CHARACTERIZATION OF STAPHYLOCOCCAL CASSETTE CHROMOSOME MEC TYPES IN METHICILLIN-RESISTANT STAPHYLOCOCCUS INTERMEDIUS STRAINS ISOLATED FROM DOGS

SCmec types in mecA-positive S. intermedius strains were characterized by using multiplex PCR method described previously by Oliveira and Lencastre [11]. A total of 100 preliminary identified S. intermedius isolates were tested to determine their species identity and mecA gene encoding methicillin resistance. The duplex PCR assay was able to identify all the strains carrying mecA gene (17%). The study showed that 4% strains identified as S. intermedius based upon their phenotypic properties do not yielded a 16S rRNA gene amplification product, indicating staphylococci other than S. intermedius. SCmec type IIIC was identified in 15 strains (88%), type IB was found only in one strain. One strain was not typable by this technique.

Key words: staphylococcus intermedius, multiplex PCR, mecA gene, MRsI, SCmec types.

Staphylococcus intermedius was first described as a new species in 1976 and was differentiated from Staphylococcus aureus and Staphylococcus epidermidis based on its biochemical and physiological characteristics [3]. This microorganism occurs as commensal bacteria on the skin and mucosal membranes of dogs. This species has also been found in cats, horses, pigeons, foxes, and other animals. In dogs it has been implicated in serious infections, such as: pyoderma, otitis externa, abscess, and infections of eyes, joints, mammary glands, respiratory tract and gastrointestinal tract [2]. In humans S. intermedius is recognized as an invasive zoonotic pathogen and has been isolated in 18% of canine-inflicted wounds [5, 8]. On the other hand it has been infrequently identified in other invasive human diseases and only a few studies have reported rare cases of infections such as bacteremia [14], infection of mastoid cavities [5], and brain abscesses [1].

Increased frequency of infections caused by S. intermedius exhibiting multi-drug resistance, including methicillin, is being observed among dogs [6, 9]. Methicillin resistance in staphylococci is mediated by the mecA gene, encoding the penicillin-binding protein 2a (PBP2a), which has reduced affinity for methicillin, oxacillin and other beta-lactam antibiotics. The mecA gene, responsible for this phenomenon...
Methicillin resistance is most often associated with a mobile genetic element termed the staphylococcal cassette chromosome mec (SCCmec). The molecular basis of methicillin resistance has been well characterized in S. aureus, but little is known about the acquisition and the organization of its genetic key, the mecA gene, in other staphylococcal species. In S. aureus several types and subtypes of Staphylococcal chromosome cassettes (SCCmec) have been classified on the basis of the different combinations of the two main parts: the mec gene complex, which encodes methicillin resistance (the mecA gene and its regulators), and the ccr gene complex, which encodes site-specific recombinases responsible for its mobility [7, 10].

In this study we developed a duplex PCR assay, which allows to conduct the simultaneous identification of Staphylococcus intermedius strains and the mecA gene detection. We also characterize SCCmec types in methicillin-resistant Staphylococcus intermedius strains isolated from dogs using multiplex PCR strategy.

**Materials and methods**

A total of 100 strains were isolated in the Diagnostic Laboratory of the Division of Bacteriology and Molecular Biology at Warsaw University of Life Sciences. The strains were cultivated from different clinical specimens taken from dogs. Strains used for control purpose include the: Staphylococcus aureus ATCC 6538, Staphylococcus intermedius ATCC 29663 and methicillin-resistant Staphylococcus aureus (MRSA) 14.002 (possessed from The National Reference Centre for Antimicrobial Susceptibility).

The isolates were identified by means of the API Staph system (BioMérieux) and additional characteristics such as type of hemolysis, colony pigment, coagulase, clumping factor and acid production from maltose. Sensitivity to methicillin was assessed using a disc diffusion test according to the Clinical Laboratory Standards Institute (CLSI), with discs containing 1 µg of oxacillin (BioMérieux). Oxacillin-resistant, mecA-positive strains were tested for resistance to other antimicrobial agents.

Bacterial cultures were grown overnight at 37 °C in 5 ml of brain-heart infusion broth (BioMérieux). Chromosomal DNA was obtained using DNA Genomic Mini (A&A Biotechnology). For DNA extraction 1.5 ml of bacterial culture was centrifugated at 6 000 g for 5 minutes. Sediment was suspended in 140 µl TE buffer with 2% glucose, containing 5 µl lysozyme (5 mg/ml, SIGMA), 2.5 µl lysostaphin (1 mg/ml, SIGMA) and 2 µl ribonuclease A (10 mg/ml, Fermentas). After incubation for 2 hours at 37 °C further part of the DNA isolation process was conducted according to the manufacturer’s instructions.

Two pairs of primers were used for the detection of mecA-positive Staphylococcus intermedius strains. One was specific for mecA gene and one for S. intermedius-specific fragment of the 16S rRNA gene. The cycling conditions were as follows: after an initial denaturation step of 94 °C for 3 min, samples completed 30 cycles of amplification (30 s of denaturation at 94 °C, 30 s of annealing at 55 °C and 1.5 min of extention at 72 °C). The final elongation was performed at 72 °C for 4 min. The primer sequences, products size and references are presented in Table 1.
The PCR products, 901 bp and 523 bp in size were separated on 1% agarose gels in Tris-Acetate-EDTA buffer, stained with ethidium bromide, visualized with UV light and analyzed using the VersaDoc Model 1000 Imaging System with Quantity One 4-4-0 software (BioRad). Typical electrophoresis pattern of mecA and 16S rRNA gene amplification product are presented on Fig. 1.

![Electrophoresis Pattern](image)

**Fig.1. PCR for the species confirmation and detection of mecA gene**
Lane 1 — negative control; lane 2 — MassRuler DNA Ladder Mix (Fermentas); lane 3 — mecA product for MRSA strain no. 14.002; lane 4 — 16S rRNA gene amplification product for *S. intermedius* ATCC 29663; lane 5 — one of the researched strains (MRSI).

The 17 mecA-positive *S. intermedius* strains were used to characterize the SCCmec types. SCCmec multiplex PCR typing assay was based on Oliveira’s method [11]. Totally 9 pairs of the primers for SCCmec types and subtypes, as well as for the mecA gene were used (tab. 2).
<table>
<thead>
<tr>
<th>Locus</th>
<th>Primer</th>
<th>Oligonucleotide sequence ((5'–3'))</th>
<th>Product size ((bp))</th>
<th>Specificity ((SCC_{mec} type))</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>CIF2 F2</td>
<td>TTCGAGTTGCTGATGAAGAAGG</td>
<td>495</td>
<td>I</td>
</tr>
<tr>
<td></td>
<td>CIF2 R2</td>
<td>ATTTACCACAAGGAACCTACCAGC</td>
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<td></td>
</tr>
<tr>
<td>B</td>
<td>KDP F1</td>
<td>AATCATCTGCCCATTGGTGATGC</td>
<td>284</td>
<td>II</td>
</tr>
<tr>
<td></td>
<td>KDP R1</td>
<td>CGAATGAGTGAAAGAAAAGG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>MECI P2</td>
<td>ATCAAGACTTGCATTCAAGGC</td>
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<tr>
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<td>CATCCTATGATAGGCTGGTC</td>
<td>342</td>
<td>I, II, IV</td>
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<tr>
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<td></td>
<td>RIF4 R9</td>
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<td>F</td>
<td>RIF5 F10</td>
<td>TTCCTAGTACAGCTGAATCG</td>
<td>414</td>
<td>III</td>
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<tr>
<td></td>
<td>RIF5 R13</td>
<td>GTCACAGTAAATCTCATCAATGC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>IS431 P4</td>
<td>CAGGTCTCTTCAGATCTACG</td>
<td>381</td>
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<tr>
<td></td>
<td>pUB110 R1</td>
<td>GAGCCATAAACACCAATAGCC</td>
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<td>H</td>
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<td>303</td>
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<td></td>
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<td>GAAGATGGGGGAAGGTTCAC</td>
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<tr>
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<td>MEC A P7</td>
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</table>

The cycling conditions were as follows: after an initial denaturation step of 94 °C for 4 min, samples completed 30 cycles of amplification (30 s of denaturation at 94 °C, 30 s of annealing at 53 °C and 1 min of extension at 72 °C). The final elongation was performed at 72 °C for 4 min.

The strains used for control purposes include the methicillin/oxacillin resistant *S. aureus* strains carrying: I, IA, IB, II, III, IIIA, IIIB, IIIC, IIID, IIIE, IIIG, IIIJ and IV type of cassette. The standard strains were obtained from National Medicines Institute, Division of Clinical Microbiology and Infection Prevention.

The PCR products were separated on 2% agarose gels in Tris-Acetate-EDTA buffer, stained with ethidium bromide, visualized with UV light and analyzed using a VersaDoc Model 1000 Imaging System with Quantity One 4-4-0 software (BioRad).
Results and discussion

All strains investigated in the present study were predominarily identified as *S. intermedius* based upon their phenotypic properties. In vitro antimicrobial susceptibility tests showed that 17 isolates were resistant to methicillin. Application of duplex PCR yielded following results as shown in Table 3.

| Methicillin susceptibility phenotypes of tested strains determined by the disc diffusion method | Results of duplex PCR assay |
|---|---|---|---|---|
| Methicillin - resistant (n=17) | 17 (17%) | 0 | 0 | 0 |
| Methicillin - susceptible (n=83) | 0 | 79 (79%) | 0 | 4 (4%) |

The result of duplex PCR assay showed that 4% of the isolates were misidentified as *S. intermedius* upon the identification of the phenotypic test. Totally of 17% of strains recognised as *S. intermedius* carrying *mecA* gene. For 79% of strains we obtained a species-specific product for the 16S rRNA gene of *S. intermedius* but no *mecA* product, as expected for methicillin-sensitive *Staphylococcus intermedius* (MSSI).

SCC*mec* type IIIC was identified in 15 *mecA*-positive strains (88%), type IB was found in one strain (6%). One isolate (6%) was not typable by this technique. The results of multiplex PCR of exemplary 7 investigated *S. intermedius* strains are presented on Fig. 2.

![mecA gene](image)

**Fig. 2.** PCR SCC*mec* profiles from methicillin-resistant *S. intermedius*

*M* — DNA molecular size marker; 1–7 — exemplary 7 investigated *S. intermedius* strains; SCC*mec* type IB — lanes 3; SCC*mec* type IIIC — lanes 1, 2 and 4 to 7.
The occurrence of *S. intermedius* strains resistant to all antimicrobials commonly used in veterinary medicine is alarming. The results of recent studies have proved that some isolates are resistant to methicillin by expression of *mecA* gene and thus the abbreviation methicillin-resistant *Staphylococcus intermedius* (MRSI) is appropriate in analogy to MRSA. The worldwide increase in the number of infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) has emphasised the need for the fast and reliable identification and typing methods. In addition to genotyping characterization of the staphylococcal cassette chromosome (SCC) *mec* type has led to better discrimination of hospital-acquired MRSA (HA-MRSA), carrying one of three types of SCCmec (type I, II, or III) and nonmultiresistant community-acquired MRSA (CA-MRSA) carrying type IV or V SCCmec. Type IV and V SCCmec are small elements that do not carry the antibiotic resistance genes other than *mecA* and has the multiple subtypes [4]. Presence of this small (IV) type of SCCmec was observed in *S. schleiferi* subsp. *coagulans* strains isolated from companion animals [12]. Worthwhile to point out that SCCmec elements lacking *mecA* have also been reported in *S. aureus* and coagulase-negative staphylococci. SCCnon-mec is located at the same chromosomal site as all SCCmec elements, and it contains a virulence factor called capsular polysaccharide 1, which makes the strain more resistant to phagocytosis [10].

In this study we found that *S. intermedius* strains isolated from dogs possessed the type I and III SCCmec, and we observed that all these strains were resistant to all beta-lactams and also to other groups of antibiotics (aminoglycosides, trimethoprim, sulfonamides, tetracyclines, macrolides, and fluoroquinolones). Their MIC values were high (data not shown). One isolate was not typable by the method used in this study, and this may indicate that there are a variety of uncharacterized SCCmec elements in staphylococcal species other than *S. aureus*.

Little concern has been voiced yet about the possibility of animal to human *mecA* gene transmission. Canine strains of *S. intermedius* have been found to harbor SCCmec elements homologuous to those carried by *S. aureus*.

Our findings suggest that the high prevalence of this resistancy vector in dogs may be the reservoirs of antibiotic resistancy genes, and may perhaps be the driving force for the generation of new staphylococcal methicillin-resistant strains. The fact that dogs are in close contact with their owners, the risk of transmission of such bacteria between animals and humans must be considered.

Canine strains of *S. intermedius* have been found to harbor SCCmec elements encoding determinants for the expression of the methicillin-resistance phenotype. The SCCmec typing strategy we used in this study detected two SCCmec elements (SCCmec type IB and IIIC). One isolate was not typable by this method.

The diversity of SCCmec types found in dogs appears similar to that seen in humans, however uncharacterized SCCmec elements in *S. intermedius* may exist.

REFERENCES

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ХАРАКТЕРИСТИКА СТАФІЛОКОККОВОЇ КАСЕТНОЇ ХРОМОСОМИ

MEC ТИПА У МЕТИЦИЛІН-РЕЗИСТЕНТНИХ ШТАМІВ

STAPHYLOCOCCUS INTERMEDIUS, ІЗОЛЮВАННИХ ВІД СОБАК

Реферат

SCCmec тип у mecA-позитивних штамів S. intermedius був охарактеризований з використанням метода мультиплексної ПЦР, описаного раніше Oliveira і Lencastre [11]. 100 попередньо ідентифікованих ізолятів S. intermedius були ізольовані для подтверджения їх видової принадлежності та для виявлення mecA гена, що кодує резистентність до метициліну. Дуплексний ПЦР аналіз дав можливість ідентифікувати усі штами, що несуть mecA ген (17%). Дослідження показали, що 4% штамів, ідентифікованих як S. intermedius на підставі їх фенотипових властивостей, не давали продуктів ампілікації гена 16S rРНК, що виявляють стафілококки відмінні від S. intermedius. SCCmec типу ПЦС була виявлена у 15 (88%) штамів, типу IV — тільки у одного штаму. Лише один штам не піддавався типуванню за допомогою даного підходу.

Ключові слова: Staphylococcus intermedius, мультиплексна ПЦР, mecA ген, MRSI, типи SCCmec.

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ХАРАКТЕРИСТИКА СТАФІЛОКОККОВОЇ КАСЕТНОЇ ХРОМОСОМИ

MEC ТИПУ У МЕТИЦИЛІН-РЕЗИСТЕНТНИХ ШТАМІВ

STAPHYLOCOCCUS INTERMEDIUS, ІЗОЛЮВАННИХ ВІД СОБАК

Реферат

SCCmec тип у mecA-положительних штамов S. intermedius был охарактеризован с использованием метода мультиплексной ПЦР, описанного ранее Oliveira и Lencastre [11]. 100 предварительно идентифицированных изолятов S. intermedius были исследованы для подтверждения их видовой принадлежности и для обнаружения mecA гена, кодирующего резистентность к метициллину. Дуплексный ПЦР анализ дал возможность идентифицировать все штаммы, несущие mecA ген (17%). Исследования показали, что 4% штаммов, идентифицированных как S. intermedius на основании их фенотипических свойств, не давали продуктов амплификации гена 16S rРНК, определяющих стафилококки отличные от S. intermedius. SCCmec типа ПЦС была обнаружена у 15 (88%) штаммов, типа IV — только у одного штамма. Лишь один штамм не поддавался типированию с помощью этого подхода.

Ключевые слова: Staphylococcus intermedius, мультиплексная ПЦР, mecA ген, MRSI, типы SCCmec.