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Original Article

Effectiveness of weekly polymerase chain reaction-based open reading frame typing analysis of all newly isolated methicillin-resistant *Staphylococcus aureus* strains for controlling nosocomial infections

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ABSTRACT

Polymerase chain reaction (PCR)-based open reading frame typing (POT) helps differentiate between bacterial strains based on the open reading frames (ORFs) of the prophage-encoding genes; multiplex PCR screening is performed to identify strains based on keeping patterns.

At our hospital, surveillance of nosocomial methicillin-resistant *Staphylococcus aureus* (MRSA) transmission is undertaken using POT to conduct molecular epidemiological analysis for all newly detected MRSA strains. In 2014, we performed POT only once a month; however, in 2015, we increased the frequency of POT to once a week, which helped us detect nosocomial transmission that would normally be difficult to detect, and thus achieve 40% reduction in nosocomial transmission, compared to that in 2014. This suggests that weekly POT screening for all MRSA strains is one of the effective methods available for minimizing nosocomial transmission of MRSA.

1. Introduction

Nosocomial infections caused by various resistant bacteria have been a source of concern in recent times. Currently, methicillin-resistant *Staphylococcus aureus* (MRSA) is the most important pathogen causing nosocomial infections. To prevent nosocomial infection, hospitals undertake regular surveillance to detect resistant bacteria, including MRSA.

At our hospital, if MRSA is isolated from newly admitted patients within 48 h of admission, we define them as "imported cases"; all other MRSA cases are grouped under "nosocomial transmission." However, not all patients undergo MRSA screening upon hospitalization, and therefore, it is very difficult to accurately assess the nosocomial transmission of MRSA. The drug susceptibility patterns of MRSA strains help us differentiate among isolates. However, some strains might have identical gene patterns, but they might show different drug susceptibility patterns; therefore, caution must be exercised when assessing nosocomial transmission based on drug sensitivity tests alone. Pulsed-field gel electrophoresis (PFGE) is a highly reliable method for assessing nosocomial transmission; however, it is time-consuming and expensive and requires specific skills [1].

The polymerase chain reaction (PCR)-based open reading frame typing (POT) method, developed by Suzuki et al. [2,3], is based on phage open reading frame (ORF) typing and can differentiate among MRSA strains. The ORFs of phage genomes lysogenized in the MRSAs are amplified in this reaction. POT assay yields results in only 4 h, and these results are converted into numerical values, which allows easy comparison of previous data and data analyzed at other facilities. Furthermore, the discriminatory power of the POT method is reported to be equal to that of PFGE [1].

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In the present study, we discuss the use of POT to analyze new MRSA strains as well as the efficacy of weekly analysis of all strains.

2. Materials and methods

2.1. Reagents and devices

To perform POT, we used a Cica Geneus Staph POT kit (Kanto Chemical Co., Ltd., Tokyo, Japan) and Cica Geneus DNA Extraction Reagent (Kanto Chemical Co., Ltd.). PCR was performed using 2720 Thermal Cycler (Thermo Fisher Scientific Co., Ltd., Waltham, MA, USA). For electrophoresis, Reliant™ Minigel TBE (Lonza Japan Co., Ltd., Tokyo, Japan) and Mupid-exU (Mupid Co., Ltd., Tokyo, Japan) were used. FAS-IV (Nippon Genetics Co., Ltd., Tokyo, Japan) was used for capturing images. Bacterial identification and drug susceptibility tests were performed with MicroScan WalkAway-96 SI (Beckman Coulter, Inc., Brea, CA, USA) using MicroScan Series Panel PC1T (Beckman Coulter, Inc.).

We performed POT according to the manufacturer's instructions to study MRSA isolates. In brief, the POT kit comprised two multiplex PCR reactions, and the output was determined by the presence of 22 targets. The band size of these products was estimated with the help of the positive control. The results are expressed as three POT scores calculated in a binary manner [2].

2.2. Frequency of POT assay to analyze MRSA strains

At our hospital, the results of microbiological tests can be viewed in in-hospital electronic medical records; however, for direct confirmation with staff, the Infection Control Team reports the results to the ward on the day MRSA is isolated, thereby enabling swift contagion countermeasures.

We began using POT for genetic analysis of strains during nosocomial MRSA outbreaks in 2010. Since 2014, however, we have been using POT to analyze all MRSA strains detected. Furthermore, in 2015, we increased the frequency of POT testing from once a month to once a week.

If MRSA strains with the same POT number are detected in the same ward, this information has to reach the medical staff in order to draw their attention to the spread of MRSA. Moreover, such data are reviewed weekly by the Infection Control Team, which proactively intervenes if there is a possibility of an outbreak.

2.3. Percentage of MRSA among S. aureus strains

To assess the effectiveness of weekly POT screening for all MRSA strains, we compared the percentage of MRSA among *S. aureus* as observed before regular testing of all strains (2013) to that with monthly (2014) and weekly testing (2015). The formula used was (Number of patients testing positive for MRSA/Number of patients testing positive for *S. aureus*) × 100.

2.4. Conventional definition of MRSA outbreak and nosocomial transmission

An MRSA outbreak has been defined as three or more cases of what is considered the same strain of MRSA within four weeks in one ward. The definition was based on a notice issued by the Health Policy Bureau, Japan's Ministry of Health, Labour and Welfare in December 2014. MRSA nosocomial transmission has been defined as the detection of two or more cases considered to be the same MRSA strain within six months in our hospital.

2.5. Transmission rate in the same ward

To assess the effects of POT screening frequency, we compared the transmission rate of MRSA strains in the same ward from January to October 2014 (monthly screening) and January to October 2015 (weekly screening). “MRSA transmission in the same ward” has been defined as two or more cases of which are considered the same POT strain of MRSA within six months in same ward.

The transmission rate was calculated using the formula (Number of cases with the same POT number in the same ward [case 2 onward]/Number of newly admitted patients with MRSA) × 100. Statistical analysis was performed using SPSS ver. 20. The yearly inward transmission rates of MRSA strains were compared using chi-square test, with $P < 0.05$ being considered statistically significant.

2.6. Definition of MRSA infection

We defined MRSA infection as follows. Bacteremia was defined as one or more positive blood cultures from patients with clinical signs of infection such as fever, chills, and sweats, with or without local signs and symptoms [4]. Pneumonia was defined as development of a new pulmonary infiltrate based on radiographic imaging in conjunction with clinical signs, and MRSA was isolated from a purulent respiratory secretion. Wound infection was defined as isolation of MRSA from a purulent fluid drained from the wound. Even if MRSA was isolated from various types of specimens, it was defined as “colonized” when there were no clinical signs of infection [5].

3. Results

3.1. Usefulness of POT assay for molecular epidemiological analysis of nosocomial MRSA strains

Molecular epidemiological analysis of newly isolated MRSA in 2014 and 2015 was performed using POT. The POT types of MRSA isolates in 2014 and 2015 are listed in Tables 1a and b. The most frequent isolate detected in 2014 was 106-137-80 (13.5%). Subsequently, 93-191-103 (9.1%) and 93-137-103 (5.6%) were isolated. Similarly, the most frequent isolate detected in 2015 was 106-137-80 (12.4%). Subsequently, 106-9-80 (4.0%) and 93-217-111 (3.6%) were isolated. The frequencies of POT types with a unique POT number were 35.3% and 38.2% in 2014 and 2015, respectively. We examined the instances of nosocomial transmission of MRSA strains isolated within 48 h of hospital admission and strains isolated after >48 h of admission. In 2014, MRSA strains with the same POT number were detected in the same ward in 76 cases, and therefore, these were suspected to be cases of nosocomial transmission. Of these 76 cases, MRSA was isolated after >48 h of admission in 57 cases (75%), whereas it was isolated within 48 h in 19 cases (25%); the latter would thus not conventionally be assessed as nosocomial transmission.

3.2. Reduction in the rate of MRSA among all S. aureus isolates

The rate of MRSA among all *S. aureus* isolates was 43.8% (271/619) in 2013, 39.5% (281/712) in 2014, and 33.5% (259/772) in 2015. The difference between the rate in 2013 and 2014 was not statistically significant; however, the rate in 2015 was significantly lower than that in 2014 (Fig. 1).