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# Molecular analysis of methicillinresistant *Staphylococcus pseudintermedius* of feline origin from different European countries and North America

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#### Sir,

Staphylococcus pseudintermedius is the most frequent causative agent of canine pyoderma and may also be associated with wound infections, urinary tract infections and otitis externa in dogs.<sup>1</sup> Although more rarely, *S. pseudintermedius* causes infections in cats and has also been identified in infections of humans.<sup>1-3</sup> The latter observation highlights the zoonotic potential of *S. pseudintermedius*. *S. pseudintermedius* appears to be able to readily accumulate antimicrobial resistance genes<sup>1,4</sup> and, in recent years, a rapid increase in methicillin resistance has been observed.<sup>5,6</sup> A recent multicentre study on methicillin

resistant *S. pseudintermedius* (MRSP) of canine origin, obtained in different European countries as well as in the USA and Canada, revealed that most of the canine strains exhibited resistance to virtually all classes of antimicrobial agents approved for veterinary use.<sup>4</sup> This represents a major therapeutic challenge for veterinarians in Europe and North America. Moreover, this multicentre study revealed that among MRSP from dogs two distinct, dominant clones—as identified by multilocus sequence typing (MLST), *spa* typing, SCC*mec* typing and SmaI PFGE—have disseminated across Europe and North America.<sup>4</sup> In contrast to the wealth of data available for MRSP from cats.

The aim of the present study was to characterize MRSP isolates from cats in different countries for their genetic relationships and antimicrobial resistance phenotypes and genotypes. Twelve epidemiologically unrelated MRSP isolates of feline origin were identified during 2006-08 in five different countries (Table 1). Eleven isolates were from clinical disease conditions, including septicaemia, urinary tract infections, nephritis, rhinitis, wound infection and pneumonia. The remaining isolate was obtained from a nasal swab of an apparently healthy cat (Table 1). All isolates were confirmed to be S. pseudintermedius by MboI digestion of a PCR-amplified internal fragment of the pta gene.<sup>4</sup> For a better comparison with data of canine MRSP isolates, MLST,<sup>7</sup> spa typing,<sup>8</sup> SmaI PFGE and SCCmec typing was performed as recently described.<sup>4</sup> MICs of 17 antimicrobial agents were determined using the VetMIC<sup>TM</sup> microdilution panels (National Veterinary Institute, Uppsala, Sweden) as previously described and evaluated using the breakpoints of the CLSI.<sup>4</sup> MICs of rifampicin, mupirocin and quinupristin/dalfopristin were determined by Etest® (AB Biodisk, Solna, Sweden). Antibiotic resistance genes were detected using either a microarray or specific PCR assays as described previously.<sup>4</sup>

All 12 feline S. pseudintermedius proved to be MRSP by oxacillin MICs of >16 mg/L and carriage of the mecA gene. Despite the diverse geographical origins, the 11 European MRSP isolates shared the same MLST type ST71, spa type t02 and SCCmec type II-III. SCCmec type II-III is a hybrid of SCCmec II (2A) from Staphylococcus epidermidis and SCCmec III from Staphylococcus aureus.<sup>9</sup> PFGE analysis identified three different patterns J, N and O among these European feline MRSP isolates. The single MRSP isolate from Canada harboured an SCCmec type V element and exhibited spa type t23, MLST type ST100 and PFGE pattern B. A comparison with the data of the multicentre study on MRSP in dogs<sup>4</sup> revealed that PFGE patterns B, N and O were exclusive to feline MRSP isolates whereas pattern J was the dominant PFGE pattern among European canine MRSP.<sup>4</sup> The Canadian feline MRSP isolate also differed largely in its resistance phenotype and genotype from the European feline MRSP isolates. It was only resistant to  $\beta$ -lactam antibiotics via mecA and blaZ, and to tetracyclines via tet(M). In contrast, the European isolates

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| Countryª | Disease condition       | SCC <i>mec</i><br>type | <i>spa</i> type | MLST type | PFGE<br>pattern | Resistance phenotype <sup>b</sup>         | Resistance genotype  |
|----------|-------------------------|------------------------|-----------------|-----------|-----------------|---|--|
| Ι        | septicaemia             | II–III                 | t02             | ST71      | J               | BLA, TET, ML, CHL, TMP, GEN, KAN, STR, FQ | mecA, blaZ, tet(K), erm(B), cat <sub>pC221</sub> , dfrG,<br>aac(6')-Ie/aph(2')-Ia, aph(3')-III, ant(6')-Ia |
| Ι        | nephritis               | II–III                 | t02             | ST71      | Ν               | BLA, TET, ML, CHL, TMP, GEN, KAN, STR, FQ | mecA, blaZ, tet(K), erm(B), cat <sub>pC221</sub> , dfrG,<br>aac(6')-Ie/aph(2')-Ia, aph(3')-III, ant(6')-Ia |
| Ι        | septicaemia             | II–III                 | t02             | ST71      | J               | BLA, TET, ML, CHL, TMP, GEN, KAN, STR, FQ | mecA, blaZ, tet(K), erm(B), cat <sub>pC221</sub> , dfrG,<br>aac(6')-Ie/aph(2')-Ia, aph(3')-III, ant(6')-Ic |
| Ι        | septicaemia             | II–III                 | t02             | ST71      | J               | BLA, TET, ML, CHL, TMP, GEN, KAN, STR, FQ | mecA, blaZ, tet(K), erm(B), cat <sub>pC221</sub> , dfrG,<br>aac(6')-Ie/aph(2')-Ia, aph(3')-III, ant(6')-Ia |
| СН       | rhinitis                | II–III                 | t02             | ST71      | J               | BLA, TET, ML, CHL, TMP, GEN, KAN, STR, FQ | mecA, blaZ, tet(K), erm(B), cat <sub>pC221</sub> , dfrG,<br>aac(6')-Ie/aph(2')-Ia, aph(3')-III, ant(6')-Ia |
| СН       | wound infection         | II–III                 | t02             | ST71      | J               | BLA, ML, CHL, TMP, GEN, KAN, STR, FQ      | mecA, blaZ, erm(B), cat <sub>pC221</sub> , dfrG, aac(6')-Ie/<br>aph(2')-Ia, aph(3')-III, ant(6')-Ia        |
| СН       | urinary tract infection | II–III                 | t02             | ST71      | J               | BLA, TET, ML, TMP, GEN, KAN, STR, FQ      | mecA, blaZ, tet(K), erm(B), dfrG, aac(6')-Ie/<br>aph(2')-Ia, aph(3')-III, ant(6')-Ia                       |
| СН       | urinary tract infection | II–III                 | t02             | ST71      | J               | BLA, TET, ML, CHL, TMP, GEN, KAN, STR, FQ | mecA, blaZ, tet(K), erm(B), cat <sub>pC221</sub> , dfrG,<br>aac(6')-Ie/aph(2')-Ia, aph(3')-III, ant(6')-Ia |
| CH       | urinary tract infection | II–III                 | t02             | ST71      | J               | BLA, TET, ML, CHL, TMP, GEN, KAN, STR, FQ | mecA, blaZ, tet(K), erm(B), cat <sub>pC221</sub> , dfrG,<br>aac(6')-Ie/aph(2')-Ia, aph(3')-III, ant(6')-Ia |
| NL       | urinary tract infection | II–III                 | t02             | ST71      | 0               | BLA, ML, CHL, TMP, GEN, KAN, STR, FQ      | mecA, blaZ, erm(B), cat <sub>pC221</sub> , dfrG, aac(6')-Ie/<br>aph(2')-Ia, aph(3')-III, ant(6')-Ia        |
| D        | healthy                 | II–III                 | t02             | ST71      | J               | BLA, TET, ML, CHL, TMP, GEN, KAN, STR, FQ | mecA, blaZ, tet(K), erm(B), cat <sub>pC221</sub> , dfrG,<br>aac(6')-Ie/aph(2')-Ia, aph(3')-III, ant(6')-Ia |
| CAN      | pneumonia               | V                      | t23             | ST100     | В               | BLA, TET                                  | mecA, blaZ, tet(M)   |

Table 1. Characteristics of the 12 MRSP isolates from cats investigated in this study

<sup>a</sup>I, Italy; CH, Switzerland; NL, the Netherlands; D, Germany; CAN, Canada.

<sup>b</sup>BLA, β-lactam antibiotics; CHL, chloramphenicol; FQ, fluoroquinolones; GEN, gentamicin; KAN, kanamycin; ML, macrolides/lincosamides; STR, streptomycin; TET, tetracyclines; TMP, trimethoprim.

A

**Research** letters

Isolate no.

E028

E029

E031

E037

E047

E051

E053

E060

E072

E076

E114

E132

exhibited three different expanded resistance phenotypes and genotypes (Table 1). All isolates were resistant to  $\beta$ -lactam antibiotics (mecA, blaZ), macrolides/lincosamides [erm(B)], gentamicin/kanamycin [aac(6')-Ie/aph(2')-Ia], kanamycin [aph(3')-III], streptomycin [ant(6')-Ia], trimethoprim [dfrG] and ciprofloxacin. Moreover, all but one isolate from Switzerland and all but two isolates from Switzerland and the Netherlands were resistant to chloramphenicol (cat<sub>pC221</sub>) and to tetracyclines [tet(K)], respectively. However, all feline MRSP isolates were susceptible to mupirocin, rifampicin, quinupristin/dalfopristin, linezolid and vancomycin, which are important for decolonization of humans or represent 'antimicrobial agents of last resort' for the treatment of methicillin-resistant *S. aureus* (MRSA) infections in humans.

A comparison with the results of the genetic analysis of canine MRSP showed that the Canadian feline MRSP differed in all characteristics, except SCCmec type V, from the dominant canine MRSP clone in North America, which is characterized by ST68 (MLST)–C (PFGE)–t06 (*spa*)–V (SCCmec).<sup>4</sup> In contrast, 9 of the 11 European feline MRSP isolates were identified as members of the previously described dominant clonal lineage among canine MRSP in Europe, which is characterized by ST71 (MLST)–J (PFGE)–t02 (*spa*)–II–III (SCCmec).<sup>4</sup> This observation strongly suggested an exchange of MRSP isolates between dogs and cats in Europe. Whether the feline MRSP isolate from Canada represents a member of a new MRSP clone with a particular tropism for cats remains to be determined.

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#### **Transparency declarations**

None to declare.

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# Molecular characterization of plasmids encoding CTX-M-15 extended-spectrum $\beta$ -lactamase associated with the ST131 *Escherichia coli* clone in Belgium

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Keywords: ESBLs, B2 phylogroup, pEK499, pC15

#### Sir,

Previous studies performed in a large number of Belgian hospitals showed the dissemination of a major clone of the virulent B2 group O25b:H4-ST131 CTX-M-15-producing *Escherichia coli*.<sup>1</sup> This *E. coli* ST131 clone has been reported worldwide and represents a major public health problem.<sup>2</sup> The present study sought to characterize *bla*<sub>CTX-M-15</sub>-containing plasmids associated with ST131 *E. coli* CTX-M-15 isolates recovered in Belgian hospitals. This specific clone was detected from clinical specimens of patients hospitalized at the Erasme hospital in Brussels since 2001, as well as in 18 other Belgian hospitals