Identification of Methicillin Resistance Genes by Fully Automated Real-Time PCR System in Suspected Cases of PJI and SSI.

Koki Abe[1], Hyonmin Choe[1], Yuta Hieda[1], Masashi Shimoda[1], Hiroyuki Ike[1], Naomi Kobayashi[1], Yutaka Inaba[1]
[1]Yokohama City University, Department of Orthopedic Surgery
Email: t196005f@yokohama-cu.ac.jp

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INTRODUCTION:
Staphylococcus aureus is frequently detected in SSIs, with MRSA reportedly accounting for approximately half of these cases. MRSA is particularly important as an initiator of SSI and PJI and has been reported to be increasing in recent years. Implant insertion is also a risk factor for SSI due to MRSA, and early diagnosis and therapeutic intervention are important when SSI or PJI is suspected after surgery.
Bacterial culture remains the gold standard for diagnosing SSI and PJI. However, there are some cases in which the bacterial culture test is negative, and it takes several days to obtain the results. Therefore, several studies have reported the usefulness of using molecular biological tests to diagnose infection. The real-time polymerase chain reaction (PCR) tests determine the presence or absence of bacteria by amplifying bacteria-specific DNA in specimens. Some tests target the MecA gene and are said to provide relatively early results and high test accuracy. However, real-time PCR testing methods involve manual techniques and hands-on time, and there is a risk of contamination. Fully automated PCR testing systems can reduce contamination and accuracy errors caused by manual techniques by performing the process fully automated. They can also reduce the time until the results are known.
Therefore, we report the results of a fully automated real-time PCR test for methicillin resistance, which is important in selecting antimicrobial agents.

METHODS:
Cases with suspected PJI or surgical site infection (SSI) submitted for bacterial culture testing at Yokohama City University Hospital from July 2020 to May 2022 were included. A comparison was made between groups in which real-time PCR tests were performed using a conventional PCR test device (LightCycler® system, Roche diagnostics) and a fully automated real-time PCR device (GEAN CUBE®, Toyobo) targeting the methicillin resistance (MecA) gene region. Bacterial culture tests were performed as follows. If the standard culture test showed no growth of bacteria, an additional culture test was performed. The presence or absence of methicillin resistance by drug susceptibility testing of the identified bacteria and the accuracy of identification of the MecA gene by the results of each PCR test were verified.

RESULTS:
A total of 484 specimens from 167 cases were included in the study. To identify the MecA gene, 209 specimens were tested by conventional real-time PCR, and 275 specimens were tested by fully automated real-time PCR (Figure 1). In terms of specimens, 176 were joint fluid specimens, 69 were in the conventional PCR method, and 77 were in the fully automated PCR. Bacterial culture tests showed methicillin resistance in 57 samples, 35 in the conventional PCR group, and 22 in the fully automated real-time PCR system. The accuracy of each MecA-positive test for identification of methicillin resistance was 0.89 for sensitivity, 0.89 for specificity with the conventional system, 0.67 for sensitivity, and 0.97 for specificity with the fully automated real-time PCR system. (Table 1). The accuracy of MRS diagnosis by the fully automated PCR system, when limited to joint fluid specimens, was 1.00 for sensitivity and 0.96 for specificity (Table 2).

DISCUSSION:
In this study, the accuracy of the conventional PCR method was compared with that of a fully automated PCR testing system. In a whole specimen comparison, the fully automated PCR test had higher diagnostic accuracy and specificity, but the sensitivity of the conventional PCR test method was higher. This may be because the fully automated PCR tester uses a machine to judge the results, whereas the conventional PCR method uses a person to judge the results. In limited to joint fluid, both sensitivity and specificity were improved, indicating that the accuracy of the test may be lower for tissue specimens. This may be because the fully automated PCR testing system is susceptible to poor judgment depending on the quality of the specimen. Compared with joint fluid, tissue specimens may contain substances that inhibit human DNA and PCR.
Finally, fully automated PCR testing systems have higher diagnostic accuracy than conventional systems. They are helpful for the early diagnosis of MRS, especially in joint fluid specimens, where the testing accuracy is very high.

Limitations: The limitations include the small number of specimens, failure to consider clinical outcomes or other laboratory findings, cases of infection with negative bacterial culture, and failure to consider criteria for testing on a fully automated PCR assay.

SIGNIFICANCE/CLINICAL RELEVANCE: We investigated the usefulness of a fully automated polymerase chain reaction (PCR) testing system for the genetic diagnosis of MRS in patients with suspected PJI or SSI. The fully automated PCR testing system had higher diagnostic accuracy than conventional PCR methods and was helpful in the early diagnosis of MRS, particularly in joint fluid specimens.

IMAGES AND TABLES:

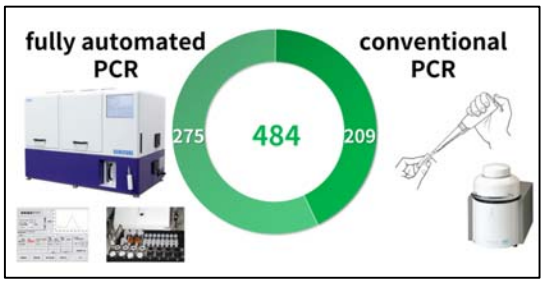

Figure.1 The number of specimens
275 specimens were tested by fully automated real-time PCR.
209 specimens were tested by conventional real-time PCR.

Table.1 Accuracy of fully automated and conventionl PCR.

	sensitivity	specificity	accuracy
conventional PCR	0.89	0.89	0.89
fully automated PCR	0.67	0.97	0.95

Table.2 Comparison with previous study

	Samples	sensitivity	specificity
Yang F., et al. J. Orthop. 2020	PJI and SAH Only Fluids (N = 99)	0.89	0.89
This study	PJI and SSI Fluids and Tissues (N = 275)	0.67	0.97
	PJI and SSI Only Fluids (N = 77)	1.00	0.96