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Using multiplex PCR as a diagnostic tool to detect methicillin resistant Staphylococcus aureus

Metisiline dirençli Staphylococcus aureus'u tespit etmek için bir tanı aracı olarak multipleks PCR kullanılması

Sadık Akgün [1], Hakan Sezgin Sayiner [2]

[1] Adiyaman University, Faculty of Medicine, Department of Medical Microbiology, Adiyaman, Turkey
[2] Adiyaman University, Faculty of Medicine, Department of Infectious Diseases and Clinical Microbiology, Adiyaman, Turkey

ORCID ID of the authors
SA: 0000-0002-1413-0450
HSS: 0000-0002-4693-3784

Abstract

Aim: The goal of this report was to deliver the methicillin resistant Staphylococcus aureus (MRSA) reports to the clinician and prevent the treatment delays, investigating the efficacy in addition to diagnostic testing and contact isolation strategies for intensive care unit (ICU) patients with MRSA.

Methods: In this report, 320 Staphylococcus aureus strains identified as coagulase positive were cultured from hospitalized ICU patients between 2015 and 2017. Wound swabs were performed and bacteria cultures were evaluated for identification and antibiotic susceptibility testing using a culture antibiogram. Among these cultures from the swabs, MRSA was identified and subsequently screened for the MecA gene using rapid Multiplex polymerase chain reaction (PCR).

Results: MRSA was detected in 67 of 320 strains, because of oxacillin resistance was detected by working with a fully automated culture antibiogram device. In addition, MRSA positivity was detected because of the high MecA gene expression in 56 of these 67 strains using rapid multiplex PCR.

Conclusion: With greater than 86% sensitivity, patients were able to get early treatment for MRSA due to the rapid screening analysis using Multiplex PCR. This method, as a diagnostic tool, may be of benefit in other diseases.

Keywords: Staphylococcus aureus, Methicillin resistant Staphylococcus aureus, Multiplex PCR, Wound culture

Öz

Amaç: Bu çalışmanın amacı, yoğun bakım ünitesi (YBÜ) hastalarında izole edilen, metisiline dirençli Staphylococcus aureus (MRSA) için tanısal test ve temas izolasyon stratejilerine ek olarak etkinliği araştırarak, MRSA raporlarını klinisyene daha hızlı bir şekilde iletmek ve tedavideki gecikmeleri önlemekti.

Yöntemler: Çalışmaya, 2015 ve 2017 yılları arasında, hastaneye yatırılmış YBÜ hastalarından alınan kültür örneklerinden izole edilen ve koagülaz pozitif olarak tanımlanan 320 Staphylococcus aureus suşu dahil edildi. Yara sürüntüleri alındı ve bakteri kültür işlemleri yapılarak, antibiyotik duyarlılık testi ve identifikasyon işlemleri için değerlendirildi. Swablardan alınan bu kültürler arasında MRSA olarak tanımlanan suşlar daha sonra hızlı Multipleks polimeraz zincir reaksiyonu (PCR) kullanılarak MecA geni için tarandı.

Bulgular: 320 suşun 67'sinde, tam otomatik kültür antibiyogram cihazı ile çalışılarak oksasilin direnci saptanarak MRSA tesbit edildi, Ayrıca hızlı Multipleks polimeraz zincir reaksiyonu (PCR) ile çalışıldığında bu 67 suşun 56'sında MecA geni tesbit edilerek, MRSA pozitifliği saptandı.

Sonuç: Multiplex PCR kullanarak hızlı tarama analizine bağlı olarak, % 86'dan fazla duyarlılık ile hastalar MRSA için erken tedavi alabildiler. Bu yöntem, bir tanı aracı olarak, diğer hastalıklarda da yararlı olabilir.

Anahtar kelimeler: Staphylococcus aureus, Metisiline dirençli Staphylococcus aureus, Multiplex PCR, Yara kültürü

Corresponding author / Sorumlu yazar:
Sadık Akgün
Address / Adres: Adıyaman Üniversitesi, Tıp Fakültesi, Tıbbi Mikrobiyoloji Bölümü, 02100, Adıyaman, Türkiye
E-mail: sakgungizem@hotmail.com

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Introduction

Staphylococcus aureus is a bacterial pathogen that causes numerous complications in humans. Methicillin-resistant Staphylococcus aureus (MRSA) has caused many problems in patients due to it multidrug resistance. MRSA strains have been detected since the early 1960s, soon after methicillin became clinically available [1,2]. MRSA continues to increase in the health facilities and it has undergone rapid epidemiological development, spreading outside the boundaries of hospitals. Due to its resistance to multiple antibiotics, new drugs options have been considered to help reduce the morbidity and mortality rates caused by MRSA [2-5].

Presently, the regulation of MRSA in hospitals is unresolved and affects hospitals worldwide. Furthermore, the use of gloves, gowns, or masks to prevent the spread of MRSA has not been proven effective [6,7]. MRSA has been estimated to contribute to ~150,000 infections in the European Union, which has resulted in 380 million euros of healthcare costs.

In order to determine the best treatment option, timely diagnosis and uncovering the antimicrobial resistance profile is very important [1,2]. Molecular methods for identification of MRSA have been based on the of the mecA gene, which is associated with methicillin resistance and encodes the penicillin-binding protein 2a (PBP-2a).

MRSA detection from non-sterile samples (i.e., nasal samples) often contain coagulase-negative staphylococci and Staphylococcus aureus, and both have the mecA gene. MRSA is differentiated from coagulase-negative staphylococci through targeting mecA from Staphylococcus aureus and femA from coagulase-negative staphylococci. There are many kits available that are suitable to determine MRSA from other methicillin sensitive bacteria [8-10].

This project was coordinated to determine a method for diagnosis of MRSA using Multiplex PCR for the mecA gene from swabs collected from ICU patients.

Materials and methods

Staphylococcus aureus was isolated from the wound swabs of 320 patients hospitalized in the ICU at our Medical Microbiology laboratory between 2015 and 2017. Initially, all of the 320 patient swab specimens were included in the study because the oxacillin resistance of coagulase positive Staphylococcus aureus strains loaded on the culture antibiogram device was unknown. The swabs were removed from the transport container and inoculated on a 5.0% sheep blood agar medium. Then, the cultures were incubated overnight at 37℃ in a bacteriological incubator. Species suspected as Staphylococcus aureus, using conventional methods (with catalase, coagulase, etc.), were examined for MRSA with a fully automated culture antibiogram (Phoenix, BD, USA) and an antibiotic sensitivity test panel (PMIC 381 BD, USA). Subsequently, multiplex real-time polymerase chain reaction (PCR) (BD-MAX, BD, USA) was evaluated as a molecular diagnostic tool to determine MRSA positivity. For the study, 2-3 Staphylococcus aureus colonies on the plates were mixed with distilled water into the tubes, vortexed at high speed for 1 minute with a table top vortexer, and mixed with PCR primers and Taq polymerase. Detection, amplification and analyses were performed using the semi-automated BD MAX platform.

In our study, statistical analyses were performed using the SPSS software (SPSS 15.0, IBM Inc., Chicago, IL, USA). Our results of continuous data analyses were given as minimum, maximum, median, and mean values, and the results of categorical (intermittent) variables as frequency and percentage.

Results

Of the 320 patients, 170 (53%) were male and 150 (47%) were female. Only 67 (20.9%) of the 320 Staphylococcus aureus strains collected were considered to be methicillin-resistant using the full-automated culture and antibiotic sensitivity test with oxacillin resistance. Thereafter, 67 strains of Staphylococcus aureus strains evaluated as MRSA by the Culture-Antibiogram device were examined by rapid PCR. Within 3 hours, mecA was detected in 56 (83.6%) of these strains and therefore reported to the clinician as MRSA. The 11 misclassified (16.4%) Staphylococcus aureus strains were reported as methicillin sensitive Staphylococcus aureus (MSSA) (Table 1).

Table 1: The number of *Staphylococcus aureus* isolated Oxacillin resistance, Non-PCR, PCR (+), and PCR (-)

Total wound culture	Men	1358 (56%)
	Women	1066 (44%)
Staphylococcus aureus isolated	320	
Oxacillin resistance	67	20.0%
PCR (+)	56	83.6%
PCR (-)	11	

Average age: 47.5, Range (oldest - youngest): 87 - 1

Discussion

In a study conducted by Lucke et al. [11] in a region with low prevalence for MRSA, 1,601 culture samples from the nose and other body regions were analyzed by culture and BDGeneOhm MRSA tests. The sensitivity, specificity, and positive and negative predictive values of the assay were found as 84.3%, 99.2%, 88.4%, and 98.9%, respectively. In a report by Widen et al. [12], BD MAX and Xpert MRSA assays were compared and good agreement (97.9%) was demonstrated. They concluded that the BD MAX MRSA assay was a reliable alternative automated system for the detection of MRSA from nares samples. In our study, the predictive values were not calculated because only the MRSA strains that were detected as culture results were tested by the PCR. However, our PCR test results were also quite high (84%) and were consistent with their study.

In a study conducted by Abbadil et al. [13] in Saudi Arabia, 55 Staphylococcus aureus strains were obtained from different hospital units. Strains were swabbed from surgical wounds (n=30) and from the other parts of the body (n=25). They performed real-time PCR on these samples and found that Staphylococcus aureus most frequently found in patients aged 21-40 years (40%). Forty five of these isolates (82%) were determined to be MRSA. With our study, the MRSA positivity in their study was found to be very close to each other.

In the study conducted by Lopez-Alcalde et al [7] they used multiplex PCR to detect MRSA directly from clinical specimens containing a mixture of staphylococci in less than an

hour. Of the 1,657 MRSA isolates tested, 1,636 (98.7%) were detected and the authors concluded that PCR is a rapid and powerful tool for MRSA detection. In our study, we found MRSA positivity close to previously reported values (84%) using the real-time multiplex PCR method.

In the study conducted by Aqel et al. [14] on nasal swabs of health care workers in Jordan, the frequency as well as phenotypic/genotypic features of MRSA was investigated. They discovered MRSA in 56 (7.8%) nasal swabs, which were all resistant to oxacillin. All samples were positive for mecA, while mecC was negative for all isolates. In our study, MRSA strains isolated from wound cultures in ICU patients were used instead of nasal swabs and subjected to real time PCR. For this reason, our numerical values were different from what they found.

In a study by Hogan et al. [15] in Madagascar, nasal MRSA carriage of a group of healthcare workers and non-medical university students was determined. They performed multiplex PCR to determine multiple methicillin resistant and sensitive strains. Of 1,548 analyzed nasal swabs, 171 (11%) were Staphylococcus aureus positive and only 20 (1.3%) were methicillin resistant. In our study, MRSA recovered from ICU patient wound swabs were found to be high (21%).

In a study conducted by Khairalla et al. [16], 1,300 samples from an Egyptian dental clinic were examined for MRSA. From the 34 mecA-positive isolates, the hand swabs from patients, nurses, and dentists were 9.8%, 6.6%, and 5%, respectively. Nasal swabs showed 11.1%, 6.7%, and 9.7%, respectively from these subjects. The environmental swabs showed 1.3% MRSA positivity. The authors reported that the results indicated augmented MRSA pathogenicity in dental wards and stressed a necessity for better surveillance/infection strategies. Because our study included MRSA strains isolated from cultured-antibiotics in patients in the ICU, the mecA gene value obtained by PCR in the studied samples was higher than in their values.

In particular, extension of antimicrobial resistance and identification may result in delays at the onset of antibiotic therapy, and thus a higher mortality and morbidity rate for patients admitted to the ICU. It may also cause a delay in contact isolation, which must be taken to prevent contamination of this microorganism with other diseases. On the other hand, it has been estimated that fast molecular tests used to remove these delays have created a usability problem. However, our study suggests that delays in treatment and isolation measures may be overcome by giving MRSA results at a lower cost and with higher sensitivity. Moreover, we believe that more work related to this issue is needed. Delays in antibiotic administration after severe sepsis recognition increases mortality. Therefore, it is important to start antibiotic treatment as soon as possible, especially in the intensive care units. For this reason, it is very important to start early treatment and diagnosis. Using the multiplex PCR method can reduce cost and time (~3 hours) as compared to culture antibiogram and antibiotic sensitivity tests, which take about 3-4 days.

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