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CLINICAL LABORATORY SCIENCE **CASE REPORT****Non-hemolytic, Mucinous, Coagulase Negative MRSA Isolated from Urine**Jae Soo Kim¹, Qute Choi², Bo Kyeung Jung³, Jong Wan Kim³, Ga Yeon Kim⁴¹Department of Laboratory Medicine, Dankook University Hospital, Cheonan, Korea²Department of Laboratory Medicine, Chungnam National University College of Medicine, Daejeon, Korea³Department of Laboratory Medicine, Dankook University College of Medicine, Cheonan, Korea⁴Department of Public Health, Dankook University Graduate School, Cheonan, Korea**소변에서 분리된 비용혈성, 점액성, 응고효소 음성 MRSA**김재수¹, 최규태², 정보경³, 김종완³, 김가연⁴¹단국대학교병원 진단검사의학과, ²충남대학교 의과대학 진단검사의학과, ³단국대학교 의과대학 진단검사의학과, ⁴단국대학교 대학원 보건학과**ARTICLE INFO**Received March 30, 2019
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Revised 3rd May 31, 2019
Accepted June 1, 2019**Key words**Coagulase-negative MRSA
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Non-hemolytic MRSA**ABSTRACT**

An 84-year-old woman presented to the emergency department with a chief complaint of pressure sores of the anus. She had a urine catheter when she showed pyuria three times but had no fever. A microscopic examination revealed many grapevine-like Gram positive strains and neutrophils. After 24 hours of urine culture on blood agar, non-hemolytic mucous colonies were found and further enlarged after 48 hours of culture. The capsules were identified after India ink stain. The catalase was positive, but the tube coagulase and latex coagulase were both negative. The *S. aureus* was identified by Vitek-2 and mass spectrometer Vitek MS V-3 IVD. The strain was confirmed by 16S rRNA gene sequencing and multilocus sequence typing (MLST). The phenotypically atypical MRSA found in the tube coagulase and latex coagulase were both negative. MRSA often show no beta hemolysis as in this case but are rarely latex coagulase-negative. We report a woman whose urine culture showed non-hemolytic, tube coagulase-negative, and latex coagulase-negative MRSA.

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INTRODUCTION

Staphylococcus aureus (*S. aureus*) is classified into facultative anaerobic Gram-positive cocci which do not form spore. *S. aureus* is positive both for catalase and coagulase test. Coagulase-negative staphylococcus (CNS) is generally considered to be less pathogenic than *S. aureus* [1]. Meanwhile, the increased prevalence of methicillin

resistant *S. aureus* (MRSA) resulting in increased antimicrobial resistance is difficult to treat [2, 3]. The *S. aureus* is the causative agent of many diseases such as skin infection, pneumonia, endocarditis and osteomyelitis. Some strains produce toxins that cause gastroenteritis, skin elasticity syndrome and toxic shock syndrome. It also causes food poisoning and is one of the most frequently isolated bacterias in patients [1]. The diagnosis is mostly based on Gram stain and culture [3, 4]. The methicillin resistance of *S. aureus* is caused by *mecA* gene which is transcribed to transformed penicillin binding protein (PBP 2a). Vancomycin is an acceptable treatment option

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for MRSA and the recent emergence of clinical isolates resistant to vancomycin has made control of *S. aureus* be difficult [5]. Newer agents such as linezolid, daptomycin, telavancin, and ceftaroline have received regulatory approval for MRSA control [6].

Here, we report the MRSA strain isolated from the urine which show unique characteristics [7-9]. The isolate was non-hemolytic, tube coagulase negative and latex coagulase negative MRSA strain.

CASE REPORT

The patient visited the emergency department on March 3, 2017. She was an 84-year-old woman with pressure sores of the anus. She was transferred from nursing hospital nearby and had dementia and the history of back surgery. She was with urine catheter when admitted and showed pyuria three times but had no fever. The urinalysis was performed; protein 2+, occult blood 3+, WBC 3+ and RBC: 10~29/High Power Field (HPF), and WBC: >100/HPF on microscopic examination. The Gram stain was performed and a large number of Gram-positive cocci clusters and >25 WBCs ($\times 1,000$ magnification) on microscope were observed. The culture on blood agar and MacConkey agar was done following standard method using 0.001 mL disposable loops and incubated at 36°C in 5% CO₂ for 24~48 hours. In the blood

agar medium, a strong mucinous colony with diameter of 2~3 mm was observed above 10⁵ CFU/mL. The mucus was further enlarged after 48 hours of incubation. We assessed the number of bacteria per 1 mL of urine. The catalase was positive but the tube coagulase and the latex coagulase were both negative (Figure 1). The capsules were confirmed by India ink stain (Figure 2). The bacterial identification and antibiotics susceptibility testing were performed using Vitek-2 (Biomerieux Clinical Diagnostic, France). The colony showed antibiotic resistance to penicillin (≥ 0.5) and oxacillin (≥ 4) and susceptibility to gentamicin (≤ 0.5) and vancomycin (≤ 0.5). The MRSA was identified by Vitek-2 and mass spectrometer Vitek MS V-3 IVD (Biomerieux Clinical Diagnostic, France). The 16S rRNA gene sequencing (Table 1, Figure 3) and Multilocus Sequence Typing (MLST) revealed *S. aureus* which is 99.9%

Figure 2. The capsules of *S. aureus* were observed by India ink stain ($\times 1000$).

A

B

C

Figure 1. Non-hemolytic, mucinous, coagulase negative MRSA isolated from urine. (A) Non-hemolytic, mucinous MRSA colony. (B) Tube coagulase negative and (C) Latex coagulase negative MRSA.

agreement with the standard strain ATCC 29213 (Table 2, Figure 4). The patient was treated with vancomycin 15-20 mg/kg IV every 8~12 hr and was discharged after culture-negative.

DISCUSSION

Many of the genes coding for extracellular toxins, enzymes, and cell surface proteins of *S. aureus* are regulated by a 510-nucleotide (nt) RNA molecule, RNAIII. These are controlled by a complex regulatory locus, an gene regulator (*agr*) [10-12], whose unique feature is control of the expression of its target genes by means of an RNA molecule (RNAIII) [13]. *agr* RNAIII acts as a positive regulator on genes which are preferentially expressed postexponentially, whereas protein A and coagulase are inhibited. The kinetics of protein A gene (*spa*) tran- scription has been investigated with *agr* and *agr* mutant strains [14] and shown to be inversely related to the level

of RNAIII. RNAIII had a strict inhibitory effect on *spa* expression. The regulation of *coa* expression is different from that of *spa*, in that coagulase expression may be modulated both positively and negatively by the *agr* system according to the growth stage. Therefore, coagulase expression appears to be both positively and negatively modulated by an *agr*-dependent mechanism [15], and the *agr*-dependent repression effect on *coa* mRNA is most probably the result of a direct binding of RNAIII to *coa* mRNA [16].

We found phenotypically atypical MRSA in that tube coagulase and latex coagulase were both negative. The

Table 1. Primers used for 16S rRNA Sequencing

Primer name	Sequence (5'-3')
27F	AGAGTTTGATCMTGGCTCAG
533F	GTGCCAGCMGCCGCGGTAA
907R	CCGTCGAATTCCTTTRAGTTT
1492R	CGGTTACCTTGTACGACTT

Table 2. Primers for Multilocus Sequence Typing (MLST)

Gene	Primer	Sequence (5'-3')
arc	Forward	TTGATTCACCAGCGGTATTGTC
	Reverse	AGGTATCTGCTTCAATCAGCG
aro	Forward	ATCGGAAATCCTATTTCACATTC
	Reverse	GGTGTGTATTAAATACGATATC
glp	Forward	CTAGGAATGCAATCTTAATCC
	Reverse	TGGTAAAATCGCATGTCCAATTC
gmk	Forward	ATCGTTTTATCGGGACCATC
	Reverse	TCATTAACACAAACGTAATCGTA
pta	Forward	GTAAAAATCGTATTACCTGAAGG
	Reverse	GACCTTTTGTGAAAAGCTTAA
tpi	Forward	TCGTTTATTCTGAACGTCGTGAA
	Reverse	TTTGCACCTTCTAACAATTGTAC
yqi	Forward	CAGCATACAGGACACCTATTGGC
	Reverse	CGTTGAGGAATCGATACTGGAAC

Figure 3. Bacterial identification using 16S rRNA sequencing. (A) Diagrammatic representation of 16S gene and relative primer positions. In addition to highly conserved primer binding sites, 16S rRNA gene sequences contain hypervariable regions that can provide species-specific signature sequences useful for identification of bacteria. (B) Part of sequencing electropherogram.

Figure 4. Multilocus sequence typing [MLST] analysis using PubMLST site (<https://pubmlst.org/>).

MRSA often show no beta-hemolysis like our case patient but rarely latex coagulase-negative. We emphasize the role of 16S rRNA gene sequencing and MLST to fully ensure correct species diagnosis especially when it shows ambiguous biochemical findings [17]. Here, we report a case of an 84-year-old woman whose urine culture showed non-hemolytic, tube coagulase-negative and latex coagulase-negative MRSA.

요약

84세 여성이 항문까지 욕창이 번져 응급실에 내원하였다. 그녀는 근처의 요양기관에서 전원되었고, 입원시 소변 도뇨관을 달고 있었으며 열은 없었다. 그람염색에서 많은 수의 포도상구균과 >25의 호중구(×1000배 렌즈)가 관찰되었다. 24시간 배양한 혈액한천배지에서 용혈이 없는 점액성의 집락이 관찰되었으며 배양 48시간에는 점액성이 더욱 확대되었다. India ink로 염색하여 협막의 모습을 관찰하였으며, 카타라제 양성, 라텍스법 혈액응고효소 음성, 시험관법 혈액응고효소 음성소견을 보였다. Vitek-2와 질량분석기인 Vitek MS V-3 IVD에서 *S. aureus*로 나타났으며, 16S rRNA 유전자 염기서열 분석 및 Multilocus sequencing typing (MLST)에서 *S. aureus*로 표준 균주인 ATCC 29213과 99.9% 일치율을 보였다.

본 증례는 용혈성이 없고, 점액성이 강하며, 라텍스법 혈액응고효소 음성, 시험관법 혈액응고효소 음성소견을 보이는 MRSA를 동정하여 이를 보고한다.

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