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ANTIBIOTIC SUSCEPTIBILITY PATTERNS OF METHICILLIN RESISTANT (MRSA) ISOLATES FROM NASAL CAVITY OF UNIVERSITY OF HEALTH SCIENCES STUDENTS

Yeonim Choi¹, Na Young Kim²

Abstract- Staphylococcus aureus and methicillin resistant S. aureus (MRSA) is a major cause of nosocomial infections and is one of the most commonly isolated bacterial species in the hospital and continues to be an important pathogens in both community and hospital-acquires infection. The purpose of this study is to investigate the carrier rate of S. aureus and MRSA in the community and antibiotics susceptibility patterns of these organisms. The identification of S. aureus and MRSA were done by the procedures in Murray's manual of Clinical Microbiology and antibiotic susceptibility patterns by the Clinical and Laboratory Standards Institute (CLSI). MRSA strains were confirms by oxacillin disk diffusion method. Sixty strains (60%) of S. aureus were isolated from the nasal specimens of 100 students in health science. Ten strains (16.6%) of 60 S. aureus were resistant to penicillin and oxacillin. Ten strains of the 60 S. aureus isolates were methicillinresistant Staphylococcus aureus (MRSA). The mecA genes in MRSA were detected by polymerase chain reaction (PCR). Community and nosocomial infections caused by methicillin-resistant Staphylococcus aureus are a significant problem worldwide. There continuous epidemiological study is to investigate the prevalence of MRSA in community acquired infections.

Keywords - methicillin resistant S. aureus (MRSA), mecA genes

I. INTRODUCTION

Staphylococcus aureus is carried in the nose of 25–35% of humans, and it is a common cause of serious and lifethreatening infections. S. aureus bacteria harboring the mecA gene are resistant to methicillin and other β -lactam antimicrobials and are referred to as methicillin-resistant S. aureus (MRSA). Methicillin-resistant Staphylococcus aureus is one of the major human pathogens both in nosocomial and community associated infections, such as skin and soft tissue infections (SSTIs), pneumonia, and bacteremia¹. Various categories of MRSA based on epidemiologic characteristics are commonly used and include healthcare-associated MRSA (HA-MRSA), community-associated MRSA (CA-MRSA) and livestock-associated MRSA (LA-MRSA). Identified risk factors associated with CA-MRSA outbreaks include sharing of personal care products, frequent skin-to-skin contact, skin abrasions and crowded living conditions².

Brook et al. (2009) examined 70 frequently used environmental surfaces in a university of 25 000 individuals for contamination of *S. aureus* and MRSA from 420 samples taken from student desktops, computer keyboards, telephone mouthpieces, water fountains, photocopy keypads, vending machines and elevators buttons over a 3-week period in 2007³. Accurate molecular epidemiological typing has become of primary importance for the classification of MRSA isolates and the control of their spread. In many laboratories, conventional methods of typing such as serotyping and bacteriophage typing have been replaced by PCR-based methods. To investigate the virulence factors of MRSA clinical isolates, SCC*mec* typing were used to analyze the relationship among the three methodologies in this study.

II. RESEARCH METHODOLOGY

One hundred students were enrolled in a study. Sample collection was accomplished by using sterile swabs(BBL CultureSwab, Sparks, MD) containing Stuart's medium inserted approximately 2 cm into one naris, rotated against the anterior nasal mucosa and repeated with same swab in second naris. Nasal swabs were collected from 100 health college students. Samples were cultured quantitatively, and MRSA isolates were confirmed by growth on selective media, coagulase testing and the presence of the *mecA* resistance gene.

The identification of *S. aureus* and MRSA were done by the procedures in Murray's manual of Clinical Microbiology and antibiotic susceptibility patterns by the Clinical and Laboratory Standards Institute (CLSI). All *S. aueus* isolates were screened for methicillin resistance by disc diffusion (6 mg/ml oxacillin) on Mueller Hinton agar with 2% NaCL. Oxacillin-resistant isolates were tested for the presence of penicillin binding protein 29 (PBP 2a) using latex agglutination kit (MRSA latex

¹ Departments of Biomedical Laboratory Science, Songho University Gangwon-do, Korea

² Departments of food culinary arts, Songho University Gangwon-do, Korea

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agglutination test, Oxoid Ltd., Hants, UK). At least one S. aureus isolate which was also PBP 2a positive from given sample was forwarded for molecular testing⁴.

Presence of mecA gene and SCCmec typing were investigated by PCR using primers and previously described conditions^{5,6}. The amplification was performed in a thermal cycler (GeneAmp® PCR System 2700, Perkin-Elmer Cetus, Boston, USA) beginning with an initial denaturation step at 94 $^{\circ}$ for 5 minutes followed by 30 cycles of 94 $^{\circ}$ for 30 seconds, 60 $^{\circ}$ for 30 seconds, and 72 °C for 30 seconds, ending with a final extension step at 72 °C for 5 minutes followed by a hold at 4 °C. The PCR products were analyzed using 2.5% agarose gel electrophoresis with 0.5 µg/ml of ethidium bromide and visualized under UV light.

III. FINDINGS

sixty strains (60%) of S. aureus were isolated from the nasal specimens of 100 students in health science. Ten strains (16.6%) of 60 S. aureus were resistant to penicillin and oxacillin. Ten strains of the 60 S. aureus isolates were methicillin-resistant Staphylococcus aureus (MRSA). Following the antimicrobial susceptibility test, SCCmec, mecA gene from presumptive MRSA strains using a multiplecx PCR(Table 1). Ten isolates were confirmed as MRSA since they carried mecA gene and SCCmec. The mecA genes in MRSA were detected by polymerase chain reaction (PCR). From another single PCR to detect SCCmec and to determine SCCmec type, observed that all ten samples were shown as SCCmec type III, IV. Community and nosocomial infections caused by methicillin-resistant Staphylococcus aureus are a significant problem worldwide. There continuous epidemiological study is to investigate the prevalence of MRSA in community acquired infections(Table 2).

Table 1. Isolation rates of MIRSA isolated from students							
	No. of stu	dents	No. of <i>S. aureus</i> isolated		No. of MRSA isolated, mecA gene		
	Male	Female	Male	Female	Male	Female	Total
100	45	55	27	33	3(5.0%)	7(11.7%)	10(16.6%)
Table 2. SCC	<i>mec</i> types of N	ARSA					
	Ν	No. of SCCmec types					
	Ι		II		III	IV	
Male	C)	0		3	4	
Female	C)	0		0	3	

IV.CONCLUSION

During the past years methicillin resistant S. aureus (MRSA) has emerged as the community acquired methicillin resistant S. aureus (CA-MRSA)⁷. CA-MRSA can be spread by close skin-to-skin contact, touching contaminated stuffs and surfaces, unsanitary and crowded living conditions and poor personal hygiene. These are the usual modes of transmission of CA-MRSA. Most of CA-MRSA strains have the virulence factor, Panton-Valentine Leukocidin (PVL), which is not frequently found in hospitalacquired MRSA strains or methicillin-sensitive S. aureus (MSSA) strains but a low incidence of MSSA was reported, and has been associated with necrotizing pneumonia and death. The most commonly known carrier of the mecA gene is the bacterium known as MRSA. The detection of PVL and methicillin resistance (mecA) genes represents a new tool to aid the early identification of CA-MRSA isolates⁸.

The study was performed on college students taking health science courses. It was shown that there is no high rate of PVLpositive mecA and SCCmec. PVL-positive isolates is more serious but CA-MRSA do always carry PVL gene. This proves that the higher rate of prevalence of the three parameters, PVL-positive, mecA and SCCmec can be acquired through exposure to unsanitary environments. From the results of this first collaborative study on the comparison of prevalence rates in the three different communities, information to elucidate on the three parameters, mecA, SCCmec and PVL gene from CA-MRSA is made available and can be useful to future researchers who wish to probe deeper on MRSA.

Community and nosocomial infections caused by methicillin-resistant Staphylococcus aureus are a significant problem worldwide. There continuous epidemiological study is to investigate the prevalence of MRSA in community acquired infections. Community infection in the hospital is important for students working at laboratory in the future, and further information is needed about the risks of Community infection in this polulation.

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