Phenotypic and molecular characterization of antimicrobial resistance in *Proteus mirabilis* isolates from dogs

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Large-scale monitoring of resistance to 14 antimicrobial agents was performed using 103 *Proteus mirabilis* strains isolated from dogs in Japan. Resistant strains were analysed to identify their resistance mechanisms. Rates of resistance to chloramphenicol, streptomycin, enrofloxacin, trimethoprim/sulfamethoxazole, kanamycin, ampicillin, ciprofloxacin, cefalothin, gentamicin, ceftoxin and cefotaxime were 20.4, 15.5, 12.6, 10.7, 9.7, 8.7, 5.8, 2.9, 2.9, 1.9 and 1.9 %, respectively. No resistance to ceftazidime, aztreonam or imipenem was found. Class 1 and 2 integrases were detected in 2.9 and 11.7 % of isolates, respectively. Class 1 integrons contained *aadB* or *aadB-catB*-like-*blaOXA10*-aadA1, whereas those of class 2 contained sat–aadA1, *dhfr1*–sat–aadA1 or none of the anticipated resistance genes. Of five distinct plasmid-mediated quinolone-resistance (PMQR) genes, only *qnrD* gene was detected in 1.9 % of isolates. Quinolone-resistance determining regions (QRDRs) of gyrA and parC from 13 enrofloxacin-intermediate and -resistant isolates were sequenced. Seven strains had double mutations and three had single mutations. Three of nine ampicillin-resistant isolates harboured AmpC-type β-lactamases (i.e. *blaCMY-2*, *blaCMY-4* and *blaOXA-1*). These results suggest that canine *Proteus mirabilis* deserves continued surveillance as an important reservoir of antimicrobial resistance determinants. This is the first report, to our knowledge, describing integrons, PMQRs and QRDR mutations in *Proteus mirabilis* isolates from companion animals.

The emergence of antimicrobial resistance in *Proteus mirabilis* isolates from companion animals has been documented (Authier et al., 2006; Pedersen et al., 2007; Grobbel et al., 2007). The development of antimicrobial resistance increases the risk of antimicrobial treatment failure in companion animals infected with *Proteus mirabilis*. In addition to an impact on animal health, the emergence of bacterial pathogens that have antimicrobial resistance may have important human public health consequences if isolates are transmitted to humans by their pets (Guardabassi et al., 2004; Lloyd, 2007). Understanding the prevalence of antimicrobial resistance among canine *Proteus mirabilis* isolates is important not only from a veterinary perspective but also from a global public health perspective. However, the status regarding the emergence of resistant *Proteus mirabilis* in companion animals remains unknown in Japan.

The spread of multidrug resistance among Gram-negative bacteria, including *Proteus mirabilis*, is a major concern.
Multidrug resistance is classified as co-resistance or cross-resistance; the former is resistance to unrelated classes of drugs and the latter is resistance to related classes of drugs (Harada & Asai, 2010). Integrons, which are a type of mobile genetic element, are often associated with co-resistance because their acquisition leads to an accumulation of antimicrobial resistance genes (Partridge et al., 2009). In contrast, resistances to fluoroquinolones and extended-spectrum β-lactams are representative of cross-resistance. Fluoroquinolone resistance is mainly acquired by modification of their target enzymes, DNA gyrase and topoisomerase IV, but it may also involve acquisition of plasmid-mediated quinolone resistance (PMQR) determinants (Fábrega et al., 2009). Extended-spectrum β-lactam resistance is mainly associated with the production of extended-spectrum β-lactamases (ESBLs), plasmid-mediated AmpC β-lactamases (PABLS) and plasmid-mediated metallo-β-lactamases (MBLs) (Rubin & Pitout, 2014). These mechanisms of multidrug resistance have been identified in human Proteus mirabilis isolates (Weigel et al., 2002; Mokracka et al., 2012) but have not been completely elucidated in isolates from companion animals.

In the present study, the susceptibility of 103 Proteus mirabilis isolates of canine origin to 14 antimicrobial agents was assessed. In addition, genetic analyses for integrons and resistance to fluoroquinolones and extended-spectrum β-lactams were used to assess the multidrug resistance status of these organisms.

METHODS

Bacterial isolates. The 103 Proteus mirabilis isolates analysed in this study were obtained from canine clinical specimens that were collected from 17 veterinary hospitals in Japan between 2003 and 2013. The specimens were isolated from various anatomical sites, which were estimated to be sites of bacterial infection by clinical veterinarians, including the ear canal (n = 41), urinary tract (n = 26), skin (n = 19), genitals (n = 7), faeces (n = 4), nasal cavity (n = 1) and eye (n = 1). Four specimens were of unknown anatomical origin. No information was available regarding previous treatment of the dogs by veterinarians, including the ear canal, which were estimated to be sites of bacterial infection by clinical veterinarians.

Antimicrobial susceptibility testing. The susceptibility of the isolates to ampicillin (AMP), cephalothin (CEP), cefoxitin (FOX), cefazidime (CAZ), cefotaxime (CTX), aztreonam (ATM), imipenem (IPM), streptomycin (STR), kanamycin (KAN), gentamicin (GEN), chloramphenicol (CHL), trimethoprim/sulfamethoxazole (TMS), ciprofloxacin (CIP) and enrofloxacin (ENR) was determined. Susceptibility testing was conducted using the agar diffusion method with E-test strips (bioMérieux) or discs (Becton Dickinson), according to the manufacturer’s instructions or the Clinical and Laboratory Standards Institute guidelines (CLSI, 2013a). The results obtained were interpreted as per the criteria contained within CLSI guidelines (CLSI, 2013b, c). When calculating resistance rates, strains with an ‘intermediate’ result to the tested antimicrobial were considered resistant. Escherichia coli ATCC 25922 was used as a quality control strain.

Detection of integrase genes and characterization of gene cassette regions. Genomic DNA from each of the isolates was prepared by suspending several colonies in 0.5 ml of water and boiling the samples for 10 min. These genomic DNA preparations were used as templates for genetic analyses conducted using a previously described PCR method designed to detect class 1, 2 and 3 integrases (Mazel et al., 2000). The integrase-positive isolates were further investigated to characterize the gene cassette captured by each integron. Amplification of the variable regions of class 1 and 2 integrons was performed by PCR with the specific primer sets (5′-CS and 3′-CS primers, and Hep74 and Hep51 primers, respectively) (Mokracka et al., 2012). Gene cassettes were identified by PCR mapping (Lévesque et al., 1995) using several primers for aminoglycoside (aadA1, aadA2, aadA5, estX and sat1/2) or trimethoprim (dfrA1, dfrA12 and dfrA17) resistance genes (LITERAKI et al., 2010) or by direct sequencing. Any ambiguous PCR results were clarified with repeat assays.

Detection of PMQR genes. Each of the strains was assessed for the presence of PMQR genes using multiplex PCR assays for the detection of qnrA, qnrB and qnrS genes (Cattoir et al., 2007), and using a simplex PCR assay for the detection of either the qnrS (Cavaco et al., 2009) or aac(6′)-Ib-cr (Park et al., 2006) gene. Any ambiguous PCR results were clarified with repeat assays. All PCR products were bi-directionally sequenced with the same primers for confirmation.

Mutations in the quinolone resistance-determining regions of gyrA and parC. For isolates with intermediate or full resistance to ENR (MIC ≥1 µg ml⁻¹), the quinolone resistance-determining regions (QRDRs) of the gyrA and parC genes were amplified by PCR with previously described primers (Weigel et al., 2002). The resulting PCR products were bi-directionally sequenced with the same primers.

Identification of β-lactamase genes. For AMP-resistant isolates (MIC ≥32 µg ml⁻¹), class A β-lactamase genes (i.e. blaTEM and blaSHV) were identified using PCR, followed by DNA sequencing, as previously reported (Kojima et al., 2005). In addition, PABLS, ESBLs and MBLs were phenotypically screened using discs of FOX (30 µg), CTX (3 µg) and meropenem (10 µg), respectively; the results were considered positive if the inhibition zone diameters were ≤18, ≤27 and ≤19 mm, respectively (Thomson, 2010; CLSI, 2013c). In PABL-positive isolates, the responsible genes were screened by multiplex-PCR (Pérez-Pérez & Hanson, 2002), amplified and then bi-directionally sequenced using specific primers (Yan et al., 2002; Literacka et al., 2004).

Statistical analysis. The prevalence of antimicrobial resistance was compared among isolation sites (i.e. ear canal, urinary tract and others) by using Fisher’s exact test. A P value less than 0.05 was considered significant.

RESULTS AND DISCUSSION

Susceptibility testing revealed that 66 of the 103 (64.7%) isolates of Proteus mirabilis were susceptible to all 14 of the antimicrobials tested. The rates of resistance to CHL, STR, ENR, TMS, KAN, AMP, CIP, CEP, GEN, FOX and CTX were 20.4, 15.5, 12.6, 10.7, 9.7, 8.7, 5.8, 2.9, 2.9, 1.9 and 1.9, respectively (Fig. 1). None of the strains tested were resistant to CAZ, ATM or IPM. A similar study of 29 canine Proteus isolates from Denmark showed higher rates of resistance to STR (41.4%) and AMP (20.7%) and lower rates of resistance to CIP (0 %) and GEN (0 %) (Pedersen et al., 2007). A study of 37 German canine and feline isolates showed higher rates of resistance to ENR (22 %)
and TMS (27 %) (Grobbel et al., 2007). In Canada (Authier et al., 2006), 64 canine strains showed a higher rate of resistance to AMP (29 %) and CEP (19 %) and lower rate of resistance to ENR (5 %). Overall, there are some differences in the prevalence of resistance in canine Proteus isolates among countries. Similar differences among countries were found with other bacterial species, including E. coli and Pseudomonas aeruginosa isolates from companion animals (Harada et al., 2007a, b). Therefore, the risk of antimicrobial-resistance among bacteria in companion animals should be understood with respect to each country.

In this study, the tested strains of Proteus mirabilis were obtained from various sites. When comparing rates of resistance to the tested antimicrobials among the isolation sites, the rate of resistance to ENR alone was significantly more prevalent in ear isolates (26.8 %) than in the isolates from urinary tract and other sites (0 and 6.3 %, respectively, \( P<0.05 \)). This possibly indicates that ear isolates have a greater risk of acquiring ENR resistance. One of the possible reasons for this is that Proteus mirabilis is frequently isolated as one of the primary pathogen of otitis externa in dogs (Lyskova et al., 2007), and thereby may increase opportunities to be exposed to the selective pressure. Veterinarians should take into account the site-specific prevalence of antimicrobial resistance in Proteus mirabilis from companion animals when selecting antimicrobial drugs.

Integrons contain one or more gene cassettes, mainly encoding resistance determinants, and have a major role in the spread of multidrug resistance in Gram-negative bacteria (Partridge et al., 2009). In this study, we investigated the prevalence of class 1, 2 and 3 integrons in canine Proteus mirabilis. Class 1 and 2 integrases (i.e. \( \text{intI1} \) and \( \text{intI2} \), respectively) were detected in 2.9 (3/103) and 11.7 % (12/103) of the Proteus mirabilis strains tested, respectively (Table 1). One isolate was positive for both these integrases. On the other hand, class 3 integrase was not detected in any of the strains. Of the three \( \text{intI1} \)-positive isolates, one had an integron carrying an \( \text{aadB}–\text{catB-like-bla}_{\text{OXA10}}–\text{aadA1} \) array (3.0 kb) in the variable regions. To the best of our knowledge, a class 1 integron of this structure was first detected in Proteus mirabilis, although the same integron has been identified in an Acinetobacter baumannii isolate from humans (Gu et al., 2007). Of 12 \( \text{intI2} \)-positive isolates, one had an integron carrying the \( \text{sat–aadA1} \) array (1.5 kb) and five had an integron carrying the \( \text{dhfri–sat–aadA1} \) array (2.2 kb). These gene arrays have been frequently identified in class 2 integrons from human isolates of Proteus mirabilis (Mokracka et al., 2012). Most of the genes detected in class 1 and 2 integrons encode resistance to aminoglycosides (i.e. KAN, GEN and STR) and trimethoprim (Schwarz & Chaslus-Dancla, 2001). Isolates harbouring integrons with these resistance genes exhibited multidrug resistance. Therefore, although class 1 and 2 integrons are relatively uncommon, they can contribute to the occurrence of multidrug resistance in canine Proteus mirabilis.

The primary mechanism of fluoroquinolone resistance is the accumulation of mutations in the QRDR of DNA gyrase and DNA topoisomerase IV (Fàbrega et al., 2009). However, the status of QRDR mutations has not yet been clarified in fluoroquinolone-resistant Proteus mirabilis strains of animal origin. In this study, sequence analysis revealed that all but one of eight ENR-resistant (MIC \( \geq 4 \mu \text{g ml}^{-1} \)) isolates had one mutation each in \( \text{gyrA} \) (at codon 83) and \( \text{parC} \) (at codon 78 or 80) (Table 2). These mutations were previously identified in human isolates (Weigel et al., 2002). On the other hand, five ENR-intermediate isolates had no mutations or one mutation only in \( \text{gyrA} \). Thus, double point mutations in QRDR are likely to play an important role in high-level resistance to ENR in Proteus mirabilis. In contrast, isolates with double point mutations were intermediate (MIC of 2 \( \mu \text{g ml}^{-1} \)) or susceptible (MIC of \( \leq 1 \mu \text{g ml}^{-1} \)) to CIP but not resistant to this drug, a previous study reported similar results (Weigel et al., 2002). These data imply that QRDR mutations have a more significant role in the acquisition of ENR resistance compared with that in the acquisition of CIP resistance.

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**Fig. 1.** Rates of resistance (percentages) to 14 antimicrobials in 103 Proteus mirabilis isolates from dogs in Japan.
Similar QRDR mutations, PMQR determinants are important mechanisms of fluoroquinolone resistance. However, the acquisition of PMQR determinants will not generally render a wild-type organism insusceptible to fluoroquinolones (Strahilevitz et al., 2009). Thus, both fluoroquinolone-resistant and -susceptible isolates were tested for major PMQR genes using PCR, followed by DNA sequencing. None of the isolates were positive for qnrA, qnrB, qnrS and aac(6')-Ib-cr genes, whereas two of 105 (1.9%) strains were positive for the qnrD gene. Several of these genes have been identified in companion animals in other countries. The qnrA, qnrB, qnrS and aac(6')-Ib-cr genes were identified in companion animals in Italy (Donati et al., 2014); the qnrB, qepA and aac(6')-Ib-cr genes in China (Ma et al., 2009); the qnrA, qnrB, qnrS, qepA and aac(6')-Ib-cr genes in Australia (Gibson et al., 2010a, b) and the qnrS, qepA and aac(6')-Ib-cr genes in the USA (Shaheen et al., 2013). To the best of our knowledge, this is the first report of the qnrD gene in bacteria isolated from companion animals, possibly because the qnrD gene is closely related to the tribe Proteaeae, which includes the genera Proteus, Providencia and Morganella (Guillard et al., 2014). Of the two qnrD-positive isolates, one isolate (i.e. strain 77) that also had QRDR mutations exhibited

Table 1. Gene cassettes of class 1 and 2 integrons and resistance phenotypes among 14 integrase-positive isolates of canine Proteus mirabilis

<table>
<thead>
<tr>
<th>Integrase class</th>
<th>Amplicon size</th>
<th>Gene cassette array</th>
<th>Resistance phenotypes*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class 1 (2)</td>
<td>0.75 kb (2)</td>
<td>aadB (2)</td>
<td>AMP, CEP, FOX, CTX, KAN, GEN (1) AMP, KAN, STR, GEN, CHL, TMS (1)</td>
</tr>
<tr>
<td>Class 2 (11)</td>
<td>None (6)</td>
<td>NA</td>
<td>AMP, KAN, STR, GEN, CHL, TMS (1)</td>
</tr>
<tr>
<td></td>
<td>1.5 kb (1)</td>
<td>sat–aadA1 (1)</td>
<td>CIP, ENR (3)</td>
</tr>
<tr>
<td></td>
<td>2.2 kb (4)</td>
<td>dhfr1–sat–aadA1 (4)</td>
<td>STR, ENR (1)</td>
</tr>
<tr>
<td>Class 1 and 2 (1)</td>
<td>3.0 kb/2.2 kb (1)</td>
<td>aadB–catB-like-blaOXA10–aadA1/dhfr1–sat–aadA1 (1)</td>
<td>AMP, KAN, STR, GEN, CHL, TMS, CIP, ENR (2)</td>
</tr>
</tbody>
</table>

Numbers in a parentheses represent the number of isolates. Underlines denote phenotypes encoded by resistance genes within class 1 and 2 integrons.
NA, Not applicable.
*Strains with an 'intermediate' result to the tested antimicrobials were considered resistant.

Table 2. Amino acid substitutions in the QRDRs of 13 enrofloxacin-resistant and -intermediate isolates of canine Proteus mirabilis

<table>
<thead>
<tr>
<th>Strain</th>
<th>GyrA</th>
<th>ParC</th>
<th>Enrofloxacin MIC (µg ml⁻¹)</th>
<th>Ciprofloxacin MIC (µg ml⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>S83I</td>
<td>S80I</td>
<td>&gt;32</td>
<td>2</td>
</tr>
<tr>
<td>66</td>
<td>S83I</td>
<td>G78D</td>
<td>&gt;32</td>
<td>2</td>
</tr>
<tr>
<td>71</td>
<td>S83I</td>
<td>G78D</td>
<td>&gt;32</td>
<td>2</td>
</tr>
<tr>
<td>74</td>
<td>S83I</td>
<td>G78D</td>
<td>&gt;32</td>
<td>2</td>
</tr>
<tr>
<td>77</td>
<td>S83I</td>
<td>S80R</td>
<td>&gt;32</td>
<td>2</td>
</tr>
<tr>
<td>95</td>
<td>S83I</td>
<td>G78D</td>
<td>&gt;32</td>
<td>2</td>
</tr>
<tr>
<td>91</td>
<td>S83I</td>
<td>S80R</td>
<td>8</td>
<td>0.500</td>
</tr>
<tr>
<td>32</td>
<td>E87K</td>
<td>–</td>
<td>4</td>
<td>0.250</td>
</tr>
<tr>
<td>19</td>
<td>–*</td>
<td>–</td>
<td>2</td>
<td>0.125</td>
</tr>
<tr>
<td>101</td>
<td>–</td>
<td>–</td>
<td>2</td>
<td>&lt;0.015</td>
</tr>
<tr>
<td>70</td>
<td>S83I</td>
<td>–</td>
<td>1</td>
<td>0.250</td>
</tr>
<tr>
<td>75</td>
<td>S83I</td>
<td>–</td>
<td>1</td>
<td>0.125</td>
</tr>
<tr>
<td>86</td>
<td>–</td>
<td>–</td>
<td>1</td>
<td>&lt;0.015</td>
</tr>
</tbody>
</table>

* No change from type strain sequence.
high-level resistance to ENR (MIC $>32 \, \mu g \, ml^{-1}$) and the other isolate was susceptible to ENR (MIC $0.25 \, \mu g \, ml^{-1}$). Thus, the prevalence of the qnrD gene should be cautiously monitored in both fluoroquinolone-resistant and -susceptible isolates of *Proteus mirabilis* in companion animals.

The prevalence of $\beta$-lactamas has been a serious problem in *Enterobacteriaceae* from companion animals (Rubin & Pitout, 2014), but this problem has been poorly studied in *Proteus* isolates. In this study, eight of nine AMP-resistant isolates harboured TEM-1 $\beta$-lactamase (Table 3). In addition, three CEF-resistant strains (strains 5, 61 and 68) harboured PABLs like *bla*<sub>CMY-2</sub>, *bla*<sub>CMY-4</sub> and *bla*<sub>DHA-1</sub>. CMY-2 has previously been detected in feline *Proteus* isolates from the Netherlands (Hordijk et al., 2013), while CMY-4 and DHA-1 were first detected in this study. Notably, the two isolates that harboured CMY-type enzymes also exhibited resistance to CTX (MIC $>4 \, \mu g \, ml^{-1}$), a third-generation cephalosporin. On the other hand, neither ESBLs nor MBLs were detected, although these types of $\beta$-lactamas have been detected in human *Proteus* isolates (Papagiannitis et al., 2012; Chong et al., 2013). These data imply that CMY-type $\beta$-lactamase deserves close attention as a factor contributing to the prevalence of *Proteus mirabilis* strains resistant to extended-spectrum $\beta$-lactams in companion animals.

In conclusion, we firstly performed large-scale monitoring of antimicrobial resistance in *Proteus mirabilis* isolates from dogs. The resistant strains encountered were analysed to identify important resistance mechanisms. The results demonstrate that resistance to the tested drugs is relatively infrequent. Furthermore, notable resistance mechanisms, including integrons, PMQRs, PABLs and QRDR mutations were identified. Therefore, canine *Proteus mirabilis* deserves continued monitoring as an important reservoir of antimicrobial resistance. This is the first report in which integrons, PMQRs and QRDR mutations were described in *Proteus mirabilis* isolates from companion animals.

### Table 3. MIC of $\beta$-lactam drugs and prevalence of $\beta$-lactamase genes among nine ampicillin-resistant isolates of canine *Proteus mirabilis*

<table>
<thead>
<tr>
<th>Strain</th>
<th>AMP</th>
<th>CEF</th>
<th>FOX</th>
<th>CAZ</th>
<th>CTX</th>
<th>ATM</th>
<th>IPM</th>
<th>$\beta$-Lactamase gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>32</td>
<td>4</td>
<td>0.125</td>
<td>0.125</td>
<td>&lt;0.016</td>
<td>0.25</td>
<td>TEM-1</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>&gt;128</td>
<td>&gt;128</td>
<td>16</td>
<td>2</td>
<td>16</td>
<td>0.250</td>
<td>1</td>
<td>TEM-1, CMY-4</td>
</tr>
<tr>
<td>13</td>
<td>&gt;128</td>
<td>4</td>
<td>2</td>
<td>&lt;0.015</td>
<td>&lt;0.125</td>
<td>&lt;0.016</td>
<td>0.50</td>
<td>TEM-1</td>
</tr>
<tr>
<td>19</td>
<td>32</td>
<td>4</td>
<td>0.125</td>
<td>0.125</td>
<td>&lt;0.125</td>
<td>0.016</td>
<td>1</td>
<td>TEM-1</td>
</tr>
<tr>
<td>39</td>
<td>32</td>
<td>4</td>
<td>4</td>
<td>&lt;0.015</td>
<td>&lt;0.125</td>
<td>&lt;0.016</td>
<td>0.50</td>
<td>TEM-1</td>
</tr>
<tr>
<td>61</td>
<td>32</td>
<td>&gt;128</td>
<td>8</td>
<td>0.125</td>
<td>&lt;0.125</td>
<td>&lt;0.016</td>
<td>1</td>
<td>TEM-1</td>
</tr>
<tr>
<td>68</td>
<td>&gt;128</td>
<td>&gt;128</td>
<td>64</td>
<td>4</td>
<td>4</td>
<td>0.125</td>
<td>1</td>
<td>TEM-1</td>
</tr>
<tr>
<td>77</td>
<td>32</td>
<td>4</td>
<td>4</td>
<td>0.125</td>
<td>&lt;0.125</td>
<td>&lt;0.016</td>
<td>0.250</td>
<td>TEM-1</td>
</tr>
<tr>
<td>77</td>
<td>32</td>
<td>4</td>
<td>4</td>
<td>0.125</td>
<td>&lt;0.125</td>
<td>&lt;0.016</td>
<td>0.125</td>
<td>TEM-1, OXA-10*</td>
</tr>
</tbody>
</table>

*The gene was identified by sequencing the variable region of a class 1 integron.

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### REFERENCES


