Prevalence and Pattern of Antimicrobial Susceptibility of Methicillin-Resistant and Methicillin-Sensitive Staphylococcus Aureus in North Jordan

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Abstract

The main goal of this study is to determine the prevalence and pattern of antimicrobial susceptibility of methicillin-resistant (MRSA) and methicillin sensitive Staphylococcus aureus (MSSA) in north Jordan. The study also aims to evaluate the (MRSA) latex screen test for the detection of (MRSA). One hundred and fifty isolates of *Staphylococcus aureus*, that were received at the clinical microbiology laboratory of the faculty of medicine at Jordan University of Science and Technology from two major hospitals in the north part of Jordan from September of 2007 to Febrauary of 2008, were included in the study. Ninety isolates were obtained from clinical specimens and sixty isolates from carrier patients. *Staphylococcus areus* strains were identified morphologically and biochemically by standard laboratory tests and procedures. Antimicrobial susceptibility was performed by the disk diffusion (Kirby-Bauer), and Minimum Inhibitory Concentration (MIC) method was used to identify methicillin resistance. The pattern of resistance to seven antimicrobials (Vancomycin, Cephalothin, Clindamycin, Cortimaxizole, Erythromycin, Gentamycine, and Penicillin G) showed that none of the intrinsic-resistant isolates were resistant to vancomycin. The majority of the isolates was recovered from anterior nares (60%) and the least from peritoneal and synovial fluid (0.7% each). The majority of isolates were recovered from surgical specimens (40%) and the minority from the ophthalmology department (1.3%). The pattern of antimicrobial succeptability was as follows: MSSA (n=123) ,MRSA (n=27), Vancomycin (100%, 100%), Caphalothin (99.2 %, 85.2%), Clindamycin (95.9 %, 88.9%), Cotrimaxizole (98.4%, 85.2%), Erythromycin (81.3%, 40,7%), Gentamycin (95.9%, 85.2%), and penicillin G (4.9%, 0%). The diffusion test showed a sensitivity of 100 and 88.9 and a specificity of 100 and 86.2, respectively. The pattern of antimicrobial susceptibility of *S. areus* differs between methicillin-sensitive and resistant isolates. Except for penicillin, most of methicillin-sensitive isolates were susceptible to nearly all antimicrobial agents used in this study.

Keywords: MRSA, prevalence, Antimicrobial susceptibility.

(J Med J 2009; Vol. 43 (1):15-20)

Received	***Accepted***
July 14, 2008	*August 28, 2008*

Introduction

The most significant problem of drug resistance among staphylococci is their resistance to the penicillins, particularly methicillin. *Staphylococcus aureus* is recognized as both community-acquired and nosocomial pathogen. [1,2] Since the 1970s, this organism has been resistant to penicillinase-resistant Beta-

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lactamase such as Methicillin, Oxacillin, and Nafcillin. [3- 6] Historically, these isolates have been referred to as methicillin-resistant *S. aureus* (MRSA), because when first noted, methicillin was the only penicillinase-resistant Beta- lactam used for in vitro testing and in vivo treatment of infection. While Oxacillin replaced methicillin, the acronym MRSA remained the common usage. [7]

Methicillin and Oxacillin resistance has been attributed to the acquisition of the mec A gene, which encodes a low affinity penicillin-binding protein (PBP-2a). [8-11] Expression of Methicillin resistance in *S. aureus* is heterogeneous. [12,13] Detection of this heteroresistant phenotype can be difficult due to various factors such as inoculum size, incubation time and temperature. pH of the medium and medium salt concentration. [14] Precise determination of Oxacillin resistance is possible by conforming the presence of the mec A gene. [15] However, this technique is impractical in routine clinical practice. Similar heteroresistant phenotype for coagulase-negative Staphylococcus has been described in the nervous system. [16, 17] Nosocomial MRSA isolates are resistant to many other non Beta-lactam agents, whereas community-acquired MRSA isolates that are resistant to Beta-lactam agents commonly remain susceptible to non Beta-lactam agents. [18] The prevalence and antimicrobial susceptibility to MRSA varies markedly from one country to another and from hospital to hospital in the same country. This study aims to investigate the prevalence and pattern of antimicrobial susceptibility of both methicillin-resistant and methicillin –sensitive *S. aureus* in order to help physicians treating related problems in formulating first line treatment (empirical) in two referral hospitals in north Jordan.

Materials and Methods

The study population was the in-patients and out-patients of two referral hospitals in north Jordan. Ninety samples were isolated from clinical specimens, whereas 60 samples were isolated from carrier patients.

Isolates of *S. aureus* were identified on the basis of colonial morphology, gram staining, fermentation of manitol and the results of catalse and tube coagulase tests.

Identification of MRSA was performed in three methods. 1) Antimicrobial susceptibility testing, 2) Agar, 3) disk diffusion method, 4) Determination of minimum inhibitory concentration (MICs) of Oxacillin by agar dilution method and 5) Slide latex agglutination test (MRSA screening). The *S. aureus ATCC* 29213 strains were used as a negative control and MRSA ATCC 4330 were used as a positive control for MRSA screening latex test.

All specimens were handled according to the standard microbiological procedures. Subjects participated in this study agreed and signed a consent form to participate in the study, which was approved by the Human Research Committee. Isolates were fresh, 18-24 hours old cultures were grown at 35 Cº. After the extraction of penicillin binding protein (PBP2) according to the manufacturer's instructions, specimens were stored at 4Cº for later testing on the same day to avoid repeated freezing and thawing.

Results

1) Prevalence of MRSA infections

Out of ninety isolates of S. aureus, 22 (24.4%) were MRSA. Nine were isolated from infected wounds, three from ear infection, three from respiratory tract infection, two from abscesses, two from urinary tract infection, two from skin lesions, and one from bone (table1). Four of the isolates (from ear and urinary tract infection) were obtained from out patients. The 18 isolates from inpatients were derived from different departments. *Staphylococcus aureus* collected from the first hospital (Prince Rahma Teaching Hospital for Children) showed higher rate of resistance to methicillin than those collected from the second hospital (Prince Bade'ah Teaching Hospital), a difference that was statistically significant, $P > 0.05$.

2) Prevalence of MRSA colonization

Out of 60 *S. aureus* strains isolated from the anterior nares, five (8.3%) were shown to be MRSA, therefore , *S aureus* isolates from *Staphylococcus* infections showed significantly higher rate of resistance to methicillin than those isolated from nose of hospitalized patients (P >0.001).

4) Agar dilution method

For MRSA infections, 22 (24.4%) out of 90 *S. aureus* isolates had oxacillin-resistance (Oxacillin MIC > 4ug/ml). Fourteen (16%) isolates were Oxacillin-resistant (Oxacillin MIC > 8ug/ml, designed as "intrinsic resistance). Eight (8.9%) isolates showed a borderline-resistance (eg. Oxacillin MIC of 4 to 8ug/ml). Concerning the nasal carriage, five (8.3%) of 60 *S. aureus* isolates had oxacillin-resistance. One (1.7%) isolate was defined as intrinsic-resistant and 4 (6.7%) isolates showed a borderline-resistance.

One hundred and twenty-three (82%) isolates had oxacillin MIC of >2 ug/ml and were classified as methicillin-sensitive. The prevalence of intrinsic and borderline resistance varies according to the source of specimen (table 1).

5) Disk diffusion method

By using oxacillin disks, organisms showing an inhibition zone of > 13mm with no microorganisms within the zone were interpreted as susceptible to oxacillin. Organisms showing an inhibition zone of <13mm, or with microorganisms growing within the inhibition zone, were interpreted as resistant. Concerning susceptibility to antimicrobials, Vancomycin was the most active antibiotic against *S. aureus* where all isolates were susceptible to this agent regardless of susceptibility to methicillin. Antimicrobial Susceptibility test is shown in table (2). Results of antimicrobial susceptibility are shown in table (3).

Table 1: The distribution of intrinsic and borderline resistance among 150 clinical isolates of S. aureus.

Source of the specimen	*No. (%) of Specimens*	*No(%) of Intrinsic - resistance*	*No. (%) of Borderline-Resistance*	*Total*
Anterior nares	*60 (40)*	*1 (1.7)*	*4 (6.7)*	*5*
Ear	*28 (18.7)*	*1 (3.5)*	*2 (7.1)*	*3*
Wound	*17 (11.3)*	*6 (35.3)*	*3 (17.6)*	*9*
Abscess	*10 (6.7)*	*1 (10)*	*1 (10)*	*2*
Urine	*8 (5.3)*	*1 (12.5)*	*1 (12.5)*	*2*
Blood	*8 (5.3)*	*0 (0)*	*0 (0)*	*0*
Bone	*4 (2.7)*	*2 (50)*	*0 (0)*	*2*
Post operation	*4 (2.7)*	*2 (100)*	*0 (0)*	*2*
Sputum	*1 (0.7)*	*0 (0)*	*1 (25)*	*1*
Skin lesion	*2 (1.3)*	*2 (100)*	*0 (0)*	*2*
Eye discharge	*2 (1.3)*	*0 (0)*	*0 (0)*	*0*
Peritoneal fluid	*1 (0.7)*	*0 (0)*	*0 (0)*	*0*
Synovial fluid	*1 (0.7)*	*0 (0)*	*0 (0)*	*0*
Total		*16*	*12*	*28*

Table (2): The pattern of antimicrobial susceptibility in 150 clinical isolates of S. aureus.

Antibiotic	*MSSA (%) (n=123)*	*MRSA (%) (n=27)*
Vancomycin	*123 (100)*	*27 (100)*
Cephalothin	*122 (99.2)*	*0(0)*
Clindamycin	*118 (95.9)*	*24 (88.9)*
Cotrimaxizole	*121 (98.4)*	*23 (85.2)*
Erythromycin	*100 (81.3)*	*11 (40.7)*
Gentamycin	*118 (95.9)*	*23 (85.2)*
Penicillin G	*6 (4.9)*	*0 (0)*

Table (3): The number of strains of S. aureus susceptible or resistant to oxacillin by two different methods.

Method	*MIC > 4 ug/ml Positive strains (n=27)*	*MIC < 2ug/ml Negative strains (n=123)*	*Sensitivity (100%)*	*Specificity (100%)*	*Efficiency of test (100%)*
Disk diffusion	*24/27*	*106/123*	*88.9*	*86.2*	*88.2*
MRSA Screen Latex Test	*27/27*	*123/123*	*100*	*100*	*100*

Discussion

In a previous study that investigated the prevalence of MRSA infection in north Jordan in 1993, [19] the prevalence was 8.8%. In this study, it appears that the prevalence of MRSA has relatively increased; almost tripled (from 8.8% to 24.4%) and they have emerged as important pathogens in north Jordan partially due to the indiscriminate use of antibiotics. There were no previous studies in north Jordan about the nasal carriage of MRSA of in-patients, and results of this study indicate low carriage. Among 60 *S. aureua* isolates obtained from anterior nares of patients, the prevalence of MRSA colonization was 1.7%. Therefore, methicillin-resistant isolates from *Staphylococcus* infections were significantly higher than those among *S. aureus* causing infection, and than those isolated from the nose of hospitalized patients ($p > 0.001$). These findings are consistent with those reported by Beilman' et al. [20] who pointed out to the increasing incidence of MRSA. Subsequent reports demonstrated the increasing prevalence of community-acquired MRSA in the United States.[21]

In a recent study by Otth et al. from Chilli, the antimicrobial susceptibility of *Staphylococcus aureus* isolated from patients and carrierswas studied. It was worrisome in it that 2% of *S. aureus* strains obtained from hospitalized patients showed intermediate resistance to vancomycin. [22] These are alarming signs that resistance is emerging in even the best drug of choice for the treatment of MRSA, which consequently emphasizes the need to investigation and research for new antimicrobials.

With the spread of (MRSA) and methicillin-resistant coagulase-negative Staphylococci in hospitals, rapid and reliable methods for their detection are needed in order to provide choice of appropriate antimicrobial therapy. [23] In this study, the MRSA latex screen test was found to be the method of choice to identify MRSA; this result is in agreement with previous studies about the suitability of latex screen test for MRSA identification. [24-26]

This study showed that the pattern of Antimicrobial Succeptability of *S. aureus* differs between methicillin-sensitive and methicillin-resistant isolates. Except for penicillin, most methicillin-sensitive isolates were susceptible to almost all antimicrobial agents used in this study.

Conclusion

The prevalence of MRSA infections in the northern part of Jordan was relatively high, while the prevalence of nasal carriage and community-acquired MRSA was low. The increasing incidence of MRSA infection nictitates the search for new antimicrobials to combat MRSA spread.

Acknowledgment

We would like to thank the Deanship of Research for their continuous support for this project.

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معدل انتشار وحساسية بكتيريا المكورات العنقودية الذهبية المقاومة والحساسة للمثيسلين في شمال الأردن

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الملخص

يهدف هذا البحث، على نحوٍ رئيس، إلى دراسة معدلات انتشار بكتيريا المكورات العنقودية الذهبية المقاومة والحساسة للمثيسلين في شمال الأردن، وكذلك تقييم الطريقة المعمول بها في المختبرات الطبية لتشخيص هذه البكتيريا وتعريفها.

قام الباحثون بعزل مئة وخمسين عينة من مختلف أقسام مستشفى الأميرة رحمة ومستشفى الأميرة بديعة والعيادات الخارجية فيهما في الفترة من 2007-2008، وقد تم تعريف هذه البكتيريا كما هو متبع في الطريقة الحيوية والشكلية طبقاً لمعايير تشخيص هذه البكتيريا في المختبرات الطبية، كما فُحصت هذه البكتيريا لمعرفة مقاومتها وحساسية فحص الشرائح المعرفة لها لأنواع عدة من المضادات الحيوية الأكثر استعمالاً في هذا المجال، وقد تبين أن هذه البكتيريا لم تكن مقاومه للمضاد الحيوي فانكومايسين، واختلفت مقاومتها للمضادات الحيوية وحساسيتها للفحص على شكل: سيفالوثين (99.2%، 85.2%)، كلندامايسين (95.9%، 88.9%)، كوتريماسيزول (98.4%، 85.2%)، إثرومايسين (81.3%، 40.7%)، جنتمايسين (95.9%، 85.2%)، وبنسلين ج (4.9%، 0%) تباعاً. إن نتائج هذه الدراسات تعطي دعماً علمياً لاختيار أولويات استخدام المضادات الحيوية وطرق الكشف عن هذا النوع من البكتيريا.

الكلمات الدالة: بكتيريا المكورات العنقودية الذهبية المقاومة والحساسة للمثيسلين، معدل انتشار، حساسية.