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The role of *mecA* and *blaZ* regulatory elements in *mecA* expression by regional clones of methicillin-resistant *Staphylococcus pseudintermedius*

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ABSTRACT

Two major regional clones of methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) have been identified in Europe and North America. They are designated multilocus sequence types (ST) 71 and 68 and contain staphylococcal chromosome cassette (SCC*mec*) types II–III and V_T, respectively. One notable difference between the two clones is a deletion in the *mecI*/*mecR1* regulatory apparatus of ST 68 SCC*mec* V_T. This deletion in analogous methicillin-resistant *Staphylococcus aureus* (MRSA) results in more responsive and greater expression of the *mecA* encoded penicillin-binding protein 2a, and is associated with SCC*mec* types occurring in community-acquired MRSA lineages. The aim of this study was to characterize *mec* and *bla* regulatory apparatuses in MRSP and determine their effects on expression of *mecA*. Seventeen *S. pseudintermedius* isolates representing nine methicillin-resistant ST lineages were screened for the presence of the repressors *blaI* and *mecI* and sensors *blaR1* and *mecR1*. The *bla* and *mec* operons for each isolate were sequenced and compared for homology between the repressor open-reading frames (ORF), sensor ORFs, and *mecA* promoter regions. A real-time reverse transcriptase PCR expression assay was developed, validated and applied to nine isolates determining the effect of oxacillin induction on *mecA* transcription. Significant differences were found in *mecA* expression between isolates with a full regulatory complement (*mecI*/*mecR1* and *blaI*/*blaR1*) and those with truncated and/or absent regulatory elements. Isolates representative of European and North American MRSP ST regional clones have dissimilar *mecA* responses to oxacillin.

1. Introduction

The *mec* and *bla* operons are modulated by two-component systems consisting of the analogous repressors *mecI* and *blaI* and sensors *mecR1* and *blaR1*, respectively (Fuda et al., 2005). BlaR1 and MecR1 are transmembrane spanning and signal transducing proteins. Acylation of BlaR1/MecR1 subsequent to external interaction with beta-lactam antibiotics is followed by autoproteolytic cleavage on the cytoplasmic side of the cell membrane. The separated intracellular portion of BlaR1/MecR1 travels to the bacterial chromosome and removes its cognate repressor BlaI/MecI via proteolysis. Once the repressor dissociates from its promoter-region binding site, transcription of the *blaZ*/*blaR1*/*blaI* and *mecA*/*mecR1*/*mecI* genes begins. The structure and function of MecI and BlaI are similar but not identical; they share 60% sequence homology (Safo et al., 2005; García-Castellanos et al., 2003). The repressors act interchangeably, binding to the promoter–operator region of either the *blaZ* or *mecA* operon. Induction of the *mecA* gene occurs only through the cognate sensor; the intracellular peptidase portion of

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transmembrane BlaR1 only has activity on BlaI, and MecR1 only cleaves MecI (McKinney et al., 2001).

The emergence of MRSP is recent relative to that of MRSA and thus far two geographic clones bearing distinct cassette types seem to predominate (Black et al., 2009; Ruscher et al., 2010; Perreten et al., 2010). SCC*mec* types II–III and V_T differ in the *mecI*/*mecR1* regulatory apparatus of *mecA*, and are analogous with the two types of cassette structures found in HA and CA-MRSA (Descloux et al., 2008; Black et al., 2009; Chambers and Deleo, 2009). Here we characterize the *mecA*/*mecI*/*mecR1*, *blaZ*/*blaI*/*blaR1*, and *mecA* promoter regions of MRSP isolates and determine the effect of regulatory gene presence or absence on the speed and abundance of *mecA* transcription.

2. Materials and methods

2.1. Bacterial isolate selection and identification

S. pseudintermedius isolates were obtained from clinical samples submitted to the University of Tennessee, College of Veterinary Medicine Clinical Bacteriology Laboratory as well as from European and North American collaborators through previous studies (Black et al., 2009; Descloux et al., 2008; Perreten et al., 2010) and used by permission of the providers. A total of 17 non-duplicate isolates from dogs were selected representing nine MLST lineages associated with methicillin resistance as an initial pool to screen for *blaI*/*blaR1* and *mecI*/*mecR1* variation. Bacterial isolation and identification procedures were those routinely used in the laboratory as previously described (Jones et al., 2007). Multilocus sequence typing provided definitive species identity of *S. pseudintermedius* with partial 16S rRNA and *pta* gene sequencing. The type strain of *S. intermedius* isolated from a pigeon (ATCC 29663) and *S. pseudintermedius* (ATCC 51874, isolated from a dog and originally designated *S. intermedius*) served as reference strains for this study.

2.2. DNA extraction

Isolates were grown on blood agar plates overnight at 37 °C and bacteria derived from a single colony were suspended in 0.5 ml of TE buffer mixed with an equal volume of glass beads and vortexed for 10 min. Supernatant in the centrifuged cell lysate was used as template DNA for PCR amplification of *mec* and *bla* regulatory genes.

2.3. PCR primers and conditions

Oligonucleotide primers specific for *S. pseudintermedius* *mecA* promoter and *blaI*/*blaR1* were based upon MRSP SCC*mec* type V_T cassette (GenBank accession no. FJ544922.1) and unpublished ST 68 genomic data, respectively (Table 1), and designed using IDT SciTools application (Integrated DNA Technologies, Coralville, IA). Primers for *mecI*/*mecR1* were adapted from Hiramatsu et al. (1992). A second *blaI* primer set (*blaI2*, Table 1), amplifying a region internal to that of *blaI1* was needed to amplify and sequence the blaI of e138 (ST 116). Conventional PCR was performed using the following parameters: 95 °C for 90 s followed by 35 cycles of 55 °C for 30 s, 68 °C for 120 s, and 94 °C for 30 s.

Table 1
Primers used with conventional PCR.

mecA promoter	
Forward	5′-CGGACGTTCAGTCATTTCTACTTC-3′
Reverse	5′-ACACCTTCTACACCTTATCAC-3′
mecI[a]	
Forward	5′-AATGGCGAAAAAGCACAACA-3′
Reverse	5′-GACTTGATTGTTTCCTCTGTT-3′
mecR1	
Forward	5′-TGGTATTTGGTTTAGTGAA-3′
Reverse	5′-GATTAGGTTTAGGCATTGA-3′
blaI 1	
Forward	5′-AGATGGAAAGCCATCTGGGA-3′
Reverse	5′-GTCTCGCAATTCTTCAATTTCTTTG-3′
blaI 2	
Forward	5′-TCTATGGCTGAATGGGATGTTATG-3′
Reverse	5′-GTCTCGCAATTCTTCAATTTCTTT-3′
blaR1	
Forward	5′-CCCAGACGGCTTTCCATCTGATAA-3′
Reverse	5′-GCCCACAGTTGAAAGTAGGTCACA-3′

[a] *mecI* primers from Hiramatsu et al. (1992).

2.4. Sequence analysis

PCR products of expected sizes were treated to destroy single-stranded DNA (ExoSap-IT, USB Corp., Cleveland, OH) and submitted to the University of Tennessee, Molecular Biology Resource Facility for DNA sequencing. PCR primers were used for direct DNA sequencing of PCR amplification products. Open reading frames for *blaI*, *blaR1*, *mecI*, *mecR1* and *mecA* promoter were compared between isolates using Lasergene® SeqMan Pro software (DNASTAR, Inc., Madison, WI).

2.5. MLST

Genetic diversity of *S. pseudintermedius* was determined from five genes (16S rDNA, *tuf*, *cpn60*, *pta* and *agrD*) as previously described (Bannoehr et al., 2007). Sequence type numbers were assigned using the key table for MLST typing of *S. intermedius* group isolates. New sequence types were assigned by the curator, Vincent Perreten (vincent.perreten@vbi.unibe.ch).

2.6. Bacterial growth and RNA extraction – time point mecA expression assay

For each isolate a 5 ml vial of BBL™ Trypticase™ Soy Broth (TSB) (Becton, Dickinson and Co., Sparks, MD) was inoculated with a single colony grown on blood agar and then incubated overnight at 37 °C in a rotary shaker at 225 rpm. Fifty microliters of overnight growth was used to inoculate four additional 5 ml TSB vials, which were subsequently incubated as indicated above for 4 h. Oxacillin (Sigma–Aldrich, St. Louis, MO) was added to a single vial at 4 h, 2 h, and 1 h prior to RNA extraction. The oxacillin concentration was 0.01 μg/ml for all induction