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Short communication

Changes in the population structure of canine methicillin-resistant *Staphylococcus pseudintermedius* in Poland

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ABSTRACT

Methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) is being reported with an increasing frequency in small animal veterinary practice. The molecular typing of MRSP isolates revealed that the dominating European multidrug-resistant lineage is the sequence type 71 (ST71), associated with staphylococcal chromosomal cassette SCC*mec* type II–III. However, the recent reports indicated the emergence of other clones. The study aimed to determine the genetic properties of MRSP isolates obtained from dogs in Poland over a ten-year period. A total of 42 clinical MRSP isolates were subjected to multilocus-sequence typing (MLST) and SCC*mec* typing. MLST typing of 42 MRSP isolates yielded six STs belonging to two major clonal complexes (CCs): CC71 and CC551, associated with SCC*mec* element II–III and V, respectively. CC71 comprising ST71 and its newly described single locus variant (SLV) ST680. The second dominating CC551was represented by ST551 and newly described SLV ST771. The other, ST258 and ST85 were detected in single MRSP isolates. This is the first report concerning MLST typing of MRSP isolates in Poland. The results confirmed the domination of ST71 among MRSP until 2015, and the emergence of ST551 in 2015. Furthermore, in 2016 ST551 was identified in the majority of the strains, indicating the changes in the population structure of MRSP in Poland. Polish clinical MRSP isolates showed a shift in the population structure during the period of 2007 and 2016. The dominating MRSP lineage until 2015 was multidrug-resistant ST71-SCC*mec*II-III. The other lineage ST551-SCC*mec*V emerged in Poland since 2015, and in 2016 was found in the majority of MRSP isolates.

1. Introduction

Methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) strains have become one of the most important bacterial pathogens in small animal veterinary medicine (Pomba et al., 2016). MRSP isolates are resistant to all beta-lactam antibiotics and generally are also multidrug-resistant, causing difficulties in selection of an effective treatment (Perreten et al., 2010; Ruscher et al., 2010; Moodley et al., 2013). Additionally, MRSP strains constitute a reservoir of resistance genes for other staphylococci (Pomba et al., 2016).

Infections caused by MRSP represent a serious challenge for small animal veterinary practice. Dogs are natural hosts of *S. pseudintermedius*, including MRSP, both types of strains are involved in purulent infections, including pyoderma, otitis externa, wound infections, and urinary tract infections (Bannoehr and Guardabassi, 2012). Occasionally, *S. pseudintermedius* may infect other animals, mainly cats (Kadlec et al., 2016). Moreover, the dog owners having close contact with their pets are at high risk of a carrier state, potentially leading to infection (Pomba et al., 2016).

Genetic characterization of *S. pseudintermedius* strains has been achieved using many different molecular biology techniques (Bannoehr and Guardabassi, 2012), among which multilocus sequence typing (MLST) (Solyman et al., 2013; Pires Dos Santos et al., 2016) is an essential method in studies concerning the population structure of isolates worldwide. MLST is the analysis of DNA sequences of seven housekeeping genes. The important advantage of MLST over other molecular typing methods is that sequence data are unambiguous and easy to compare between different laboratories via global database available on the internet. Another method used for differentiation of MRSP isolates is the staphylococcal chromosomal cassette typing. The *mecA* gene is located on the bacterial chromosome, on a mobile genetic element − staphylococcal chromosomal cassette (SCC*mec*). SCC*mec* elements differ in their genetic structure. Several types of SCC*mec* elements (II–III, III, IV, V, VII), as well as non-typable elements, have been described in MRSP (Descloux et. al., 2008; Black et al., 2009; Perreten et al., 2010, 2013). The results of MLST revealed that the sequence type 71 (ST71) is the most common among MRSP in Europe, whereas ST68 is the most prevalent in North America (Black et al., 2009; Perreten et al., 2010;

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Table 1
Characteristics of 42 methicillin-resistant *Staphylococcus pseudintermedius* isolates.

Isolate ID	Origin	Type of clinical sample	MLST type	SCC*mec* type
523/07	Dog	Skin swab	ST71	II–III
460/07	Dog	Urine	ST71	II–III
637/07	Dog	Urine	ST71	II–III
1071/07	Dog	Skin swab	ST71	II–III
1716/07	Dog	Wound swab	ST71	II–III
1143/09	Dog	Throat swab	ST71	IV
1443/09	Dog	Pseudoarthrosis swab	ST71	II–III
1750/09	Dog	Ear swab	ST71	II–III
2477/09	Dog	Skin swab	ST71	II–III
2985/09	Dog	Skin swab	ST71	II–III
3033/09	Dog	Skin swab	ST71	II–III
87/10	Dog	Skin swab	ST71	II–III
182/10	Dog	Skin swab	ST71	II–III
937/10	Dog	Wound swab	ST71	II–III
1030/10	Dog	Urine	ST71	II–III
2311/10	Dog	Urine	ST71	II–III
2481/10	Dog	Urine	ST71	II–III
279/11	Dog	Internal organs	ST71	II–III
293/11	Dog	Skin swab	ST71	II–III
300/11	Dog	Skin fistula	ST71	II–III
590/11	Dog	Ear swab	ST71	II–III
1034/11	Dog	Internal organs	ST258	IV
1941/11	Dog	Wound swab	ST71	II–III
975/12	Dog	Ear swab	ST71	II–III
1297/12	Dog	Urine	ST71	II–III
1869/12	Dog	Skin swab	ST71	II–III
43/13	Dog	Skin swab	ST85	Non-typable
231/13	Dog	Urine	ST71	IV
464/13	Dog	Urine	ST71	II–III
722/13	Dog	Conjunctival swab	ST71	II–III
813/13	Dog	Wound swab	ST71	II–III
857/13	Dog	Wound swab	new ST680	II–III
899/13	Dog	Skin swab	ST71	II–III
320/15	Dog	Ear swab	ST71	II–III
354/15	Dog	Wound swab	ST551	V
578/15	Dog	Skin swab	ST551	V
36/16	Dog	Ear swab	ST551	V
232/16	Dog	Tracheal swab	ST551	V
417/16	Dog	Skin swab	ST71	II–III
597/16	Dog	Vaginal swab	new ST771	V
598/16	Dog	Ear swab	ST551	V
630/16	Dog	Skin swab	ST551	V

Ruscher et al., 2010). The latest reports indicate towards further diversification of the MRSP worldwide (Pires Dos Santos et al., 2016). However, there is a lack of data on MRSP isolates from Poland. The identification of the STs dominating in Poland will improve the understanding of the clonal similarity of MRSP isolates.

The study was conducted to assess the genetic properties of clinical methicillin-resistant *S. pseudintermedius* isolates obtained from dogs in Poland over a ten-year period. MLST and SCC*mec* typing were used to compare the properties of MRSP isolates from Poland with those from other countries.

2. Materials and methods

A collection of 42 MRSP strains isolated between 2007 and 2016 was analyzed using MLST scheme and SCC*mec* typing. MRSP were not isolated in 2008 and 2014. Strains were isolated from 42 different dogs with infections at different body sites. The isolates were obtained from clinical samples submitted to the Microbiological Diagnostic Laboratory, Faculty of Veterinary Medicine, Warsaw University of Life Sciences-SGGW, from the area of Warsaw. The characteristics of the 42 MRSP isolates is shown in Table 1. The identification of *S. pseudintermedius* strains was based on standard bacteriologic methods and confirmed by the *nuc* PCR (Sasaki et al., 2010). Methicillin resistance was determined by the *mecA* gene amplification (Strommenger et al., 2003).

MLST typing was performed for all 42 isolates in accordance with the method based on the analysis of seven housekeeping genes (Solyman et al., 2013). Obtained sequences were compared to known alleles at each locus using the MLST website (http://pubmlst.org/spseudintermedius). Sequence types were assigned by comparison with the allele sequences present in the PubMLST database. New types detected in this study were submitted and assigned by the curator of the PubMLST database.

SCC*mec* types I–VI were identified using the method described by Kondo et al. (2007). The presence of SCC*mec* element type II–III was examined using the method based on Descloux et al. (2008).

3. Results

Among 42 clinical isolates recognized as MRSP, MLST typing demonstrated the occurrence of six sequence types. The proportion of isolates of different STs in years covered by the study is shown in Fig. 1. The most common ST was ST71, detected in 32 (76.1%) strains. The second most frequently recognized sequence type was ST551, confirmed in 6 (14.3%) isolates. However, it is worth noting that ST551 was reported among MRSP strains isolated only in the year 2015 and 2016. Two STs (ST258 and ST85) were detected in single isolates. Another two sequence types obtained in single isolates, ST680 and ST771, were new types assigned in the PubMLST database. The newly described ST680 and ST771 represented single locus variants (SLV) of ST71 and ST551, respectively. Therefore, two major clonal complexes, CC71 and CC551, were found among examined isolates.

The number of MRSP isolates with each identified SCC*mec* element, and the associated STs, are shown in Table 2. The predominant SCC*mec* type was II–III (n = 31), followed by V (n = 7). The SCC*mec* type II–III was associated with ST71 and its single locus variant ST680. Similarly,

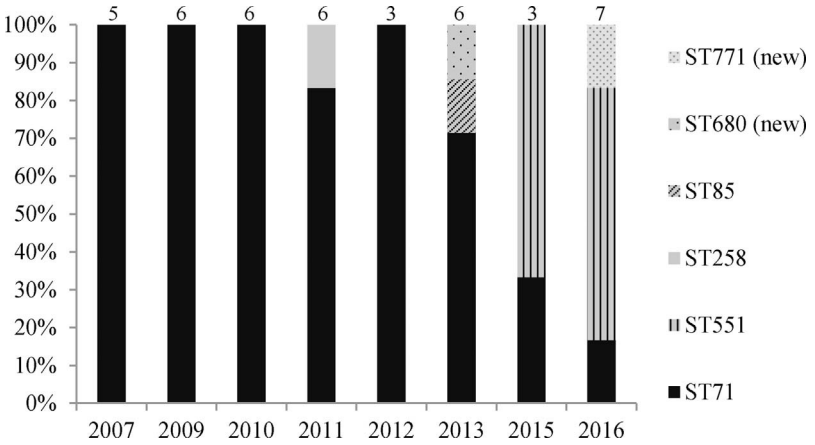

Fig. 1. The proportion of sequence types (STs) of methicillin-resistant *Staphylococcus pseudintermedius* in the period from 2007 to 2016. Numbers at the top of the columns indicate the number of isolates.