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Colonization of methicillin-resistant Staphylococcus aureus (MRSA) among health workers at Al-Hussein teaching hospital in Al-Nasiriyah city, Iraq

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Abstract

Hospital-acquired, methicillin-resistant Staphylococcus aureus is a common cause of nosocomial diseases among hospitalized patients. Healthcare, workers can transmit these bacteria from patients to another causing nosocomial disease. HA-MRSA can cause pneumonia, endocarditis, osteomyelitis, and brain abscesses. Unfortunately, colonization and prevalence of HA-MRSA in our health system are not estimated or studied yet and our study is the first to assist colonization of HA-MRSA among health-care workers at Al-Hussein Teaching Hospital in AL Nasiriyah city, Iraq. Since MRSA can be transmitted from patients to another via health workers who in touch with patients, our aim comes to study and report the colonization of HA-MRSA among health workers in post-surgical wards, intensive care units (ICU) and cardiac care units (CCU).

On 2019 we conducted a study that included 60 participants of health-care workers who work in the post-surgical wards, intensive care units, and cardiac care units. Nasal and skin swabs were collected from each participant using transporting media (Amies swab) and cultured on Mannitol Salt Agar, blood agar and Mueller-Hinton agar. To confirm the identification of the isolated colonies, we use the VITEK 2 system.

Nasal collected swabs showed high growth of MRSA 42 (40%), while skin swabs showed growth of 15 samples (25%). Isolated bacteria were identified as MRSA by mannitol salt agar and cefoxitin diffusion disk test since this test is considered the best marker for mecA mediated methicillin resistance. Staphylococcus aureus that grew and fermented mannitol salt agar (yellow color) and showed zone ≤ 21 mm for cefoxitin disk (30 µg) diffusion test was considered as MRSA.

Antibiotics sensitivity test has been conducted to explorer the resistant of MRSA for antibiotics and selection of antibiotics was subjected to the clinical laboratory standards institute (CLSI) by using the VITEK 2 system.

الخلاصـــــــــــــة:

المكورات العنقودية المقاومة للمضاد الحيوى (الميثاسيلين) هى احدى المسببات الشائعة لعدوى المستشفيات بين المرضى الراقدين. الأشخاص العاملين فى المستشفيات بإمكانهم نقل البكتريا من مريض الى اخر عبر التماس مما يؤدى الى انتشار العدوى فى المستشفيات للمرضى الراقدين. تسبب البكتريا المقاومة العديد من الامراض والتى تتضمن ذات الرئة والتهاب صمامات القلب والتهاب العظام إضافة الى خراجات الدماغ.

انتشار واستعمار هذا النوع من البكتريا داخل المستشفيات لم يتم دراسته مسبقا لذا تعد الدراسة الحالية هى الأولى التى تتولى دراسة انتشار هذه البكتريا بين العاملين فى مستشفى الحسين التعليمى فى مدينة الناصرية \ العراق فى ردهات الجراحة و العناية المركزة.

تضمنت الدراسة شمول ٦٠ مشترك من العاملين فى المستشفى المذكور فى ردهات الجراحة والعناية المركزة بالإضافة ردهات العناية القلبية. تم جمع العينات من التجويف الانفى ومن الجلد لكل عامل صحى باستخدام المسحات المزودة بوسط مغذى للحفاظ على البكتريا من التلف. بعد اكمال جمع العينات تم زراعتها على أوساط الدم **blood agar** و وسط المانيتول **Mannitol salt agar** إضافة الى استخدام جهاز التشخيص **VITEK2** لتشخيص البكتريا.

أظهرت النتائج عزل البكتريا المقاومة من مسحات التجويف الانفى **nasal swab** بمقدار ٤٦ عزلة (٤٠٪) من أصل ٦٠ مسحة. اما بالنسبة الى المسحات المأخوذة من الجلد فقد تم عزل الجلد **skin swab** فقد تم عزل ١٥ (٢٥٪) من أصل ٦٠ مسحة. تم تأكيد العزلات باستخدام الوسط الزرعى **mannitol salt agar** اذ تخمر البكتريا الوسط فيتحول الى اللون الأصفر ولتأكيد ان البكتريا مقاومة تم استخدام فحص أقراص **Cefoxitin disk (30µg)** إذا تم اعتبار العزلة مقاومة للميثاسيلين إذا كان قياس حلقة التثبيط اقل او يساوى ٢١ مليمتر.

تم اجراء اختبار الحساسية الدوائية على العزلات المقاومة لبيان مدى ضراوة البكتريا المنتشرة بين العاملين وتم اعتماد المعهد القياسى للمختبرات السريرية لاختيار المضادات الحيوية باستخدام جهاز **VITEK 2 system**.

Introduction:

Hospital-acquired, Methicillin-resistant *Staphylococcus aureus* (HA-MRSA) has been a common cause of nosocomial infections in the hospitals including healthcare-associated infection (Pathak and others, 2010; Yamasaki and others, 2019). MRSA is a strain of *Staphylococcus aureus* which contests semi-synthetic penicillin-like (methicillin, nafcillin, and oxacillin). Also, it contests cephalosporins, aminoglycosides, quinolones, and macrolides so MRSA showed multidrug-resistant (van Hal and others, 2007). Moreover, infection with MRSA has been reported among dialysis patients who interacted with the healthcare system (Wang and others, 2010).In addition, MRSA showed a high incidence of infection in many European and American hospitals, the percentage has ranged from 29% to 35% (Stapleton and Taylor, 2002; Tenover and others, 2001). Furthermore, 50% of hospital-acquired HA-MRSA were recovered from patients in intensive care units while 40% were recovered from non-ICU patients (Pathak and others, 2010; Tenover and others, 2001; Verma and others, 2000).

HA-MRSA involves in many nosocomial infections in hospitalized patients including pneumonia, endocarditis, osteomyelitis, septic arthritis, meningitis, brain and spinal epidural abscess (Liu and others, 2011). Due to the resistance of antibiotics, MRSA management needs to get concern and improve personal hygiene by washing hand and narrowing possibility of spreading it in hospitals. It is important to recognize the difference between colonization and infection. Colonization indicates the presence of the organism without symptoms of illness. Due to the exposure, the health workers are more likely colonize to MRSA than persons in the general population which showed 20% to 30% (Cespedes and others, 2002).Studies showed the screening of MRSA among health workers and taking the managements have a positive impact regarding outbreak and reduced spreading diseases caused by MRSA among hospitalized patients (Ben-David and others, 2008; Blok and others, 2003).

Since there was no study showed the prevalence and colonization of methicillin-resistant *Staphylococcus aureus* among health workers in Iraqi hospitals. Aim of this study came to elucidate the prevalence of MRSA among health workers at Al-Hussein teaching hospital in Al-Nasiriyah, Iraq and how can that effects spreading diseases among hospitalized patients during health care provide.

Materials and methods

Samples collection:

Samples were collected from 60 health-care workers who work in the post-surgical wards, intensive care units at Al-Hussein Teaching Hospital in Al-Nasiriyah city on 2019. Both gender male and female were included in this

study. Two samples were collected from each participant, one from nasal and the second from the skin. All samples were collected and transported to the main lab using transporting media (Amies swab).

Culturing and isolating of MRSA:

Collected samples were directly cultured on a blood agar plate. The growth of isolated bacteria was further tested by coagulase test to confirm *Staphylococcus aureus* bacteria. Then cultured on mannitol salt agar to further identified and confirm the growth of *Staphylococcus aureus* since this species can ferment mannitol and turning the media to the yellow because increase the acidity of the media. Data showed that the major mechanism of resistance of MRSA to β-lactam antibiotics is due to the acquisition of the mecA gene encoding an additional penicillin-binding protein (Hartman and Tomasz, 1984). The next step was to confirm the resistance of isolated bacteria which presumptively identified as *Staphylococcus aureus* through using cefoxitin disk diffusion test. This test is considering the best test to induce the *mecA* gene when compared to oxacillin and methicillin (Bonjean and others, 2016). Isolated *Staphylococcus aureus* bacteria were inoculated on Mueller-Hinton agar and cefoxitin disks (30 µg) were placed on the surface and incubated at 37C^{o} overnight. Next day, the inhibiting zone were measured using an electronic ruler and zones that showed more-than or equal to 22 mm considered susceptible to cefoxitin disks (30 µg). In addition, extra confirmation to the identification of *Staphylococcus aureus* with antibiotic sensitivity test was conducted using the VITEK 2 compact automated device (bioMerieux) using gram-positive and AST cards. Selection of antibiotics is subjected to the recommendation of the clinical and laboratory standard institute (CLSI, 2019).

Statistical analysis

Our hypothesis regarding the colonization of MRSA is that nasal colonization of MRSA is totally independent of skin colonization for the same participant. In another way, the colonization of MRSA in nasal not necessarily has to face itscolonization in the skin for the same participant. GraphPad Prism software (version 6.01) used to calculate Chi-square and P-value< 0.05 was considered significant (Sun and others, 2019).

Results

Colonization of MRSA

Our current data showed the prevalence of MRSA among health-care workers at Al-Hussein teaching Hospital. Out of 120 collected swabs, 60 nasal and 60 skin for each participant has been collected, MRSA colonization of nasal samples were 24 samples (40%) out of 60 nasal collected samples. While skin swabs the prevalence was 15 positive samples (25%) out of 60 skin samples. Our hypothesis relied on that the nasal

colonization is independent of skin colonization in the same participant. Meaning, colonization of MRSA in the nasal sample is not necessarily faced it colonization of MRSA in skin sample. Chi-square statistical analysis between nasal and skin colonization in the same participant showed no significant difference with *P* value (0.079) (Figure 1). That means the nasal colonization is totally independent of skin colonization in the same person.

Figure (2): Cefoxitin disk diffusion test (30 µg) on Mueller-Hinton agar showing growth (resistance) of MRSA against Cefoxitin disk (FOX= Cefoxitin; susceptible: > 22 mm).

	Nasal	Skin	Marginal Row Totals
MRSA (Positive)	24 (19.5) [1.04]	15 (19.5) [1.04]	39
MRSA (Negative)	36 (40.5) [0.5]	45 (40.5) [0.5]	81
Marginal Column Totals	60	60	120 (Grand Total)

The chi-square statistic is 3.0769. The *p*-value is .079411. This result is *not* significant at $p < .05$.

Figure (1): Colonization of MRSA among health-care workers. Nasal samples showed higher colonization 24 (40%) compared to skin samples 15 (25%).Statistical analysis showing that nasal colonization is totally independent from skin colonization.

Antibiotics susceptibility test:

Antibiotics sensitivity tests were conducted using the VITEK 2 compact automated device (bioMerieux). Gram-positive colorimetric identification and antibiotic cards have been inoculated with MRSA suspension (0.50-0.63) McFarland turbidity range. Antibiotics selection is subjected to the clinical laboratory standards institute (CLSI), USA, 2019. VITEK 2 results confirmed that the isolated bacteria are MRSA according to the resistance of Beta-Lactamase, Cefoxitin Screen which showed (100%) (Table: 1).

Antibiotics	S	R	I	Antibiotics	S	R	I
Beta-Lactamase		39 (100%)		Linezolid	39 (100%)		
Cefoxitin Screen		39 (100%)		Teicoplanin	34 (86.9%)	3 (7.8%)	2 (5.3%)
Benzylpenicillin		39 (100%)		Vancomycin	15 (38.4%)		24 (61.6%)
Oxacillin		38 (97.5%)	1 (2.5%)	Tetracycline	18 (46.1%)	21 (53.9%)	
Gentamicin	21 (53.8%)	15 (38.4%)	3 (7.8%)	Tigecycline	39 (100%)		
Tobramycin	21 (53.8%)	15 (38.4%)	3 (7.8%)	Rifampicin	26 (66.6)	13 (33.4%)	
Levofloxacin	13 (33.3%)	20 (51.2%)	6 (15.5%)	Trimethoprim/ Sulfamethoxazole	30 (76.9%)	9 (23.1%)	
Moxifloxacin	20 (51.2%)	4 (10.4%)	15 (38.4%)				
Erythromycin	17 (43.5%)	22 (56.5%)					
Clindamycin	17 (43.5%)	20 (51.2%)	2 (5.3%)				

Table (1): Antibiotics susceptibility test (AST) for staphylococcus Methicillin resistance (MRSA) showing percentage of sensitivity and resistance of antibiotics. S: Sensitive, R: Resistant and I: Intermediate.

Discussion:

Since MRSA involved in many nosocomial infections among hospitalized patients and health-care workers and showed the transition of this bacteria from patients to another during hospitalization, we sought to focus on this part and figure out a solution and instruction which can reduce prevalence MRSA in hospitals. MRSA is characterized by beta hemolytic on blood agar as showing in (figure: 2) and resist to cefoxitin (figure: 3) that features give that bacterium a significant virulence impact to cause diseases.

Figure (2): Methicillin resistant staphylococcus (MRSA) cultured on blood agar, showing the beta hemolysis of RBCs.

Previous publications data revealed that nasal carriage of MRSA in general population was 6.3% and 17.8%, while among health care workers the prevalence of MRSA was 18.2% and 43.8% (Hartman and Tomasz, 1984; Yamasaki and others, 2019). On the other hand, a study revealed that 29.7% of the third year of medical student carried of *Staphylococcus aureus* but not MRSA (Treesirichod and others, 2013). A study that carried in Oman, data showed 311 participants enrolled, nasal colonization with HA-MRSA was found in 47 individuals (15.1%). HA-MRSA was also isolated from the cell phone surfaces in 28 participants (9.0%), 5 participants (1.6%) showed positive results both from their nasal swabs and from their cell phones. Antibiotic resistance to erythromycin (48%) and clindamycin (29%) (Pathare and others, 2016). These data were relatively close to our date that showed 56.2% and 51.2 % for erythromycin and clindamycin respectively. Furthermore, vancomycin-resistant was 9.3% of isolated MRSA from nasal carriage (Pathare and others, 2016). Our results showed no resistance of isolated MRSA to vancomycin antibiotics among health workers. Another study showed that MRSA resistance to wide range of antibiotics which

involved Gentamycin 36 (100%), Rifampin 10 (27.78%), Ciprofloxacin 36(100%), Moxifloxacin 36(100%), TMP 9(25%), Vancomycin (0%), Clindamycin 35(97.22%) and Linezolid 1(2.78%) (Kaur and Chate, 2015). These results were approximately close to our as showed in (Table: 1).Differentiation among our results and others might be involved first, the difference of geographical regions that study been conducted. Second, environmental causes also can be involved since we know our health system is totally different from that in Europe and USA in ways of treatment options, diagnosis processes and treatment. Finally, quality of medicine and types of antibiotics that used is also might be another reason since evaluation requirement at another countries are highly subjected to the restricted protocols and evaluation before release to market compare to our country.

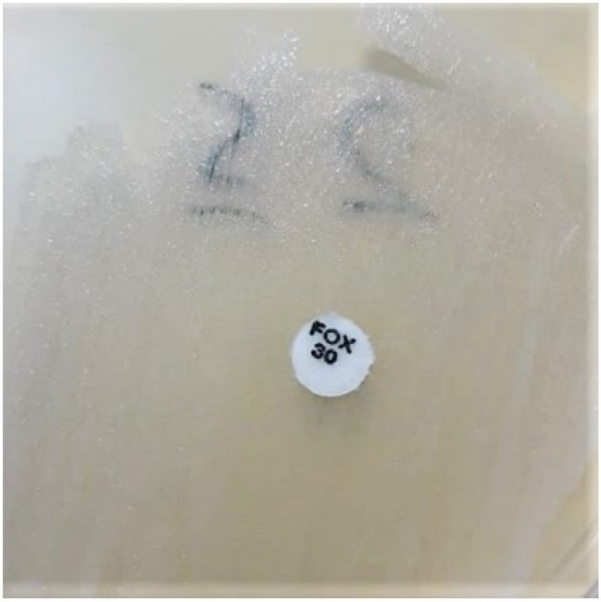

Figure (3): Cefoxitin disk diffusion test (30 µg) on Mueller-Hinton agar showing growth (resistance) of MRSA against Cefoxitin disk (FOX= Cefoxitin; susceptible: > 22 mm).

Conclusion:

Since the prevalence of MRSA among health workers in our health system in Iraq is not estimated, we tried to explorer the colonization of this type of microorganism and need more studies to study the real effects of MRSA on the diseases outbreak inside hospitals. Our study suggested that should be a sanitizing requirement and hand hygiene and must be followed by all health provider including nurses and physicians. We highly recommended that all health workers should follow WHO cleaning, disinfection and sterilization instructions. Also, we notice that Al-Hussein teaching hospital in Al-Nasiriyah city is overloaded and that increases chances of contamination of wards. We strongly recommended reducing the number of patient's visitors and the city need another hospital to contain the population. On the other hand, disinfection and sterilization process should be conducted by trained workers that involved in infections and disease control courses and that is not found in our health system since all disinfection and sterilization processes are done by not training workers. Furthermore, all disposable equipment or parts like oxygen masks must be discarded after one use and cannot be reuse to another patient because that might increase the colonization and prevalence of MRSA among patients and health worker since the health workers provide health care for patients.

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