Genotyping and Quantitative Analysis of Biofilm Formation by Methicillin-resistant Staphylococcus aureus (MRSA) Strains Isolated from Patients with Orthopaedic Infections

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BACKGROUND:

Methicillin-resistant Staphylococcus aureus (MRSA) is one of the common pathogens involved in orthopaedic infections. Device-related infections are difficult to cure. However, epidemiological analysis and genotyping of biofilm formation among clinical MRSA isolates from orthopaedic infections have not previously been studied.

OBJECTIVE:

To evaluate genotyping and quantitative analysis of biofilm formation among MRSA isolates from patients with orthopaedic infection and asymptomatic carriers.

METHODS:

Eighty-one (81) strains from infection sites and blood cultures (device-related surgical site infections (SSI), 23; device-nonrelated infections, 58) and 73 MRSA strains isolated from the nasal cavity of asymptomatic outpatients were examined.

A Staphylococcal cassette chromosome mec (SCCmec), which contains mecA as the methicillin resistance determinant, was classified into type I to IV by multiplex PCR 1; a mec-HVR based on the size of the mec-associated hypervariable region was classified into type A to F by PCR 2; an accessory gene regulator (agr) gene linked to expression of many virulent factors was classified into type I to IV by multiplex PCR 3; and, a Staphylococcal Protein A gene (spa) was classified according to the repeat region of the spa gene by DNA sequence analysis 4.

Biofilm formation was quantified using microtiter plate assay 5. Bacterial strains were incubated in a 96-well polystyrene microtiter plate. The cells were decanted and the wells washed. Adhesive cells were stained 0.1% crystal violet and resolved by 95% ethanol. Absorbance was measured at 595 nm by use of an ELISA plate reader and the biofilm index was defined at OD595.

RESULTS:

The incidences of genotype SCCmec type II (63.0% vs. 38.4%, p=0.002), mec-HVR type D (56.8% vs. 32.9%, p=0.003), and agr-2 (63.0% vs. 39.7%, p=0.004), spa t002 (46.9% vs. 20.5%, p=0.0006) was significantly higher in the infection group than in the nasal colonization group, and SCCmec type IV (31.5% vs. 17.3%, p=0.04), mec-HVR type E (43.8% vs. 25.9%, p=0.02), and agr-1 (46.6% vs. 28.4%, p=0.02) was significantly higher in the nasal colonization group than in the infection group. However, there was no difference in the incidence of genotype between the device-related SSI group and the device-nonrelated infection group.

The mean biofilm index of agr-2 strains was significantly higher than that of agr-1. (mean ± SD, 0.55±0.57 vs. 0.29±0.46, p=0.005) (Fig. 1) No difference in the mean biofilm index was observed between the infection group and the nasal colonization group. However, in the device-related SSI group, the incidence of strongly biofilm-producing strains (OD595 >0.50) almost reaching the 90th percentile value of the biofilm index in agr-1 strains as weakly biofilm-producing strains was significantly higher than that in device-nonrelated infection group (43.5% vs. 19.0%, p=0.03). (Fig. 2)

DISCUSSION:

The results of this study suggest that MRSA genotyping was relevant to the pathogenesis of orthopaedic infections. The agr system is linked to a quorum-sensing system to control the regulation of many virulent factors via cell-to-cell communication releasing signal molecules, and agr genotypes was especially relevant to pathogenesis and biofilm formation.

The level of biofilm formation was associated with device-related SSI, but other factors in addition to agr genotypes are expected to be linked to biofilm formation causing device-related SSI and these remain to be examined in the future.

REFERENCES:


Fig.1 Comparison of biofilm formation among the three(3) agr types.
* p=0.005 (Mann-Whitney’s U test)

Fig.2 Incidence of strongly biofilm-producing (biofilm index >0.50) strains.
A: device-related SSI group (n=23), B: device-nonrelated infection group (n=58), C: nasal colonization group (n=73)
* p=0.03 (chi-square test for independence)