

## Two high-risk clones of carbapenemase-producing *Klebsiella pneumoniae* that cause infections in pets and are present in the environment of a veterinary referral hospital

Michael Brilhante<sup>1,2</sup>, Stefanie Gobeli Brawand<sup>1</sup>, Andrea Endimiani<sup>3</sup>, Helene Rohrbach<sup>4</sup>, Sonja Kittl<sup>1</sup>, Barbara Willi<sup>5</sup>, Simone Schuller<sup>4</sup> and Vincent Perreten<sup>1\*</sup>

<sup>1</sup>Institute of Veterinary Bacteriology, University of Bern, Bern, Switzerland; <sup>2</sup>Graduate School of Cellular and Biomedical Sciences, University of Bern, Bern, Switzerland; <sup>3</sup>Institute for Infectious Diseases, University of Bern, Bern, Switzerland; <sup>4</sup>Department of Clinical Veterinary Medicine, University of Bern, Bern, Switzerland; <sup>5</sup>Clinic for Small Animal Internal Medicine, University of Zurich, Zurich, Switzerland

\*Corresponding author. E-mail: vincent.perreten@vetsuisse.unibe.ch

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**Objectives:** Infections with carbapenem-resistant Enterobacterales (CRE) are an emerging problem in pets and a major threat to public health. We determined the genetic relationships among carbapenemase-producing *Klebsiella pneumoniae* (CPKp) strains causing infections in hospitalized pets in a veterinary clinic and those found in the environment.

**Methods:** WGS was performed with both the Illumina and Nanopore platforms. Searches of genetic features were performed using several databases and bioinformatics tools, and phylogeny was assessed by whole-genome MLST (wgMLST) using SeqSphere and SNP calling with Snippy.

**Results:** WGS analysis of the CPKp strains identified all environmental and almost all animal strains as the high-risk clone ST11, with the exception of two strains that belonged to ST307. All CPKp belonged to novel complex types (CTs) and carried a conjugative 63 kb IncI plasmid encoding the carbapenemase gene *bla*<sub>OXA-48</sub>, yersiniabactin and other virulence factors. Although all CPKp ST11 strains carried additional similar IncR plasmids harbouring multiple antimicrobial resistance genes (ARGs), such as the plasmid-mediated *bla*<sub>DHA-1</sub> AmpC gene, some structural variations were observed. The two ST307 strains carried identical 156 kb MDR IncFIB(K) plasmids with several ARGs, including the *bla*<sub>CTX-M-15</sub> ESBL gene. Both wgMLST and cgSNP analysis confirmed that CPKp strains of the same ST were genetically highly related independent of the source of isolation.

**Conclusions:** This study demonstrated that the clinical CPKp strains were highly related to those contaminating the clinical environment. These findings confirmed nosocomial spread and highlight veterinary hospitals as a source of CPKp, which may further spread to animals, the environment and humans.

### Introduction

Modern small animal veterinary hospitals provide advanced diagnostic and therapeutic interventions for companion animals (CAs) with complex injuries or illnesses. The care of such patients often involves the use of antimicrobials such as  $\beta$ -lactam antibiotics as well as critically important antimicrobials such as fluoroquinolones and extended-spectrum cephalosporins.<sup>1</sup> Carbapenems can also be used 'off label' in veterinary practice in some countries (including Switzerland) to treat infections with MDR bacteria<sup>1</sup> and the use of such broad-acting antimicrobials can foster selection pressure for MDR bacteria such as carbapenem-resistant Enterobacterales (CRE) in small-animal hospital environments.

One of the world's most important nosocomial pathogens among Enterobacterales is *Klebsiella pneumoniae*.<sup>2</sup> Some carbapenemase-producing *K. pneumoniae* (CPKp) strains belonging to clonal groups CG258 (including ST11) and CG307 are among the high-risk clones with the highest antimicrobial resistance reported worldwide.<sup>3</sup> The majority of infections caused by *K. pneumoniae* are opportunistic healthcare-associated infections (HAIs) in which severity and mortality can dramatically increase if carbapenem resistance<sup>4</sup> and/or virulence factors (e.g. yersiniabactin)<sup>5</sup> are present. The yersiniabactin siderophore system is strongly associated with invasive infections in humans<sup>5</sup> and carbapenem resistance is associated with a 2-fold increase in the mortality rate in infections caused by CPKp compared with

carbapenem-susceptible *K. pneumoniae* (CSKp) strains (42% versus 21%, respectively).<sup>4</sup>

Resistance to carbapenems is usually mediated by carbapenemases, which are classified into class A, B or D.<sup>6</sup> Group D represents a diverse group of OXA enzymes,<sup>7</sup> in which the 'phantom' OXA-48-like enzymes display low carbapenemase activity, making their detection challenging.<sup>8</sup> OXA-48-like enzymes have been increasingly reported in Enterobacteriales from animals in Europe,<sup>9,10</sup> the USA<sup>11</sup> and northern Africa<sup>12</sup> and are usually carried by mobile genetic elements (MGEs) such as plasmids.<sup>3</sup> Due to this recent worldwide expansion of CRE to the veterinary setting<sup>13-16</sup> and the increasing dissemination of these pathogens in European human healthcare systems,<sup>17</sup> the necessity for broader surveillance, including genomic characterization of outbreak investigations and tracking of sources of infections in both humans and animals, becomes crucial.<sup>18</sup> WGS-based typing methods like whole-genome MLST (wgMLST) and core-genome SNP (cgSNP) analysis provide the high degree of resolution power necessary for such investigations.<sup>19,20</sup> WgMLST offers more standardization and reproducibility while cgSNP offers higher resolution for ST-specific studies.<sup>20</sup>

In 2018, several cases of CPKp infections were diagnosed in CAs (dogs and cats) hospitalized in a veterinary referral hospital in Switzerland. In this same hospital, CSKp that produced the plasmidic cephalosporinase (pAmpC) DHA-1 caused two outbreaks, in 2011 and 2012.<sup>21</sup> The recent screening of this clinical environment revealed the presence of CPKp in different locations and on devices,<sup>22</sup> prompting us to determine the genetic relatedness among the clinical and environmental CPKp strains as well as with the former DHA-1-producing CSKp.<sup>21</sup> We performed WGS using Illumina and Oxford Nanopore Technologies (ONT) to investigate their MGEs associated with antibiotic resistance and virulence and to explore their phylogenetic relationships using wgMLST and cgSNP.

## Methods

### Bacterial strains, biochemical and molecular tests

Fifteen CPKp strains were isolated by our diagnostic unit between August 2018 and August 2019 from animal infection sites in five dogs and four cats treated at a veterinary referral hospital in Switzerland. Two of the animals (a dog and a cat) were admitted more than once, with a time interval of at least 3 weeks between admissions. Among the total 15 CPKp strains found, 8 were obtained from the two animals that were admitted multiple times. Clinical CPKp strains were isolated from urine (9/15), respiratory tract fluids (3/15), abdominal fluid (1/15), pus (1/15) and bile (1/15). Additionally, 1 CPKp strain obtained from a case of cat gut carriage and 15 CPKp strains isolated from the clinical environment during the same time period (August 2018,  $n=15$ ) were included in the study.<sup>22</sup> The 15 environmental CPKp were obtained from an ECG device, a heating mat, a surgical stand, the emergency floor, transport trolleys, a telephone, an infusion pump (right side), leashes/chains, a medication box, animal boxes, a scale for dogs, ICU floor and gutters, outdoor grass, an outdoor doormat and labcoat buttons. Four nosocomial CSKp ST11 strains reported in 2015 from the same clinic were chosen based on clonality according to PFGE and the year of isolation for WGS comparative analysis.<sup>21</sup>

The isolation, selection and identification of clinical CPKp strains were performed as previously described<sup>1</sup> and the environmental strains were obtained from samples collected using transport swabs (Sarstedt AG & Co. KG, Nümbrecht, Germany) as described.<sup>22</sup> Carbapenemase production was assessed using the Blue-Carba test<sup>23</sup> and positive strains were tested for the presence of *bla*<sub>OXA-48-like</sub> genes by PCR,<sup>8</sup> before performing WGS.

### AST

The MICs of 16 antibiotics were determined by broth microdilution using Sensititre EUVSEC and EUVSEC2 plates (Thermo Fisher Scientific, Waltham, USA). The MIC results were interpreted using EUCAST criteria,<sup>24</sup> except for nalidixic acid, sulfamethoxazole and tetracycline, for which CLSI criteria were used.<sup>25</sup>

### Conjugation assays by filter mating

Conjugation assays were performed at both 37°C and room temperature (RT) as previously described.<sup>26</sup> The same *Escherichia coli* strain, J53dR, was used as the recipient, and both the 18KM1950 and 18KM2445b CPKp strains were used as donors. Transconjugants (TCs) were subjected to double antibiotic selection on Mueller-Hinton agar (MH, OXOID) containing sodium azide (100 mg/L) and imipenem (0.5 mg/L) at 37°C for 24 h under aerobic conditions. TCs were confirmed by both species identification and PCR targeting *bla*<sub>OXA-48-like</sub> genes.<sup>8</sup>

### WGS and in silico analysis

All strains were sequenced using short-read Illumina (Illumina Inc.) NovaSeq 6000 technology (2 × 150 bp paired-end). Fourteen selected strains [18KM1950, 18KM2445b, 18KM2517, 18KM2813, 19KM28, 19KM57, 19KM267, 19KM1053 and 19KM1109 (infections); AR142\_2b (carriage); EAN23 and EAN40 (environmental); and 06KM907 and 11KM256 (CSKp)] were also sequenced, as previously indicated, using MinION (ONT).<sup>1</sup> Genomic DNA for Illumina sequencing was obtained as described previously,<sup>1</sup> while the guanidium thiocyanate-based method was used for ONT sequencing.<sup>27</sup>

The assembly of Illumina reads was performed using SPAdes (v3.12.0); contigs were then annotated with PROKKA v1.12 and used for *in silico* analysis.<sup>28</sup> Hybrid assembly with Unicycler (v0.4.8)<sup>29</sup> was performed for the strains sequenced using both technologies ( $n=14$ ), except for 18KM2445b, for which assembly was conducted using the ONT assembly and Illumina polishing pipeline (<https://github.com/nanoporetech/ont-assembly-polish>) with an extra step of manual curation by read mapping long and short reads. Read mapping was performed with Geneious software v10.1.3 (Biomatters Ltd) to verify the presence of relevant genetic structures obtained from hybrid assemblies in strains sequenced only with Illumina technology. The 14 circular genomes were deposited in GenBank and annotated with the NCBI pipeline. Online tools available at the Center for Genomic Epidemiology (<http://www.genomicepidemiology.org/>) were used to detect known antibiotic resistance genes (ARGs) (ResFinder 3.0) and plasmid replicons (PlasmidFinder 1.3) and for MLST (MLST 2.0). Virulence genes, capsules, integrative conjugative elements and transposable elements were identified using the Pasteur (<https://bigsdbs.pasteur.fr/klebsiella/klebsiella.html>) and VFDB (<http://www.mgc.ac.cn/VF/>) databases, the Kaptive online tool (<http://kaptive.holtlab.net/>),<sup>30</sup> the Kleborate database (<https://github.com/katholt/Kleborate>)<sup>31</sup> and ISFinder (<https://isfinder.biotoul.fr/>),<sup>32</sup> respectively. The additional putative capsule synthesis locus of *K. pneumoniae* ST307 from KP616 (CP026495, genes C2861\_20465 to C2861\_20520) was used as a reference.<sup>33</sup> Easyfig and BLAST Ring Image Generator (BRIG)<sup>34</sup> were used to perform map comparisons of plasmids (based on BLASTn).

### Accession numbers

The full genome assemblies of the 14 *K. pneumoniae* strains are available from NCBI BioProject PRJNA539891.

### Phylogenetic analysis

A well-defined and stable scheme available in SeqSphere+ (Ridom GmbH, Münster, Germany), comprising 2365 targets or genes of the *K. pneumoniae*

core genome (cgMLST) and 2526 genes of the *K. pneumoniae* accessory genome (wgMLST; total of 4891 targets), was used for the analysis (<https://www.cgmlst.org/ncs/>).<sup>19</sup> All circular and complete assemblies of *K. pneumoniae* strains ST11 ( $n = 67$ ) and ST307 ( $n = 10$ ) were downloaded from GenBank on 5 January 2020. The ST11 *K. pneumoniae* strains included from NCBI were as follows: KP38731 (GenBank accession no. CP014294), BJCFK909 (CP034123), FDAARGOS\_444 (CP023941), L388 (CP029220), L39\_2 (CP033954), SCKP020079 (CP029384), WCHKP020037 (CP036371), WCHKP015625 (CP033396), WCHKP015093 (CP036300), WCHKP115068 (CP036365), WCHKP115069 (CP033405), WCHKP2080 (CP036361), WCHKP2 (CP028542), WCHKP3 (CP031721), WCHKP36 (CP028583), WCHKP649 (CP026585), XJ-K1 (CP032163), 675920 (CP033242), C1398 (CP034420), C789 (CP034415), KP58 (CP041373), 160111 (CP029689), 721005 (CP022997), 911021 (CP022882), CR-HvKP1 (CP040533), CR-HvKP4 (CP040539), CR-HvKP5 (CP040545), F1 (CP026130), F127 (CP026140), F138 (CP026149), F5 (CP026132), F44 (CP025461), F77 (CP026136), FDAARGOS\_443 (CP023933), GD4 (CP025951), GSU10-3 (AP018671), KP69 (CP025456), L482 (CP033960), L491 (CP029226), KSH203 (CP034327), SCKP020143 (CP028548), SWU01 (CP018454), TVGHCRE225 (CP023722), WCHKP020030 (CP028793), WCHKP020098 (CP036305), WCHKP040035 (CP028797), WCHKP8F4 (CP027068), KP65 (CP044258), B12(AN) (CP026155), 16\_GR\_13 (CP027036), AR\_0079 (CP028994), Kp\_Goe\_821588 (CP018692), Kp\_Goe\_822917 (CP018438), WCHKP7E2 (CP028806), AR\_0049 (CP018816), ATCC BAA-2146 (CP006659), AR\_0146 (CP021685), AR\_0148 (CP021950), K66-45 (CP020901), CCRI-22199 (CP035540), CAV1392 (CP011578), JM45 (CP006656), SCKP020046 (CP028783), CRKP I (CP037927), HS11286 (CP003200), JS187 (CP025466) and KP18-29 (CP034249). The ST307 *K. pneumoniae* strains included from NCBI were KP1766 (CP025146), KP1768 (CP025140), NR5632 (CP025143), ST307PT03 (CP022919), ST307PT02 (CP022922), ST307PT01 (CP022924), ST307PT04 (CP022916), 616 (CP026495), I72 (CP034281) and WCGKP294 (CP046612). All genomes met the quality threshold of 90% good targets. Genomes were loaded and searched for previously defined target sequences using the built-in BLAST. The visualization of phylogenetic distance was achieved by using the allelic profile of all strains to generate a neighbour-joining tree using SeqSphere+ with the parameters 'pairwise ignoring missing values; % column difference' for the distance calculation. A genetic distance threshold of 0.0045 for wgMLST was used to separate closely related strains from unrelated strains.<sup>19</sup> In addition, the cgMLST complex type (CT) was determined by SeqSphere+ to identify isolates with very similar cgMLST profiles. The CT distance threshold was 15 cgMLST allele differences, which was coupled with a cluster alert quality threshold of at least 90% good cgMLST targets. cgSNPs were called using Snippy v4.4.5 (<https://github.com/tseemann/snippy>) with strains 18KM1950 (GenBank accession no. CP039936) and 18KM24445b (CP039951) used as references for ST11 and ST307, respectively. Recombination was filtered using Gubbins v2.3.4. The obtained alignments were used for the phylogenetic tree calculation with IQ-TREE v1.6.12, in which the K3P model and ascertainment bias correction (ASC) were used for SNPs-only alignments, together with the ultrafast bootstrap option (-bb = 1000; 1000 replicates). The consensus trees were visualized in iTOL (<https://itol.embl.de/>).

## Results

### MDR CPKp strains belonged to two high-risk, virulent clones

All the CPKp detected in the clinical environment ( $n = 15$ ) as well as the majority of CPKp isolated from infection sites in CAs ( $n = 14/15$ ) belonged to ST11, except for two strains that belonged to ST307 (Figure 1).

All ST11 strains presented the same KL105 capsular type and O2V2 serotype, while the ST307 strains displayed the KL102 capsular type and O2V2 serotype. Regardless of the ST, all CPKp

strains from this clinic carried the conserved pathogenicity factors associated with type 1 and 3 fimbriae (*fimABCDEFGHIK* and *mrkABCDFJIH*, respectively) and enterobactin (*entABCDEF*, *fepABCDG*, *fes* and *ybdA*). They also contained the virulence factors yersiniabactin (*ybtAUTE*, *fyuA*, *irp1*, *irp2* and *ybtPQXS*) and the *rscAB* genes, which up-regulate *cps* gene expression. Yersiniabactin was always embedded in an ICEKp4 structure with similarities to the *ybt10* locus (typical of ICEKp4), together with a type IV secretion system and other accessory genes as previously described.<sup>31</sup> Despite the fact that all CPKp strains harboured the same ICEKp4 structure, the allelic profile of the yersiniabactin cluster was different between ST11 and ST307 for the genes *irp1*, *irp2* and *fyuA*.

The CPKp strains commonly harboured acquired ARGs conferring resistance to carbapenems (*bla<sub>OXA-48</sub>*), third-generation cephalosporins (*bla<sub>DHA-1</sub>* in all ST11 except five strains, and *bla<sub>CTX-M-15</sub>* in ST307), aminoglycosides [*aac(6')-Ib-cr*, *aac(3)-IVa* in ST11 strains except for 19KM1109 and *aac(3)-IIa* in ST307 strains], fluoroquinolones [*aac(6')-Ib-cr*], penicillins (*bla<sub>OXA-1</sub>*) and sulphonamides (*sul1* in ST11 and *sul2* in ST307) (Figure 1). All CPKp strains also harboured chromosomal mutations leading to a Ser83Leu substitution in GyrA and a Ser80Ile substitution in ParC, which are both associated with high-level fluoroquinolone resistance.<sup>35</sup> Antimicrobial susceptibility to cefotaxime and ceftazidime varied in some of the CPKp ST11 strains (due to expression of the *bla<sub>DHA-1</sub>* gene in most strains), as did susceptibility to tetracycline, for which no ARG was found (Table S1, available as [Supplementary data](#) at JAC Online). CPKp ST11 strains also contained the additional ARGs *catB3* (chloramphenicol), *mph(A)* (macrolides) and *arr-3* (rifampicin), which were not present in ST307. In contrast to the ST11 strains, the CPKp ST307 strains were additionally resistant to cefepime (*bla<sub>CTX-M-15</sub>*), tetracycline [*tet(A)*] and trimethoprim (*dfra14*) (Table S1). While all CPKp remained susceptible to colistin, the CPKp ST11 strains were additionally susceptible to tetracycline (except 19KM57 and 19KM267) and cefepime. CPKp ST307 was additionally susceptible to chloramphenicol and tigecycline.

Phylogenetic analyses of the CPKp using wgMLST demonstrated a close genetic relationship (genetic distance <0.0045) among all 29 CPKp ST11 strains (Figure 2) and between the two CPKp ST307 strains (Figure 3). This high genetic relatedness was also supported by the low number of cgSNP differences shared amongst the CPKp strains from the animal infection sites and the clinical environment ( $\leq 8$  cgSNPs for ST11 and 5 cgSNPs for ST307). The obtained cgSNP phylogenetic trees (Figures S1 and S2) were highly congruent with the wgMLST trees (Figures 2 and 3). These highly genetically related CPKp strains from the Swiss veterinary context were distant from other human and environmental ST11 and ST307 strains and belonged to the new CTs 5188 and 5189, respectively. Genetic distances from wgMLST are provided in Data sheets S1 and S2 for ST11 and ST307, respectively.

### CPKp carried the conjugative IncL OXA-48 plasmid and similar MDR plasmids

All CPKp strains contained identical 63 kb IncL plasmids (pMBR\_OXA-48) that harboured *bla<sub>OXA-48</sub>* within a Tn1999.2 transposon (Figure S3).<sup>36</sup> Conjugation assays revealed that pMBR\_OXA-48 was transferable to the recipient *E. coli* strain J53dR1 at both RT and 37°C, with similar conjugation frequencies ( $4 \times 10^{-5}$  donors per recipient cell) regardless of the donor used. The comparison of



**Features**

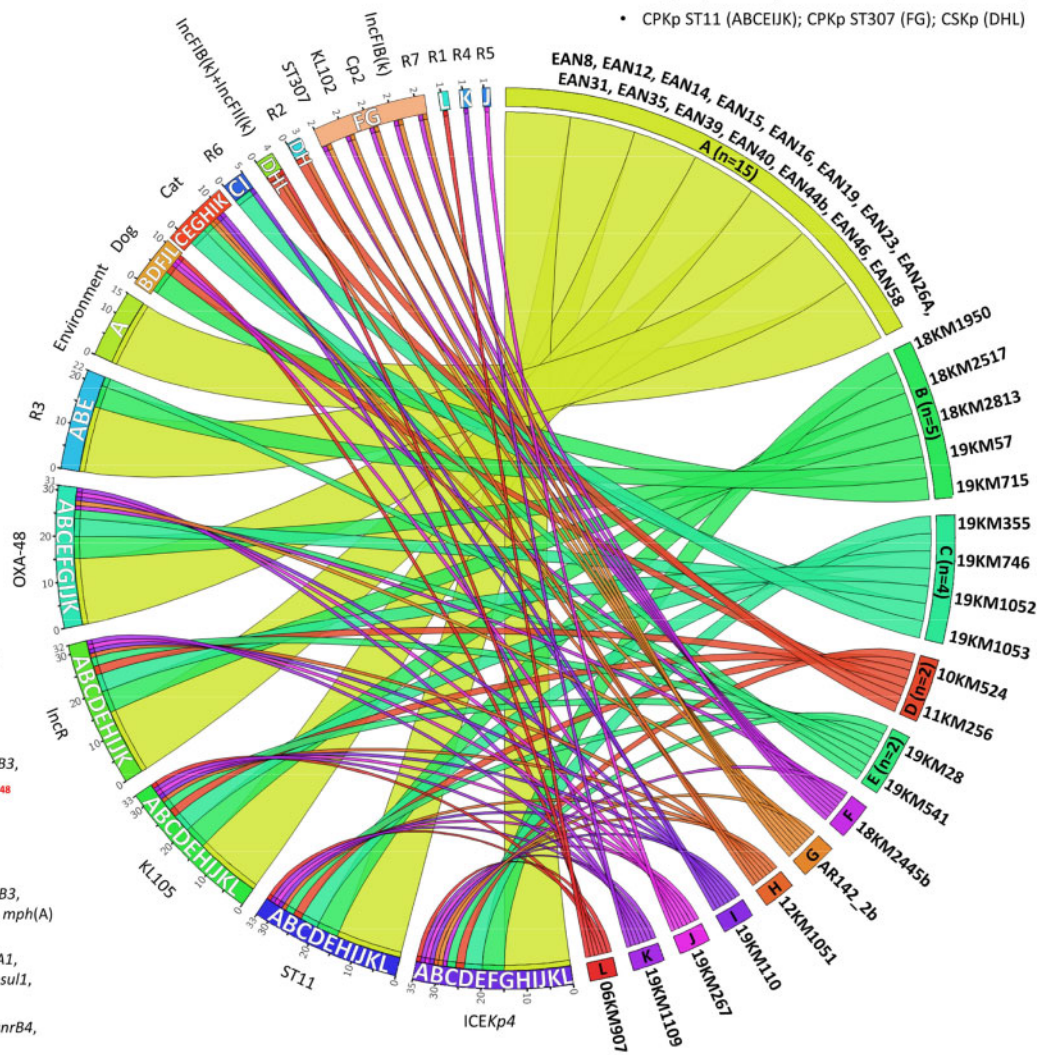
- Source: Dog, cat, environment
- Sequence types: ST11, ST307
- Capsular type: KL105, KL102
- Second capsular locus (Cp2)
- ICEKp4 (yersiniabactin)
- Incompatibility groups: IncR, IncFIB(K)-IncFII(k), IncFIB(k)
- Carbapenemase OXA-48
- Resistance genotype: R1-R7

**Isolates (n=35)**

- 12 groups of *K. pneumoniae* (ABCDEFGHIJKL)
- CPKp ST11 (ABCEIJK); CPKp ST307 (FG); CSKp (DHL)

**Resistance genotype**

- R7:** Common + *aac(3)-IIa*, *strA-B*, *qnrB1*, *bla<sub>SHV-28</sub>*, *bla<sub>TEM-1b</sub>*, *sul2*, *tet(A)*, *dfrA14*, *bla<sub>CTX-M-15</sub>* and *bla<sub>OXA-48</sub>*
- R6:** Common + *aadA2*, *aac(3)-Iva*, *catB3*, *bla<sub>SHV-11</sub>*, *arr-3*, *sul1-2*, *mph(A)* and *bla<sub>OXA-48</sub>*
- R5:** Common + *aadA2*, *aac(3)-Iva*, *catB3*, *bla<sub>SHV-11</sub>*, *bla<sub>DHA-1</sub>*, *arr-3*, *sul1-2*, *bla<sub>OXA-48</sub>* and *mph(A)*
- R4:** Common + *aadA2*, *catB3*, *qnrB4*, *bla<sub>SHV-11</sub>*, *bla<sub>DHA-1</sub>*, *arr-3*, *sul1*, *mph(A)* and *bla<sub>OXA-48</sub>*
- R3:** Common + *aadA2*, *aac(3)-Iva*, *catB3*, *qnrB4*, *bla<sub>DHA-1</sub>*, *bla<sub>SHV-11</sub>*, *arr-3*, *sul1-2*, *mph(A)* and *bla<sub>OXA-48</sub>*
- R2:** Common + *aadA2*, *aph(3')-Ia*, *catA1*, *catB3*, *bla<sub>DHA-1</sub>*, *qnrB4*, *bla<sub>SHV-11</sub>*, *arr-3*, *sul1*, *dfrA12* and *mph(A)*
- R1:** Common + *aadA2*, *catA1*, *catB3*, *qnrB4*, *bla<sub>SHV-11</sub>*, *bla<sub>DHA-1</sub>*, *arr-3* and *sul1*
- Common:** *aac(6')-Ib-cr*, *oqxAB*, *fosA*, *bla<sub>OXA-1</sub>*

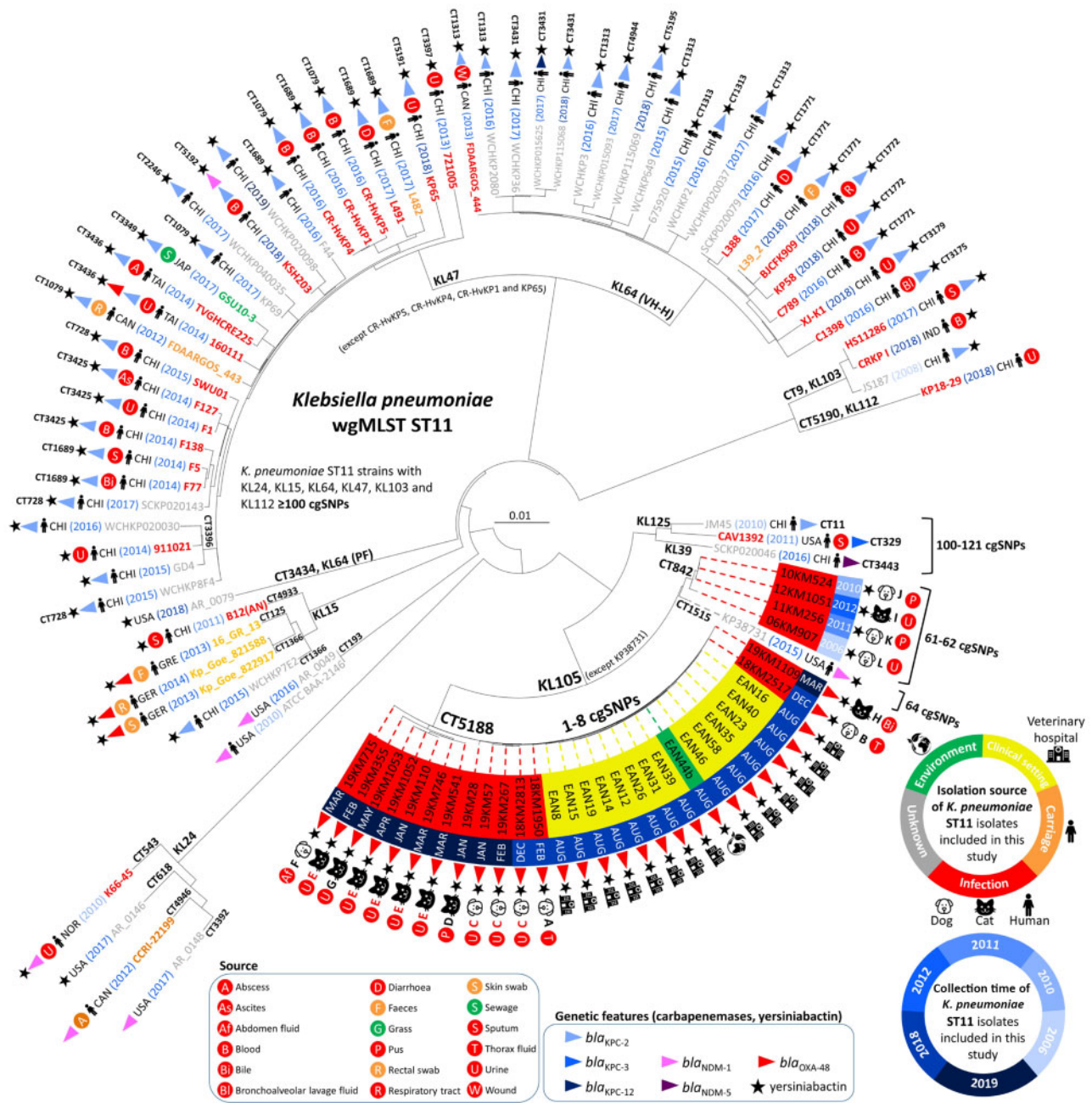


**Figure 1.** Features of CPKp (n = 31) and CSKp (n = 4) included in this study. Circos diagram showing the 35 strains (right) and the connection to their respective features (left). The strains were clustered into 12 groups (A, B, C, D, E, F, G, H, I, J, K and L) based on their similarity (rounded rectangles and black bold font, right side). When a strain group presents a specific feature, the letter representing the group is displayed within the rounded rectangle of the respective feature (white bold font, left side). The resistance genotypes of the strains were divided into seven classes, as displayed in the legend; red and bold formatting highlight the most relevant genes. The outer edge of the rounded rectangle of each feature shows how many strains possess that feature. This figure appears in colour in the online version of JAC and in black and white in the printed version of JAC.

pMBR\_OXA-48 with other IncL plasmids revealed strong structural resemblance among them (e.g. conjugation loci and *bla<sub>OXA-48</sub>*) except for IS1 transposases.<sup>37</sup>

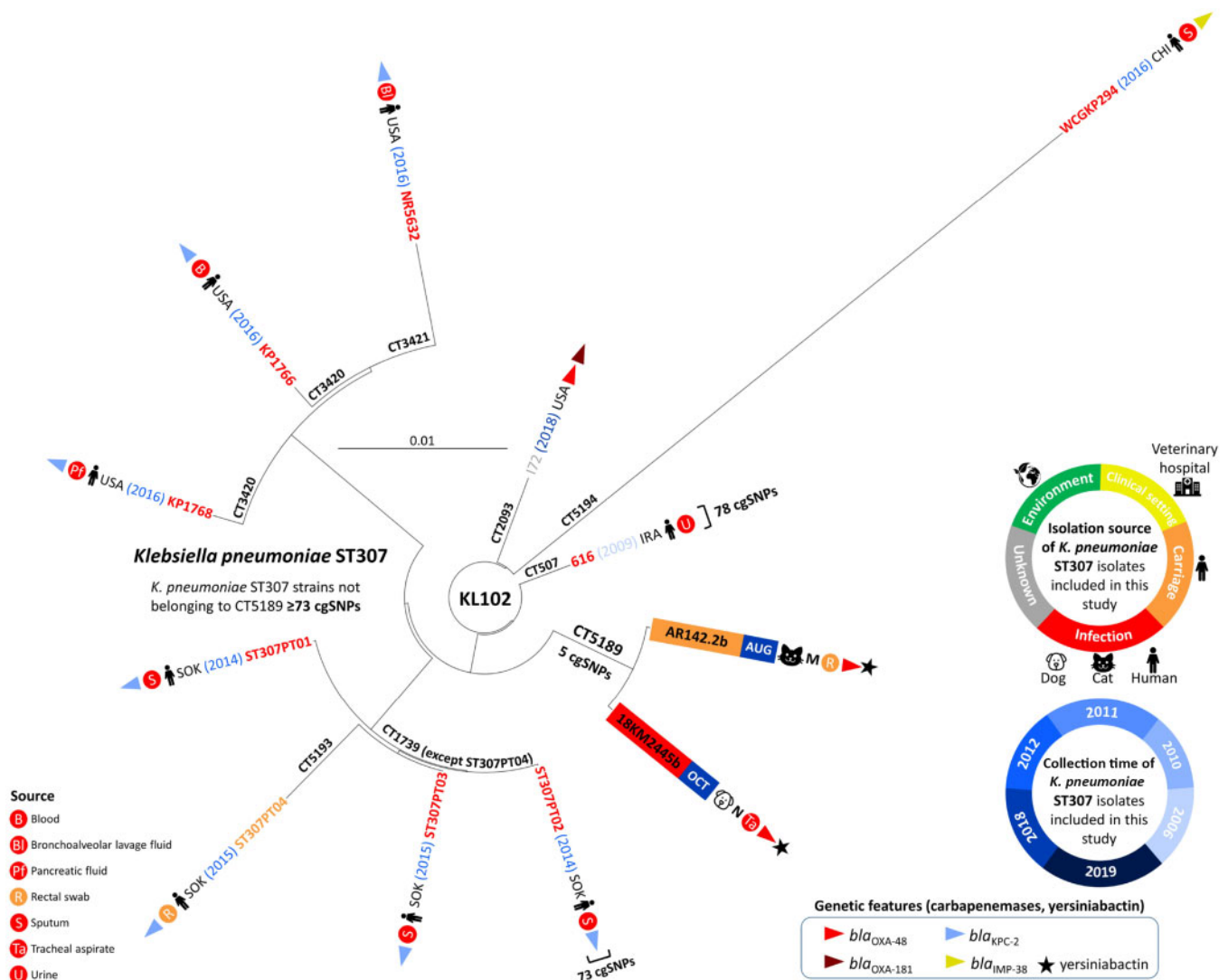
CPKp ST11 harboured similar IncR plasmids, which differed slightly in structure and ARG content (Figure 4). For instance, some IncR plasmids lacked the *bla<sub>DHA-1</sub>* gene, which was correlated with cefotaxime and ceftazidime susceptibility in these five strains. Otherwise, most CPKp ST11 strains (23 out of 29) carried highly similar 55 to 56 kb MDR IncR plasmids (pMBR\_DHA-1\_1C and pMBR\_DHA-1\_2C) (Figure 4). The ARGs present in these IncR plasmids were located within a nearly 25 kb ISCR1 element, except for

the *mph(A)* gene. The ISCR1 element was composed of a class I integron structure exhibiting one 5' conserved region (*intI1*), a variable region VR1 [*aac(6')-Ib-cr*, *bla<sub>OXA-1</sub>*, *catB3* and *arr-3*], two 3' conserved regions (*qacΔ1* and *sul1*), a second variable region VR2 (*sap* operon, *qnrB4*, *psp* operon and *bla<sub>DHA-1</sub>*) and a common region (ISCR1). The structural differences observed in the IncR plasmids were always related to the ISCR1 element, including a duplication in the common region (pMBR\_DHA-1\_2C), loss of the *sap* operon, *qnrB4*, the *psp* operon and hypothetical proteins (pMBR\_DHA-1\_1CV2) and loss of the whole VR2 region (pMBR\_DHA-1\_1CV3) (Figure 4).



**Figure 2.** Phylogenetic neighbour-joining tree based on the wgMLST of CPKp ST11 causing nosocomial infections ( $n=14$ ), environmental CPKp ( $n=15$ ), CSKp ( $n=4$ ) and other *K. pneumoniae* from the NCBI database ( $n=67$ ) with their respective features. The CPKp described in this study are highlighted in arcs and they are coloured depending on their source and year of isolation, as shown by the legend. Carbapenemase genes are displayed as triangles and the colour relates to specific genes (as displayed by the legend). Details regarding the source are shown according to the legend. Regarding the capsular type, a minimum score of ‘good’ (PF, perfect; VH, very high; H, high) from KAPTIVE was applied as a threshold; when the result was lower, the capsular type was not included in the figure. CTs are displayed for every strain included in the analysis. The cgSNPs were included for the CPKp strains included in this study (1–8 cgSNPs), the closest relative human strain (KP38731) and the strains belonging to the closest clade (JM45, CAV1392 and SCKP020046). The other *K. pneumoniae* strains were obtained from NCBI ( $n=67$ ) using the search tool assembly. Only submissions with fully assembled chromosomes were included in the study (accessed on 5 January 2020). The tree was generated and drawn using SeqSphere+ (v601) for the comparison of 4894 genes among all the genome sequences included ( $n=100$ ) based on ‘pairwise ignoring missing values; % column differences’. NOR, Norway; CHI, China; GER, Germany; GRE, Greece; CAN, Canada; TAI, Taiwan; JAP, Japan. This figure appears in colour in the online version of JAC and in black and white in the printed version of JAC.





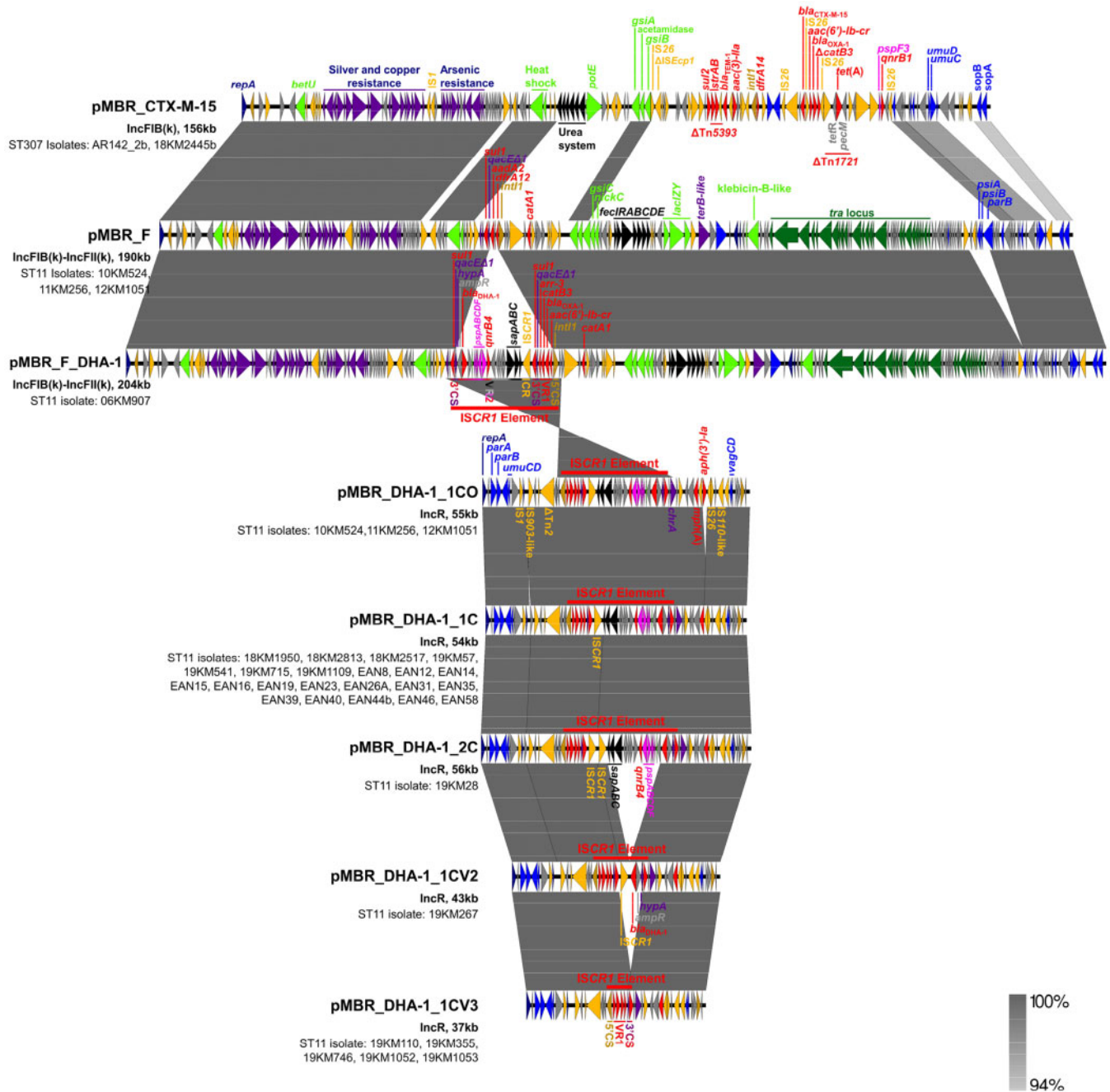
**Figure 3.** Phylogenetic neighbour-joining tree based on the wgMLST of CPKp ST307 causing nosocomial infections ( $n=1$ ), carriage CPKp ( $n=1$ ) and other *K. pneumoniae* from the NCBI database ( $n=10$ ) with their respective features. The CPKp described in this study are highlighted in arcs and they are coloured depending on their source and year of isolation. Carbapenemase genes are displayed as triangles and the colour relates to specific genes (as displayed by the legend). Details regarding the source are shown according to the legend. Regarding the capsular type, all strains included herein exhibited KL102. CTs are also displayed for every strain included. The other *K. pneumoniae* isolates were obtained from the NCBI database ( $n=10$ ) using the search tool assembly. Only submissions with fully assembled chromosomes were included in the study (accessed on 5 January 2020). The tree was generated and drawn using SeqSphere+ v601 for the comparison of 5124 genes among all the genome sequences included ( $n=12$ ) based on 'pairwise ignoring missing values; % column differences'. CHI, China; IRA, Iran; SOK, South Korea. This figure appears in colour in the online version of JAC and in black and white in the printed version of JAC.

The two ST307 strains harboured the identical 156 kb IncFIB(K) MDR plasmid pMBR\_CTX-M-15 (Figure 4). Plasmid pMBR\_CTX-M-15 harboured almost all of the ARGs in a complex 50 kb structure with eight copies of IS26 that flanked all 11 ARGs, generating four major ARG fragments. The first fragment was composed of *sul2*, *strAB*, *bla*<sub>TEM-1</sub>, *aac(3)-IIa* and *dfrA14*; the second of *bla*<sub>CTX-M-15</sub>; the third of *aac(6')-Ib-cr* and *bla*<sub>OXA-1</sub>; and the fourth of *tet(A)* and *qnrB1*.

### Genetic comparison between CPKp and CSKp ST11

The CSKp strains were highly genetically related among themselves, belonged to the same CT842 and clustered away from the

ST11 CPKp CT5188 (Figure 2). CSKp also differed from CPKp in the presence of IncFIB(K)-IncFII(K) plasmids and the different allelic profiles of two alleles within the yersiniabactin locus. Despite such divergence, CPKp ST11 and CSKp ST11 (Figure 1) shared similar genetic features, such as their ARG content [not applicable for *bla*<sub>OXA-48</sub>, *aac(3)-IVa*, *aph(3')-Ia* and *dfrA12*] and virulence content. Compared with CPKp ST11, three CSKp ST11 strains (except 06KM907) also contained similar IncR plasmids harbouring the ISCR1 element but with the additional ARG *aph(3')-Ia* (Figure 4). In strain 06KM907, the ISCR1 element was present in the 204 kb IncFIB(K)-IncFII(K) plasmid.



**Figure 4.** Linear comparison map of the MDR plasmids found in CPKp and CSKp strains from this study. The figure was obtained from Easyfig and the minimal size for BLASTn comparisons was set at 2000 bp to facilitate the visualization of the structural similarities and differences. The classical ISCR1 element is composed of the 5' conserved region (5'CS), variable region 1 (VR1), common region, variable region 2 (VR2) and the 3' conserved region (3'CS). Genes are indicated with arrows and are coloured depending on their gene functional classification as follows: ARGs are red; transposases are orange; integrases are dark orange; genes related to conjugation are dark green; genes related to increased fitness are light green; disinfectant and heavy metal resistance genes are purple; phage-related genes are pink; partition, modification and stability systems are in blue; replication genes are dark blue; putative genes that can increase virulence are black; and other genes are grey. This figure appears in colour in the online version of *JAC* and in black and white in the printed version of *JAC*.

## Discussion

Highly genetically related virulent clones of CPKp ST11 and ST307 were found in the environment of a veterinary clinic in Switzerland

and at infection sites in hospitalized animals over several months. These CPKp strains were resistant to most classes of antibiotics except for colistin, which is the last resort antibiotic for the treatment

of CPKp infections in humans.<sup>38</sup> Additionally, these CPKp strains contained specific factors that can enhance their virulence, such as the infrequently found siderophore yersiniabactin<sup>31</sup> and a second capsular cluster that was present only in CPKp ST307.<sup>33</sup>

The presence of such virulent high-risk clones of CPKp in CAs and the clinical environment poses an imminent threat to public health. Such virulent CPKp strains may be transferred to other animals, owners and healthcare professionals, as already observed for carbapenemase-producing *E. coli*.<sup>39</sup> Consequently, animals can disseminate CPKp to non-clinical environments after hospitalization and re-introduce these bacteria into clinics.

In human medicine, CPKp colonization is known to be frequently associated with the development of HAI;<sup>40</sup> therefore, the screening of patients who are at risk at the time of admission is a recommended infection control procedure.<sup>41</sup> Likewise, thorough CPE screening (i.e. rectal sampling) at the time of admission should be performed for animals at risk and screening should also be performed for all hospitalized animals at discharge. By implementing these screening procedures, together with One Health-driven genomic surveillance and better infection control procedures,<sup>22</sup> it should become possible to prevent and disrupt this dissemination chain.

Our results showed that human and veterinary clinical environments share the same medical burden of high-risk CPKp clones ST11 and ST307. In Germany, CPKp-producing OXA-48 has also been found in CAs,<sup>42,43</sup> including ST11.<sup>43</sup> However, CPKp ST307 has never been described in CAs so far.<sup>42,43</sup> Worldwide, high-risk clones ST11 and ST307 frequently harbour epidemic/transmissible plasmids (e.g. IncL) containing carbapenemase-encoding genes such as *bla*<sub>OXA-48</sub> and *bla*<sub>KPC</sub>.<sup>3</sup> Although, in Switzerland, the epidemiology of human CPKp remains poorly studied, OXA-48 was the most frequent carbapenemase found in Enterobacterales and its detection has been increasing from 2013 to 2017.<sup>44</sup>

To date, the lack of WGS-based data hinders any possible connection between human and animal CPKp strains in Switzerland. Nevertheless, the concordant phylogenetic results generated by wgMLST and cgSNP revealed that the strains of this veterinary setting belonged to specific sublineages that are different from the pandemic ST11 and ST307 strains of human or environmental origin available from NCBI. These results also agree with previous studies that wgMLST can be useful for rapid and reproducible WGS-based outbreak investigations.<sup>19,20</sup> Our CPKp ST11 belonged to the unique sublineage associated with capsular type KL105, which was not found in the other *K. pneumoniae* ST11 strains available in NCBI. However, capsular type KL105 has been recently detected in *K. pneumoniae* ST11 persisting in Portuguese human medical institutions over years,<sup>45</sup> suggesting that this capsular type may enhance the ability of ST11 strains to survive and persist in clinical settings; this may be supported by other factors that improve its survival in hostile environments. For instance, yersiniabactin has been linked to higher persistence in iron-depleted environments,<sup>46</sup> which together with other pathogenicity factors, such as type 1 and 3 fimbriae and capsules, can explain the survival of CPKp in the environment of this healthcare setting.<sup>47</sup> Furthermore, CPKp shared genes that can increase tolerance to quaternary ammonium compounds (frequently found in disinfectants),<sup>48</sup> and the *mucAB* genes in pMBR-OXA-48, which can increase survival under UV exposure.<sup>49</sup>

Despite not being found in the clinical environment, our CPKp ST307 strains shared genes previously found in other ST307 strains.<sup>33</sup> These genes contribute to resistance to osmotic stress (*betU*),<sup>50</sup> heat shock stress (*clpK*)<sup>51</sup> and exposure to heavy metals,<sup>52,53</sup> which hint at the capacity of these strains to persist in different aggressive environmental conditions. The CPKp ST307 strains belonged to the dominant capsular type KL102 (Figure 3)<sup>3</sup> and this high-risk clone is becoming the most prevalent MDR human clone in several countries.<sup>33,54,55</sup>

Hence, more genome-based studies are needed to understand the dissemination and colonization dynamics of CPKp in humans and CAs in Switzerland, since cross-contamination with owners<sup>56</sup> and veterinary healthcare professionals has already been demonstrated.<sup>39</sup>

Carbapenem resistance in the veterinary setting is driven by the clonal dissemination of high-risk clones containing conjugative *bla*<sub>OXA-48</sub>-harbouring plasmids.<sup>57</sup> However, the comparison of the WGS sequences of CPKp with those from CSKp clones previously associated with infections in this clinic demonstrated that these CSKp strains did not acquire the carbapenemase-encoding IncL plasmid. Although the origin of CPKp cannot be determined, our results clearly showed the clonal dissemination of new CTs (CT5188 and CT5189) of high-risk clones of CPKp in the veterinary setting. Additionally, the identification of two high-risk clones sharing the same pMBR\_OXA-48 plasmid can be a sign of a much broader spread of these carbapenemase-containing plasmids in this setting.

Our study provides WGS-based confirmation of the nosocomial spread of CPKp in a veterinary setting, which is sustained by the high genetic relatedness shared among the clones and by the carriage of very similar MGEs. We demonstrated that veterinary clinics play an important role as a hotspot of CPE,<sup>1</sup> adding a new piece to the large puzzle of CPKp epidemiology.<sup>18</sup> The convergence of virulence genes with life-threatening ARGs in high-risk clones emphasizes the need for strict infection prevention and control procedures and genomic surveillance to avoid the further spread of such pathogens to other animals, the environment and humans.<sup>22</sup>

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## Transparency declarations

None to declare.



## Supplementary data

Figures S1 to S3, Table S1 and Data sheets S1 and S2 are available as Supplementary data at JAC Online.

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