowest. Antimicrobial susceptibility testing of all *S. aureus* isolates is crucial for treatment of MRSA. Further studies are required to detect the risk factors of the acquisition of MRSA.

KEYWORDS: *Staphylococcus aureus*, CA-MRSA, Nasal carriage, Iraq.

1. INTRODUCTION

Staphylococcus aureus is a coagulase-positive, catalase-positive, and gram-positive bacterium. *S. aureus* constitutes one of the most important causes of nosocomial infections. *S. aureus* is naturally commensal bacteria inhabiting the anterior nares in one out of every three individuals (Kluytmans *et al.*, 1997). *S. aureus* nasal carriage is persistent in about 20% of the population, occurring more commonly in children than in adults and it is intermittent in about 60% of the population (VandenBergh *et al.*, 1999). Methicillin resistant *Staphylococcus aureus* (MRSA) was almost exclusively a problem in hospitals and other health-related settings. But, currently it is the cause of frequent outbreaks in the general public causing significant mortality. In consequences, the descriptive terminology now refers to community-associated MRSA (CA-MRSA) and health care-associated MRSA (HA-MRSA) (David and Daum, 2010). The nasal carriage of MRSA is well known to represent a major risk factor for transmission and subsequent infection of these bacteria (Wertheim *et al.*, 2005). MRSA is currently endemic and even epidemic, in many hospitals, long-term care centers and publics (David and Daum, 2010). MRSA strains could be distributing into the healthcare centers rather than the other way around (Carleton *et al.*, 2004). It is worth mentioning that the CA-MRSA is further virulent than its hospital counterpart (Braga *et al.*, 2014). Published data about MRSA colonization in healthy children in Iraq is limited. The aims of this work were to study the prevalence of *S. aureus* and CA-MRSA in the anterior nares of healthy pupils at primary schools in Zakho city, Kurdistan region, Iraq and to study their susceptibility to different antibiotics.

2. MATERIALS AND METHODS

This community-based cross sectional study was directed to assess the occurrence and antibiotic susceptibility pattern of *S. aureus* and MRSA among primary school children. A total of 316 students aged 8 to 12 years from different primary schools were recruited for this study from December 2015 to July 2016 following standards microbiological methods. Sixteen students (5%) were excluded from this study because of hospitalization and admission to healthcare facilities. The included pupils (300) were scanned for incidence of *S. aureus* and MRSA. Sixty two percent (186/300) of the participants were males and others were females. Swabs were moistened with sterile distilled water and then nasal swabs were taken from anterior nares of the pupils. Sample swabs were inoculated into transport medium and transported within an hour of its collection to laboratory for further analysis. This study was carried out with the approval of ethics committee in the School of Sciences, University of Zakho.

2.1 Data Collection for Epidemiological Risk Factors

Pre-designed structured questionnaires were used to collect the epidemiological risk factors related to medical history and demographic. It was including: Existence of respiratory infection, antibiotic usage in the past four weeks, surgery, hospitalization of self or family member for the previous year, residence in a long-term care facility within one year of the MRSA culture date, indwelling catheter through the time of culture and history of previous MRSA infection, presence of chronic disease and having member of healthcare staff in the family.

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2.2 Laboratory Analysis of *S. aureus* and MRSA Isolates

Nasal swabs were examined for *S. aureus* and MRSA. Swab samples were directly inoculated on mannitol salt agar and incubated for 24 hours at 35°C. Positive suspected colonies were identified as *S. aureus* strains based on Gram stain, morphology and biochemical tests (catalase and coagulase) (Gerard et al., 2010).

2.3 Antimicrobial Susceptibility Testing

Staphylococcus aureus isolates were screened for antibiotics resistance using agar dilution assay methods according to the recommendations of the Clinical Laboratory Standards Institute (CLSI) (CLSI, 2006).

The bacterial isolates were screened for antibiotic susceptibility on Muller-Hinton agar plates (Oxoid Limited, Hampshire, England) with different antibiotics (Table 1) and BHI agar plates were used for vancomycin resistance.

Table 1. Antimicrobial agents used in this study

| Antimicrobial group | Antimicrobials (Abbreviation) | MIC (µg/mL) |
|----------------------------|-------------------------------|-------------|
| Beta-Lactams (Penicillins) | Oxacillin (Oxa) | 6 |
| Quinolones | Ciprofloxacin (Cip) | 4 |
| Aminoglycosides | Amikacin (Amk) | 64 |
| Glycopeptides | Vancomycin (Van) | 6 |
| Tetracyclines | Doxycycline (Dox) | 16 |

3. RESULTS

A total of 300 primary school children with 186 (62%) boys and 114 (38%) girls were included. A total of 90 (30%) of the isolates were grown on mannitol salt agar with a golden-yellow color were considered as potential *S. aureus* colonies. The identification as an *S. aureus* colony was confirmed by gram stain, catalase and coagulase tests. The antimicrobial susceptibility test was carried out and 4% (12/300) of the isolates were shown to be resistant to oxacillin (i.e., MRSA). The *S. aureus* nasal colonization was 25.3% (47/186) in male students and 37.7% (43/114) in female students. The MRSA nasal colonization in male and female students were 3.8 (7/186) and 4.4% (5/114) respectively. Fortunately, all isolates were sensitive to Vancomycin, Doxycycline, Amikacin and Ciprofloxacin.

4. DISCUSSION

Staphylococcus aureus is a main bacterium colonizing humans with a noticeable level of emerging resistance to most existence antimicrobial drugs (Cohen, 2007). Nasal *S. aureus* have been associated in community infections such as soft tissue infections (Kluytmans et al., 1997) and hospital infections like bacteremia (Holton et al., 1991). CA-MRSA classically causes skin and soft-tissue infections, however several patients with CA-MRSA develop serious conditions such as endocarditis, pneumonia, septic arthritis or osteomyelitis (David and Daum, 2010).

In the present study, about 300 healthy primary school children aged between eight to twelve years were enrolled. The nasal carriage of *S. aureus* was 90 (30%). This result was slightly different with studies reported in Iraq and neighboring countries. For example, the present finding showed that the *S. aureus* colonization has increased compared to previous studies in Iraq (18.4%) (Habeeb et al., 2014). *S. aureus* colonization in different studies varied from 27.1% to 37% in Iran and Turkey respectively (Yildirim et

al., 2007, Nikfar et al., 2015). The variation in colonization with *S. aureus* might be due to fact that there are several factors that play roles in spreading of *S. aureus* and MRSA such as age, lacking hygiene lifestyles, crowded living environments, direct skin-to-skin contact, hospitalization, drug abuse and frequent antibiotic exposure, sharing of individual objects (Braga et al., 2014).

In this study, the MRSA isolation rate was found to be 12 (4%). Other studies in different countries showed variation in the MRSA colonization. The nasal colonization of MRSA was 0.8% in USA (Kuehnert et al., 2006), 4.6% in Saudi (Alaklobi et al., 2015), 5% in Turkey (Yildirim et al., 2007) and 7.1% in Jordan (Alzoubi et al., 2014). The spread of MRSA is increasing and it has become problematic worldwide. This spread can be potentially reduced by controlling of the risk factors such as antibiotic use, admission to an intensive care unit and hospitalization, invasive indwelling tools, mechanical ventilation (Cohen, 2007).

In current study, the prevalence of *S. aureus* nasal carriage was higher in male students. This result in agreement with previous studies showing that *S. aureus* nasal carriage rate was higher in males than females (Sedighi et al., 2011, Ugwu et al., 2016). While other studies showed that female carriage rates were higher than males (Rijal et al., 2008, Kejela and Bacha, 2013). There are difference between gender in susceptibility toward pathogens and in response of the adaptive immune, which has in part a physiological basis in reproductive hormone modulation of immune defense, microbial virulence, and cell physiology (Humphreys et al., 2015).

Antibiotic resistance is increasing globally. This increasing, especially to commonly used antimicrobials, has important clinical implications for the empirical use of antimicrobial drugs (Assafi et al., 2015, Polse et al., 2016). The MRSA isolates had a broad range of different antibiotic-resistance patterns (Adwan et al., 2013). Fortunately, all MRSA strain in this study showed susceptibility toward tested antibiotics. Although there is the potential for increased resistance, tested antibiotics can be important options that can be used for eradication of MRSA colonization.

5. CONCLUSION

This study concluded that the incidence of *S. aureus* and MRSA is comparable with studies carried out in other places. Measures are required to keep the emergence and transmission of these pathogens to a minimum. Antimicrobial susceptibility testing of all clinically *S. aureus* isolates is crucial for treatment of MRSA. Further studies using advanced techniques such as PCR are required to identify the risk factors of carrying MRSA.

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كورتيا ليكولينى:

تبت گوتنى كو (ستافلوكوكس اوربوس) ئيك ژ وان جوړه بهكترياييت زيانبهخشن كو نه خوشى دغه هېنت مروغان، و ستافلوكوكس اوربوس سهرجاويهكى سهرهكى يه بو نه خوشيا دفتى ل دف مروقى. نه ف كه كولينه هاتيه كرن سهر رېژا بهربلاقبوونا نه خوشيا دفتى ب بهكترياو ستافلوكوكس اوربوس و بهكترياو مارسا ل بچووكين قوتابخانا سهره تاي ل باژېرئ زاخو، ههرېمن كوردستان، عيراقن. ساميل هاتنه وهرگرتن ژ دفتن (ب سواپى) ژ ۳۰۰ قوتابين قوتابخانين سهره تاي كو ژېي وان دناقه را ۸-۱۲ ساليي دا بين. و ساميل ئينانه تاقېگه هې و جانن سهر (الوسط المانيتول) پاشان هندك تاقېكرن دى سهر هاتنه كرن وهكى (صبغة كرام، اختبار الكتاليز، اختبار التخثر و اختبار الحساسية للمضادات الحيوية على وسط هنتون اكار). هات دياركرن كو ۳۰% (۳۰۰/۹۰) ژ قوتابين قوتابخانين سهره تاي كو كهل خو راتكرن بهكترياو ستافلوكوكس اوربوس. و نه خوشيت دفتى ب بهكترياو مارسا گه هشته ۴% (۳۰۰/۱۲) ناف قوتابيان دا. و همى نه خوشيت مارسا اويت ديار بوين ل فئ كه كوليني دا حساس بين بو فان (فانكوماييسين، دوکسى سايكلين، اميكاسين و سبروفلاكسين). ل فئ كه كوليني دا دياربوو رېژا توشبوونى ب بهكترياو ستافلوكوكس اوربوس و مارسا دگونجيت له گهل وان كه كوليني ل هندك جهيت دى هاتينه نه نجام دان. (اختبار الحساسية لمضادات الميكروبات) بو همى ستافلوكوكس اوربوس بهنته كرن بو جارسهركرنا نه خوشيا مارسا. ل كه كوليني پاشه روژى دا يا پيتقى يه وان كارتېكرين دبه نه گه رې وهرگرتنا مارسا بهنته دهست نيشانكرن.

الخلاصة

تعتبر المكورات العنقودية (*Staphylococcus aureus*) واحدة من اكثر الكائنات الامراضية التي تصيب الانسان نجاحا وتكيفا. الاصابة الانفية المكورات العنقودية هي من المصادر الرئيسية للاصابة المكتسبة من المجتمع. ان الهدف الرئيسي لهذه الدراسة هو دراسة مدى انتشار الاصابة الانفية لبكتريا المكورات العنقودية و المكورات العنقودية المقاومة للميثيسلين وتقييم حساسيتها للمضادات الحيوية بين الاطفال في المدارس الابتدائية في مدينة زاخو، اقليم كوردستان، العراق. اخذت المسحات الانفية من مجموع ۳۰۰ تلميذ في المدارس الابتدائية باعمار ۸-۱۲ سنوات. المسحات الماخوذة من الانف تم معاملتها وفقا للمزارع البكتريولوجية القياسية وتم تحديد العزلات عن طريق تخمر المانيتول، صبغة كرام، اختبار الكتاليز و اختبار التخثر. اختبار الحساسية للمضادات الحيوية تم على الوسط الغذائي مولر هنتون اكار. ۳۰% (300/ ۹۰) من اطفال المدارس الابتدائية كانوا حاملين لبكتريا المكورات العنقودية. الاصابة الانفية ببكتريا مارسا كانت ۴% (300/ ۱۲) بين المشاركين. جميع عزلات المارسا كانت حساسة للفانكوماييسين، دوکسى سايكلين، اميكاسين و سبروفلاكسين. هذه الدراسة بينت انه مدى حدوث بكتريا المكورات العنقودية و بكتريا مارسا هي مطابقة للتقارير في الاماكن الاخرى. قياسات يجب ان توخذ للحفاظ على نشوء وانتقال هذه السلاسلات للحد الادنى. اختبار الحساسية لمضادات الميكروبات لكل عزلات المكورات العنقودية هي اساسية لعلاج المارسا. دراسات مستقبلية مطلوبة لتحديد عوامل الخطورة لاكتساب المارسا.