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Third-Generation-Cephalosporin-Resistant *Klebsiella pneumoniae* Isolates from Humans and Companion Animals in Switzerland: Spread of a DHA-Producing Sequence Type 11 Clone in a Veterinary Setting

Nadia Wohlwend,[a] Andrea Endimiani,[b] Thierry Francey,[c] Vincent Perreten[a]

Institute of Veterinary Bacteriology, Vetsuisse Faculty, University of Bern, Bern, Switzerland[a]; Institute for Infectious Diseases, Faculty of Medicine, University of Bern, Bern, Switzerland[b]; Small Animal Clinic, Vetsuisse Faculty, University of Bern, Bern, Switzerland[c]

Characterization of third-generation-cephalosporin-resistant *Klebsiella pneumoniae* isolates originating mainly from one human hospital ($n = 22$) and one companion animal hospital ($n = 25$) in Bern (Switzerland) revealed the absence of epidemiological links between human and animal isolates. Human infections were not associated with the spread of any specific clone, while the majority of animal infections were due to *K. pneumoniae* sequence type 11 isolates producing plasmidic DHA AmpC. This clonal dissemination within the veterinary hospital emphasizes the need for effective infection control practices.

The rapid spread of multidrug-resistant (MDR) *Klebsiella pneumoniae* has led to major concerns in hospitals (1). During the past few years, cases of infections caused by *K. pneumoniae* strains resistant to clinically important classes of antibiotics, including third-generation cephalosporins (3GCs), have also been reported in companion animals (2–6). 3GCs represent important antibiotics for the treatment of serious infections caused by *K. pneumoniae* before the use of last-resort carbapenems. Transmission of 3GC-resistant *K. pneumoniae* (3GC-R-*Kp*) between companion animals and humans represents a possible threat to both human and animal health (7). This prompted us to determine which resistance determinants and clonal lineages are associated with 3GC-R-*Kp* obtained primarily from one human hospital as well as from one companion animal clinic in Bern, Switzerland.

Isolates were selected based on decreased susceptibility to cefotaxime or ceftazidime (MICs of both, ≥1 μg/ml) (8). Species identification was confirmed by using matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry (Bruker Daltonik) (9). The human isolates consisted of all 3GC-R-*Kp* isolates from patients admitted to different wards of the same hospital (hospital 1 [H1]) between 2013 and 2014 ($n = 21$), except one (5208.51) which came from H2. *K. pneumoniae* isolates were predominantly recovered from urine and less frequently from blood and biopsy specimens (Table 1). Multilocus sequence typing (MLST) (http://www.pasteur.fr/recherche/genopole/PF8/mlst/) and XbaI pulsed-field gel electrophoresis (PFGE) profiles (contour-clamped homogeneous electric field [CHEF] DR-III apparatus [Bio-Rad]; run time, 18.5 h; gradient, 6 V/cm; initial switch time, 2.2 s; final switch time, 54.2 s; angle, 120°) (10) revealed that the human isolates differed genetically from each other; each patient was infected with a unique strain that exhibited a distinct PFGE profile, and all of the isolates belonged to different sequence types (ST) except two isolates that were of ST101 and three that were of ST873 (Fig. 1). The absence of clonal spread of *K. pneumoniae*, even within the same ward, is suggestive of infection control best practices at the hospital. Such diversity indicates that resistance may emerge independently of the wards in different *K. pneumoniae* lineages, e.g., through the acquisition of resistance to 3GCs.

In contrast, only two different clonal lineages (ST11, $n = 21$, and ST1463, $n = 4$) were found among the animal isolates, which gathered into two PFGE clusters (Fig. 1). ST1463 represents a novel lineage, so far detected only in veterinary settings in Switzerland. All of the 3GC-R-*Kp* isolates from companion animals (22 dogs and 3 cats) admitted to the same clinic (A1) between 2006 and 2012 were included in the study, except for one of ST11 and one of ST1463, which came from two other clinics (A2 and A3) (Fig. 1). Only one dog was hospitalized because of pneumonia. The others were admitted for non-*K. pneumoniae*-associated diseases, including 7 outpatients (6 dogs and 1 cat) with one ($n = 2$) or multiple ($n = 5$) visits during the study period, 9 dogs hospitalized for leptospirosis treatment with dialysis, and 3 dogs and 2 cats hospitalized for injuries that required surgical interventions (Table 2). Infections developed after they had been treated with intensive care, including after placement and manipulation of indwelling venous and urinary catheters. Such invasive interventions are already known to be critical for the development of *K. pneumoniae* septicemia and urinary tract infections (UTIs) in humans (11–13). One dog hospitalized in another clinic was found to be a carrier of the same ST11 clone (KM60/13). This dog had a history of hospitalization in the first clinic (A1) 7 years before,

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Address correspondence to Vincent Perreten, vincent.perreten@vetsuisse.unibe.ch.

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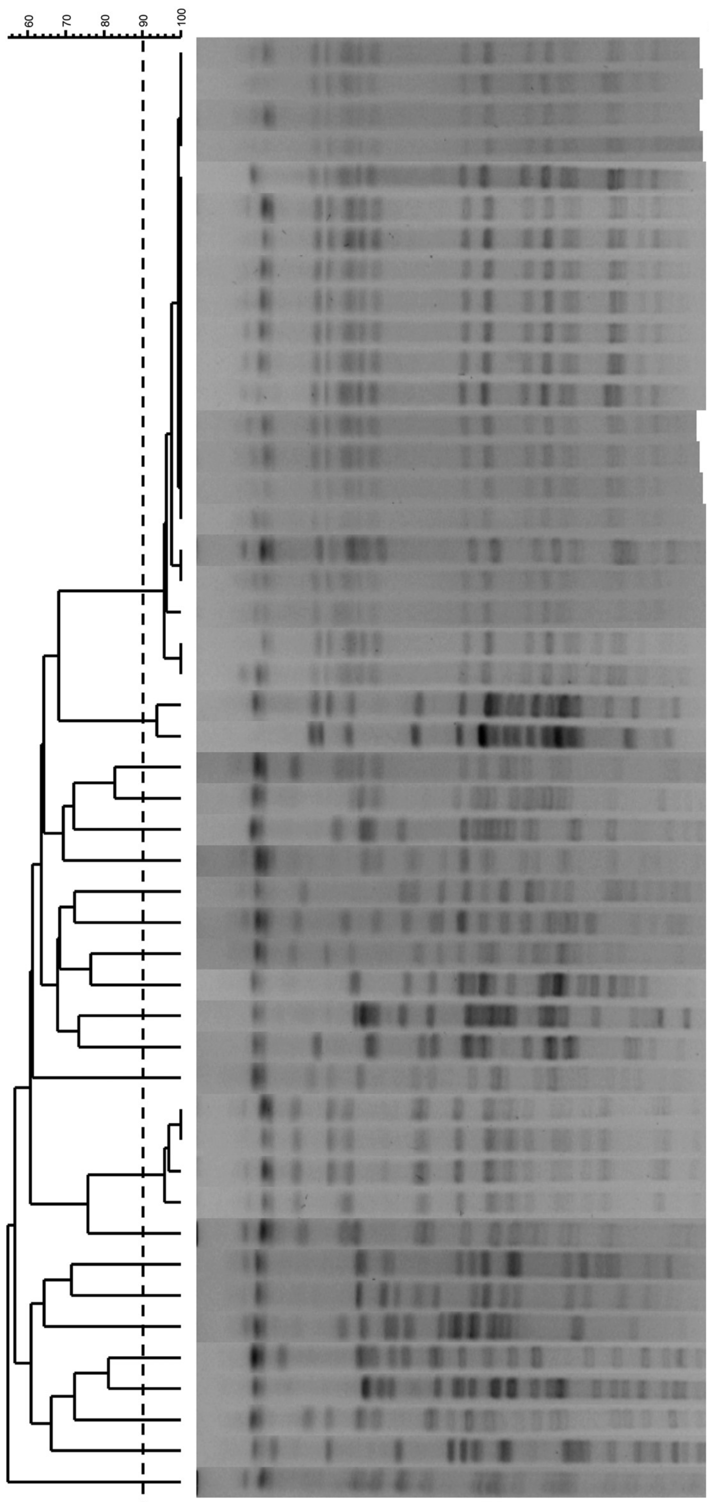
TABLE 1 Antimicrobial resistance profile and genetic characteristics of *K. pneumoniae* isolates from human infection sites[a]

Strain	Source	Hospitalization required? (ward)	Resistance phenotype	ST	Carbapenemase gene	ESBL/pAmpC gene(s)	Other *bla* gene(s)[b]	Other antibiotic resistance gene(s) and integrons[c]	Amino acid substitution(s) in: GyrA	ParC
5203.77	Blood culture	Yes (emergency room)	3GCs NAL TMP TET KAN SMX	290		*bla*$_{\text{CTX-M-15}}$	*bla*$_{\text{TEM-1}}$, *bla*$_{\text{OXA-1}}$	*aac(6′)-Ib-cr*, *sul1*, *dfrA12*, *mrx-mphA*, *tet*(A), *intI1*		
5204.79	Urine	No (SOC; ambulatory)	3GCs GEN CIP NAL TMP	147		*bla*$_{\text{CTX-M-15}}$		*aac(3)-IIc*, *dfrA14*	Ser83Ile	Ser80Ile
5107.16	Urine	Yes (urology/gynecology)	3GCs GEN TMP STR TET SMX	17		*bla*$_{\text{CTX-M-14}}$	*bla*$_{\text{TEM-1}}$, *bla*$_{\text{LAP-2}}$	*aac(3)-IIc*, *qnrS1*, *dfrA1*, *strB*, *sul2*, *tet*(A)		
5009.73	Urine	No (SOC; palliative medicine)	3GCs CIP (I) TMP STR TET SMX KAN	873		*bla*$_{\text{CTX-M-15}}$	*bla*$_{\text{TEM-1}}$, *bla*$_{\text{OXA-1}}$	*qnrB*, *aac(6′)-Ib-cr*, *dfrA14*, *strA*, *strB*, *sul2*, *tet*(A), *intI1*		
5206.76	Urine	Yes (urology)	AmpC TET SMX	262		ND	*bla*$_{\text{SHV-110}}$			
5012.10	Urine	Yes (medicine, abdominal surgery)	3GCs STR SMX	697		*bla*$_{\text{CTX-M-3}}$	*bla*$_{\text{TEM-1}}$	*strB*, *sul2*		
5208.51	Catheter	Unknown (external laboratory)	AmpC FEP MERO GEN AMP CIP NAL KAN	16	*bla*$_{\text{NDM-1}}$			*aac(6′)-Ib*	Ser83Phe, Asp87Asn	Glu84Lys
4906.28	Tracheobronchial exudate	Yes (ICU)	3GCs GEN CIP NAL TMP STR TET SMX	101	*bla*$_{\text{OXA-48}}$	*bla*$_{\text{CTX-M-15}}$	*bla*$_{\text{TEM-1}}$, *bla*$_{\text{OXA-1}}$	*aac(3)-IIc*, *aadA1*-like, *qnrB*, *aac(6′)-Ib-cr*, *dfrA14*, *strA*, *strB*, *sul2*, *tet*(A), *intI1*	Ser83Tyr, Asp87Ala	Ser80Ile
5012.64	Urine	Yes (emergency room)	3GCs FEP GEN CIP NAL KAN	101		*bla*$_{\text{CTX-M-15}}$	*bla*$_{\text{TEM-1}}$, *bla*$_{\text{OXA-9}}$	*aadA1*-like	Ser83Tyr, Asp87Gly	Ser80Ile
5011.44	Biopsy specimen	Yes (orthopedics)	AmpC CHL TET SMX	14		ND	*bla*$_{\text{SHV-28}}$	*tet*(D), *intI1*, Δ*ompK36*		
5108.48	Blood culture	Yes (oncology)	3GCs FEP GEN TMP TET SMX	742		*bla*$_{\text{CTX-M-1}}$, *bla*$_{\text{CTX-M-14}}$	*bla*$_{\text{LAP-2}}$	*aac(3)-IIc*, *qnrS1*, *dfrA1*, *sul1*, *tet*(A)		
5003.76	Biopsy specimen	Yes (orthopedics)	3GCs TMP STR TET SMX KAN	391		*bla*$_{\text{CTX-M-15}}$		*aph(3′)-Ia*, *dfrA7*, *strA*, *strB*, *sul2*, *tet*(D)		
5109.57	Urine	Yes (gynecology)	3GCs CIP TMP TET SMX	48		*bla*$_{\text{CTX-M-15}}$, *bla*$_{\text{CTX-M-14}}$	*bla*$_{\text{TEM-1}}$, *bla*$_{\text{OXA-1}}$	*qnrB*, *aac(6′)-Ib-cr*, *dfrA14*, *sul2*, *tet*(A)		
5004.63	Urine	Yes (cardiology)	3GCs GEN CHL TMP STR TET SMX KAN	39		*bla*$_{\text{CTX-M-15}}$	*bla*$_{\text{TEM-1}}$, *bla*$_{\text{OXA-1}}$	*aac(3)-IIc*, *aph(3′)-Ia*, *aadA1*-like, *aadA4*-like, *aac(6′)-Ib-cr*, *catA1*, *dfrA1*, *dfrA17*, *strA*, *strB*, *sul1*, *sul2*, *mrx-mphA*, *tet*(A), *intI1*		
5206.62	Blood culture	Yes (oncology)	3GCs GEN CIP (I) TMP STR TET SMX	985		*bla*$_{\text{CTX-M-15}}$	*bla*$_{\text{TEM-1}}$, *bla*$_{\text{OXA-1}}$	*aac(3)-IIc*, *qnrB*, *aac(6′)-Ib-cr*, *dfrA14*, *strB*, *sul2*, *tet*(A), *intI1*		
5004.42	Urine	Yes (ICU)	3GCs CIP NAL	15		*bla*$_{\text{CTX-M-15}}$			Ser83Phe, Asp87Ala	Ser80Ile
5011.36	Blood culture	Yes (oncology)	AmpC TMP SMX CHL	133		*bla*$_{\text{DHA-1}}$		*qnrB*, *catA1*, *sul1*, *intI1*		
5112.24	Urine	Yes (urology)	3GCs GEN TMP STR TET SMX	983		*bla*$_{\text{CTX-M-15}}$	*bla*$_{\text{TEM-1}}$, *bla*$_{\text{OXA-1}}$	*aac(3)-IIc*, *qnrB*, *dfrA14*, *strB*, *sul2*, *tet*(A)		
5010.55	Urine	Yes (medicine)	3GCs TMP STR TET SMX KAN	37		*bla*$_{\text{CTX-M-1}}$		*aph(3′)-Ia*, *dfrA7*, *strA*, *strB*, *sul2*, *tet*(D)		
5112.15	Urine	Yes (urology)	3GCs GEN CHL STR SMX	873		*bla*$_{\text{CTX-M-14}}$	*bla*$_{\text{SHV-27}}$, *bla*$_{\text{LAP-2}}$	*aac(3)-IIc*, *qnrS1*, *strA*, *strB*, *sul2*		
5205.14	Urine	Yes (medicine)	3GCs CIP CHL NAL TMP STR SMX	873		*bla*$_{\text{CTX-M-14}}$	*bla*$_{\text{SHV-27}}$, *bla*$_{\text{LAP-2}}$	*qnrS1*, *dfrA12*, *dfrA13*, *strA*, *strB*, *sul2*		
6531	Urine	Yes (urology/gynecology)	AmpC SMX	454-like		*bla*$_{\text{DHA-1}}$	*bla*$_{\text{OXA-1}}$	*qnrB*, *strA*, *strB*, *sul1*		

[a] ST, sequence type; 454-like, ST was assigned based on the combination of six alleles, since the *tonB* allele could not be amplified by PCR; SOC, specialized outpatient clinic; ICU, intensive care unit; 3GCs, 3rd-generation cephalosporins; AmpC, 3rd-generation cephalosporins and β-lactamase inhibitors; FEP, cefepime (4th-generation cephalosporin); CIP, ciprofloxacin; CIP (I), ciprofloxacin intermediate; CHL, chloramphenicol; NAL, nalidixic acid; TMP, trimethoprim; SMX, sulfamethoxazole; KAN, kanamycin; GEN, gentamicin; STR, streptomycin; TET, tetracycline; ND, no gene detected.

[b] Non-ESBL SHVs were not reported.

[c] Genes and functions: *aadA*, streptomycin adenylyltransferase; *aph(3′)-Ia*, kanamycin *O*-phosphotransferase; *aac(3)-IIc*, gentamicin acetyltransferase; *qnr*, quinolone resistance protein; *aac6′-Ib-cr*, variant of aminoglycoside *N*(6′)-acetyltransferase–ciprofloxacin-modifying enzyme; *aac(6′)-Ib*, *N*-acetyltransferase; *cat*, chloramphenicol acetyltransferase; *dfr*, dihydrofolate reductase for trimethoprim resistance; *sul*, dihydropteroate synthetase for sulfonamide resistance; *mrx-mphA*, macrolide inactivation resistance protein-phosphotransferase; *tet*, tetracycline efflux; *str*, streptomycin phosphotransferase; *intI1*, integrase.



Strain	ST	Origin	Clinic	Specimen
KM1170/11	11	dog	A1	blood culture
KM1140/12	11	dog	A1	blood culture
KM1312/11	11	dog	A1	blood culture
KM1126/12	11	dog	A1	blood culture
IMD842/11	11	dog	A1	urine
KM256/11	11	dog	A1	pus
KM987/12	11	dog	A1	urine
KM2/11	11	dog	A1	blood culture
KM141/10	11	dog	A1	bronchoalveolar lavage
KM524/10	11	dog	A1	pus
KM686/10	11	cat	A1	urine
KM1051/12	11	cat	A1	urine
KM1152/11	11	cat	A1	urine
KM1209/11	11	dog	A1	urine
KM1096/12	11	dog	A1	blood culture
210237006	11	dog	A1	urine
KM917/11	11	dog	A1	blood culture
KM1350/12	11	dog	A1	pus
KM60/13	11	dog	A2	genital swab
KM907/06	11	dog	A1	urine
KM900/11	11	dog	A1	blood culture
5112.15	873	human	H1	urine
5205.14	873	human	H1	urine
5206.76	262	human	H1	urine
5010.55	37	human	H1	urine
5109.57	48	human	H1	urine
5012.10	697	human	H1	urine
5107.16	17	human	H1	urine
5208.51	16	human	H2	catheter
5009.73	873	human	H1	urine
6531	454like	human	H1	urine
5004.42	15	human	H1	urine
5112.24	983	human	H1	urine
5011.36	133	human	H1	blood culture
KM899/09	1463	dog	A1	urine[1]
KM822/09	1463	dog	A1	urine[1]
KM749/09	1463	dog	A3	urine
KM65/09	1463	dog	A1	urine
5204.79	147	human	H1	urine
4906.28	101	human	H1	tracheobronchial exudate
5012.64	101	human	H1	urine
5011.44	14	human	H1	biopsy
5003.76	391	human	H1	biopsy
5206.62	985	human	H1	blood culture
5108.48	742	human	H1	blood culture
5004.63	39	human	H1	urine
5203.77	290	human	H1	blood culture

[1]same animal

FIG 1 Dendrogram of XbaI PFGE patterns of *Klebsiella pneumoniae* isolates from human and animal origins. Cluster analysis was made with BioNumerics software version 7.1 (Applied Maths, Belgium) by the unweighted-pair group method using average linkages (UPGMA) and with Dice comparison settings (optimization, 1.5%; position tolerance, 1.5%). The cutoff for determining clonality was ≥90% (21).

where it may have acquired the *K. pneumoniae* strain and was still colonized; alternatively, this clone was also present in the second clinic (A2). Long-term persistence (for years) of *Enterobacteriaceae* is a well-known phenomenon which may contribute to the spread of nosocomial isolates into the community, as well as increase the risk of infection in cases requiring hospitalization and clinical intervention (14). In this regard, studies on risk factors for MDR bacterial infections are needed in order to apply specific

TABLE 2 Antimicrobial resistance profiles and genetic characteristics of *K. pneumoniae* isolates from animal infection sites[a]

Strain	Source	Hospitalization required? (reason)	Resistance phenotype	ST	ESBL/pAmpC gene(s)[c]	Other antibiotic resistance genes and integrons[d]	Amino acid substitution in: GyrA	ParC
KM907/06	Dog urine	Yes (esophagus perforation)	AmpC CIP CHL NAL SMX	11	bla_{DHA-1}	*aadA2*-like, *qnrB*, *aac(6′)-Ib-cr*, *catA1*, *catB3*-like, *sul1*, *arr1*, *intI1*	Ser83Ile	Ser80Ile
KM60/13	Dog genital swab	No (OPP)	AmpC CIP CHL NAL SMX KAN	11	bla_{DHA-1}	*aph(3′)-Ia*, *qnrB*, *aac(6′)-Ib-cr*, *catB3*-like, *sul1*, *mrx-mphA*, *arr1*, *intI1*	Ser83Ile	Ser80Ile
KM1350/12	Dog pus	Yes (cruciate ligament rupture)	Same as for KM60/13	11	bla_{DHA-1}	Same as for KM60/13	Ser83Ile	Ser80Ile
IMD842/11	Dog urine	Yes (stomach ulcer)	AmpC CIP CHL NAL TMP SMX KAN	11	bla_{DHA-1}	*aph(3′)-Ia*, *aadA2*-like, *qnrB*, *aac(6′)-Ib-cr*, *catA1*, *catB3*-like, *dfrA12*, *sul1*, *mrx-mphA*, *arr1*, *intI1*	Ser83Ile	Ser80Ile
KM256/11	Dog pus	No (OPP; wound care after accident)	Same as for IMD842/11	11	bla_{DHA-1}	Same as for IMD842/11	Ser83Ile	Ser80Ile
KM2/11	Dog blood culture	Yes (intoxication)	Same as for IMD842/11	11	bla_{DHA-1}	Same as for IMD842/11	Ser83Ile	Ser80Ile
KM141/10	Dog BAL fluid	Yes (pneumonia)	Same as for IMD842/11	11	bla_{DHA-1}	Same as for IMD842/11	Ser83Ile	Ser80Ile
KM1051/12	Cat urine	Yes (urolithiasis)	Same as for IMD842/11	11	bla_{DHA-1}	Same as for IMD842/11	Ser83Ile	Ser80Ile
KM900/11	Dog blood culture	Yes (leptospirosis)	Same as for IMD842/11	11	bla_{DHA-1}	Same as for IMD842/11	Ser83Ile	Ser80Ile
KM917/11	Dog blood culture	Yes (leptospirosis)	Same as for IMD842/11	11	bla_{DHA-1}	Same as for IMD842/11	Ser83Ile	Ser80Ile
KM1152/11	Cat urine	Yes (feline lower urinary tract disease)	Same as for IMD842/11	11	bla_{DHA-1}	Same as for IMD842/11	Ser83Ile	Ser80Ile
KM1170/11	Dog blood culture	Yes (leptospirosis)	Same as for IMD842/11	11	bla_{DHA-1}	Same as for IMD842/11	Ser83Ile	Ser80Ile
KM1312/11	Dog blood culture	Yes (leptospirosis)	Same as for IMD842/11	11	bla_{DHA-1}	Same as for IMD842/11	Ser83Ile	Ser80Ile
KM1140/12	Dog blood culture	Yes (leptospirosis)	Same as for IMD842/11	11	bla_{DHA-1}	Same as for IMD842/11	Ser83Ile	Ser80Ile
KM1209/11	Dog urine	OPP (immunosuppression)	Same as for IMD842/11	11	bla_{DHA-1}	Same as for IMD842/11	Ser83Ile	Ser80Ile
KM524/10	Dog pus	Yes (hit by car)	Same as for IMD842/11	11	bla_{DHA-1}	Same as for IMD842/11	Ser83Ile	Ser80Ile
KM1096/12	Dog blood culture	Yes (leptospirosis)	Same as for IMD842/11	11	bla_{DHA-1}	Same as for IMD842/11	Ser83Ile	Ser80Ile
KM987/12	Dog urine	No (OPP; protein-losing enteropathy)	Same as for IMD842/11	11	bla_{DHA-1}	Same as for IMD842/11	Ser83Ile	Ser80Ile
KM1126/12	Dog blood culture	Yes (leptospirosis)	Same as for IMD842/11	11	bla_{DHA-1}	Same as for IMD842/11	Ser83Ile	Ser80Ile
KM686/10	Cat urine	No (OPP; fibrosarcoma)	Same as for IMD842/11	11	bla_{DHA-1}	Same as for IMD842/11	Ser83Ile	Ser80Ile
210237006	Dog urine	Yes (hit by car)	Same as for IMD842/11	11	bla_{DHA-1}	Same as for IMD842/11	Ser83Ile	Ser80Ile
KM899/09[b]	Dog urine	Yes (leptospirosis)	3GCs CIP CHL NAL TMP SMX KAN STR	1463	$bla_{CTX-M-1}$	*aph(3′)-Ia*, *aadA2*-like, *dfrA12*, *strB*, *sul1*, *sul2*, *mrx-mphA*	Ser83Ile	Ser80Ile
KM749/09	Dog urine	No (OPP; UTI)	AmpC, CIP CHL NAL TMP SMX KAN STR	1463	bla_{CMY-2}, $bla_{CTX-M-1}$	*aph(3′)-Ia*, *aadA2*-like, *dfrA12*, *strB*, *su11 sul2*	Ser83Ile	Ser80Ile
KM65/09	Dog urine	No (OPP; UTI)	AmpC CIP CHL NAL TMP SMX KAN STR TET	1463	bla_{CMY-2}, $bla_{CTX-M-1}$	*aph(3′)-Ia*, *aadA2*-like, *dfrA12*, *strB*, *sul1*, *sul2*, *tet*(A)	Ser83Ile	Ser80Ile
KM822/09[b]	Dog urine	Yes (leptospirosis)	Same as for KM899/09	1463	bla_{CMY-2}, $bla_{CTX-M-1}$	Same as for KM899/09	Ser83Ile	Ser80Ile

[a] OPP, outpatient pet; UTI, urinary tract infection; ST, sequence type; 3GCs, 3rd-generation cephalosporins; AmpC, 3rd-generation cephalosporins and beta-lactamase inhibitors; CIP, ciprofloxacin; CHL, chloramphenicol; NAL, nalidixic acid; TMP, trimethoprim; SMX, sulfamethoxazole; KAN, kanamycin; STR, streptomycin; TET, tetracycline.

[b] KM899/09 and KM822/09 were isolated from the same animal.

[c] All strains also had the bla_{OXA-1} gene.

[d] Genes and functions: *aadA*, streptomycin adenylyltransferase; *aph(3′)-Ia*, kanamycin *O*-phosphotransferase; *qnrB*, quinolone resistance protein; *aac6′-Ib-cr*, variant of aminoglycoside *N*(6′)-acetyltransferase– ciprofloxacin-modifying enzyme; *cat*, chloramphenicol acetyltransferase; *dfrA12*, dihydrofolate reductase for trimethoprim resistance; *sul*, dihydropteroate synthetase for sulfonamide resistance; *mrx-mphA*, macrolide inactivation resistance protein-phosphotransferase; *arr1*, rifampin ADP ribosyltransferase; *tet*, tetracycline efflux; *str*, streptomycin phosphotransferase; *intI1*, integrase.

TABLE 3 Characteristics of 3rd-generation-cephalosporin-resistant *E. coli* recipients after conjugation with *K. pneumoniae* strains or electrotransformation with *K. pneumoniae* plasmid DNA[a]

K. pneumoniae donor	Origin	*E. coli* transformant	Transformation	Resistance phenotype	Carbapenemase gene	ESBL/pAmpC gene	Other *bla* gene(s)	Other antibiotic resistance genes and integrons[b]	Replicon	Inc group
5012.10	Human	NW5A	C	3GCs STR SMX		$bla_{CTX-M-3}$	bla_{TEM-1}	*sul2*, *strB*	FII	IncFII
5109.57	Human	NW8C	C	3GCs		$bla_{CTX-M-14}$			FII	IncFII
KM749/09	Dog	NW10A	C	AmpC STR		bla_{CMY-2}			I1	IncI1-alpha
		NW10F	C^I, E	3GCs CHL TMP STR SMX KAN		$bla_{CTX-M-1}$		*aph(3′)-Ia*, *aadA2*-like, *dfrA12*, *sul1*	R	not assigned
KM256/11	Dog	NW11C	NC, E	AmpC STR SMX KAN		bla_{DHA-1}	bla_{OXA-1}	*aph(3′)-Ia*, *qnrB*, *aac6′-Ib-cr*, *catB3*-like, *mrx-mphA*, *arr-1*, *intI1*	R	Not assigned
KM899/09	Dog	NW12A	NC, E	3GCs TMP STR SMX KAN		$bla_{CTX-M-1}$		*aph(3′)-Ia*, *aadA2*-like, *dfrA12*, *sul2*	R	Not assigned
4906.28	Human	NW15C	C	3GCs GEN TMP STR TET SMX		$bla_{CTX-M-15}$	bla_{OXA-1}	*aac(3)-IIc*, *aadA1*-like, *strB*, *qnrB*, *aac(6′)-Ib-cr*, *dfrA14*, *sul2*, *tet*(A), *intI1*	FIIk	IncF
		NW4906	C	3GCs	bla_{OXA-48}				L/M	IncL/M
5010.55	Human	NW18A	C	3GCs SMX		$bla_{CTX-M-1}$		*sul2*	X1	IncX1
5203.77	Human	NW5203	C	3GCs TET TMP KAN SMX		$bla_{CTX-M-15}$	bla_{OXA-1}, bla_{TEM-1}	*aac(6′)-Ib-cr*, *dfrA12*, *sul1*, *mrx-mphA*, *tet*(A), *intI1*	FIIk	IncF

[a] C, conjugative; C^I, conjugative only together with the bla_{CMY-2} plasmid (IncI1); NC, nonconjugative; E, plasmid transformation by electroporation; Inc, incompatibility group; ST, sequence type; 3GCs, 3rd-generation cephalosporins; AmpC, 3rd-generation cephalosporins and beta-lactamase inhibitors; CHL, chloramphenicol; TMP, trimethoprim; SMX, sulfamethoxazole; KAN, kanamycin; GEN, gentamicin; STR, streptomycin; TET, tetracycline.
[b] Genes and functions: *aadA*, streptomycin adenylyltransferase; *aac(3)-IIc*, gentamicin acetyltransferase; *aph(3′)-Ia*, kanamycin *O*-phosphotransferase; *qnrB*, quinolone resistance protein; *aac6′-Ib-cr*, variant of aminoglycoside *N*(6′)-acetyltransferase–ciprofloxacin-modifying enzyme; *cat*, chloramphenicol acetyltransferase; *dfr*, dihydrofolate reductase for trimethoprim resistance; *sul*, dihydropteroate synthetase for sulfonamide resistance; *mrx-mphA*, macrolide inactivation resistance protein-phosphotransferase; *arr-1*, rifampin ADP ribosyltransferase; *tet*(A), tetracycline efflux; *strB*, streptomycin phosphotransferase; *intI1*, integrase.

population measures to prevent the dissemination of epidemic strains into animal clinics.

Antimicrobial susceptibility was determined using the microdilution ESB1F and EUMVS2 plates (TREK Diagnostic Systems) according to Clinical and Laboratory Standards Institute (CLSI) guidelines and interpretative criteria (8). Antibiotic resistance genes were detected using AMR08 ArrayStrip microarrays (15) together with the HybridizationPlus kit (Alere Technologies GmbH). Carbapenemases, extended-spectrum β-lactamases (ESBLs), plasmid-mediated AmpC (pAmpCs), and amino acid substitutions in the fluoroquinolone resistance-determining region (QRDR) of ParC and GyrA were further identified by sequence analysis of translated sequences obtained from PCR products (see Table S1 in the supplemental material). Eighteen of 21 isolates of ST11 had identical resistance profiles and contained the same resistance genes, including the pAmpC $bla_{\text{DHA-1}}$ gene, emphasizing the spread of a specific clone in a veterinary setting. The three other ST11 isolates had the same genes but lacked the kanamycin resistance gene *aph(3′)-Ia* and/or the trimethoprim resistance gene *dfrA12* (Table 2). Of note, isolate KM907/06, the least resistant strain, was isolated in 2006, whereas the other strains were isolated between 2010 and 2013. ST1463 isolates displayed resistance profiles similar to those of ST11 isolates, with additional resistance to streptomycin. However, resistance to 3GCs was associated with $bla_{\text{CMY-2}}$ and/or $bla_{\text{CTX-M-1}}$ (Table 2).

The resistance profiles of the 3GC-R-*Kp* isolates from humans were highly diverse. Resistance to 3GCs was associated predominantly with the presence of $bla_{\text{CTX-M}}$ genes. Only two isolates carried $bla_{\text{DHA-1}}$. Two isolates were resistant to carbapenems and harbored $bla_{\text{NDM-1}}$ and $bla_{\text{OXA-48}}$ (Table 1). Unlike with the 3GC-R-*Kp* isolates from animals, all of which were resistant to quinolones, only 9 of 22 human isolates exhibited decreased susceptibility to this class of antibiotics (Table 1). These isolates also did not contain the rifampin resistance gene *arr-1*. However, tetracycline resistance associated with *tet*(A) and *tet*(D), as well as gentamicin resistance [*aac(3)-IIc*], was frequent in human isolates. Resistance to sulfonamides, trimethoprim, chloramphenicol, and streptomycin was also common in human isolates, associated either with the same resistance mechanisms as those detected in animal isolates or with others (Table 1).

Selected isolates were used to determine, by PCR (PBRT kit; Diatheva), the Inc group of plasmids carrying $bla_{\text{DHA-1}}$, $bla_{\text{CTX-M-1}}$, $bla_{\text{CTX-M-3}}$, $bla_{\text{CTX-M-14}}$, $bla_{\text{CTX-M-15}}$, and $bla_{\text{OXA-48}}$ after electroporation into *Escherichia coli* TOP10 (Invitrogen) and filter mating conjugation using either *E. coli* JF33 (Rif^r) or *E. coli* J53 (sodium azide resistant; kindly provided by L. Poirel) as the recipient (16). Transformed cells were selected on Mueller-Hinton II agar containing either 70 μg/ml ampicillin, 2 μg/ml cefotaxime, or 0.5 μg/ml meropenem. The $bla_{\text{DHA-1}}$ and $bla_{\text{CTX-M-1}}$ genes from animal isolates were associated with nonconjugative R plasmids. R plasmids are commonly found in *K. pneumoniae* isolates (17) and play an important role as collectors of resistance genes in both human and companion animal settings (2, 3, 17, 18). The $bla_{\text{CMY-2}}$ gene detected in ST1463 isolates was linked to I1 plasmids belonging to ST2 as determined by plasmid MLST (pMLST) (19), a combination already observed in *E. coli* from dogs in Denmark (20). CMY-2/IncI1 plasmids of ST2 were also found in *E. coli* from dogs hospitalized in clinic A1 (data not shown), suggesting a possible plasmid exchange between *K. pneumoniae* and *E. coli* within the same clinic. Otherwise, in the human isolates, the different *bla* genes were associated with diverse plasmids, further underlining the high genetic diversity of the isolates (Table 3). The $bla_{\text{NDM-1}}$ gene could not be transferred by either electrotransformation or conjugation. Additional resistance genes, but not the $bla_{\text{CMY-2}}$- and $bla_{\text{CTX-M-14}}$-containing plasmids, were simultaneously transferred with the different *bla* genes (Table 3).

Although exchange of MDR *K. pneumoniae* strains between humans and pets have been suggested by several studies (2, 6), we did not detect any clones or plasmids shared by isolates from humans and pets. The only common *bla* genes detected in both hosts consisted of $bla_{\text{CTX-M-1}}$ and $bla_{\text{DHA-1}}$. However, $bla_{\text{CTX-M-1}}$ was found on different plasmids in isolates from animals and humans. Only $bla_{\text{DHA-1}}$ seemed to be associated with the same plasmid type, R, in both human and animal isolates, but these plasmids were apparently not conjugative. Nevertheless, a *K. pneumoniae* isolate of ST11 producing DHA-1 appeared to have a particular affinity for veterinary settings, as it has also been found in hospitalized animals in Spain (3). Of note, none of the animals were infected with the zoonotic clones ST15-CTX-M-15 and ST101-CTX-M-15, recently reported in companion animals in France, Germany, and Italy (2, 4, 6). However, these clonal lineages were present among the human isolates of our study. The emergence of predominant *K. pneumoniae* clones in different animal clinics in different geographical regions indicates that several specific lineages are able to persist and disseminate in animal clinical environments.

In conclusion, this study revealed the absence of epidemiological links between the 3GC-R-*Kp* isolates from settings of human and veterinary medicine in Switzerland. However, the results clearly demonstrated nosocomial spread of MDR *K. pneumoniae* in veterinary settings, and they emphasize the importance of hospital infection control best practices being used as has been suggested for many years in human contexts.

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