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Multilocus sequence typing of *Ornithobacterium rhinotracheale* isolated from pigeons and birds of prey revealed new insights into its population structure

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ABSTRACT

The sudden emergence of *Ornithobacterium rhinotracheale* (ORT) in commercially raised poultry species and its presence in non-galliform birds raise important epidemiological issues about the role of interspecies transmission. In the present study, 21 ORT strains isolated from pigeons and from birds of prey were analyzed using the recently established multilocus sequence typing (MLST) scheme. Results were compared to MLST sequence data available from ORT strains isolated mainly from turkeys and chickens, but also single strains from pheasant, guineafowl and rook.

The pigeon-derived ORT strains (n=11) were closely related amongst themselves representing their own cluster distant from ORT strains of non-columbiform avian hosts. ORT strains isolated from birds of prey (n=10) revealed a higher genetic heterogeneity that corresponded well to their host family relationships but grouped within the two mainly poultry-based clusters. None of these strains had a sequence type identical to strains investigated previously. However, three strains isolated from common kestrels and a single strain from a turkey vulture shared one or two out of seven gene loci, respectively, with strains of turkey and chicken origin.

The MLST results of ORT isolated from pigeons and birds of prey likely reflect evolutionary bacterial host adaptations but might also indicate a potential for interspecies transmission. Definite conclusions should be drawn carefully as so far a few strains from non-galliform birds were analyzed by MLST. By extending the number of ORT isolates and the range of potential avian hosts, the MLST database can provide a valuable resource in understanding transmission dynamics.

1. Introduction

Ornithobacterium rhinotracheale (ORT) is an important poultry pathogen, named after its first isolation from the respiratory tract of different bird species (Hafez, Kruse, Emele, & Sting, 1993; Vandamme et al., 1994). The infection is mostly associated with respiratory signs, an increase in mortality, reduced growth rates, a drop in egg production as well as high condemnation rates in slaughtering leading to considerable economic losses for poultry farmers. Beside turkeys and chickens and minor poultry species, ORT has been detected in non-galliform birds. In rooks, the bacterium was isolated in 1983 (Hafez & Vandamme, 2011) – 11 years before its formal description as a novel bacterial genus within the family Flavobacteriaceae (Vandamme et al., 1994). In Taiwan, Iran and Germany, ORT has been isolated from domesticated and feral pigeons (Chou, Lin, Chen, & Tsai, 2009; Mirzaie & Hassanzadeh, 2013; Mirzaie, Hassanzdeh, Bozorgmehrifard, & Banani, 2011; Tsai & Huang, 2006; Warkentin, Kohls, & Hafez, unpublished). In Taiwan, three additional isolates have been collected from two ostriches and a wild Asian crested goshawk (Chou et al., 2009). Moreover, a single case of interspecies transmission of ORT from galliform birds to nestling falcons was determined in a breeding farm via the cockerels they were fed (Hafez & Lierz, 2010).

Shortly after its first detection, ORT was isolated from domesticated poultry in several countries from various geographical origins, e.g. Germany, Belgium, South Africa (Vandamme et al., 1994), Canada (Joubert et al., 1999), Brazil (Canal et al., 2005), Taiwan (Tsai & Huang, 2006) and Japan (Sakai et al., 2000). The global presence of ORT and its sudden emergence as an important poultry pathogen raises various epidemiological questions. To date, the origin of ORT

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and its clinical importance in non-galliform species is unknown. Wild birds have been suggested as reservoir hosts and carriers of ORT but their role in transmission of the bacterium is not fully understood (Amonsin et al., 1997; Chou et al., 2009; Welchman et al., 2013).

Much effort has been put into the isolation and characterization of ORT in order to understand its population structure and to address epidemiological questions (Amonsin et al., 1997; Chou et al., 2009; Numee, Hauck, & Hafez, 2012; Empel, & Hafez, 1999). Some authors included strains from pigeons or from other non-galliform birds in their analyses and detected higher genetic variation in ORT strains from wild birds than in those from domesticated poultry (Amonsin et al., 1997; Chou et al., 2009). A study from Taiwan showed that based on 16S rRNA gene analysis, strains from pigeons were clearly separated from the remaining ORT strains (isolated from chickens, turkeys, guineafowl and rooks) and were combined in a distinct cluster (Tsai & Huang, 2006).

Recently, we established a novel multilocus sequence typing (MLST) scheme for ORT in which, in a stepwise fashion, we gain more insights into the genetic diversity and evolution of the bacterial species (Thieme, Mühldorfer, Gad, Lüschow, & Hafez, 2016). Our MLST results indicated an overall clonal population structure among strains isolated from poultry from various geographic origins. This present study aims to continue and extend this research by comparing existing MLST data with new results of ORT strains isolated from pigeons and from different birds of prey.

2. Material and methods

2.1. Bacterial strains

In a recent publication, 14 different MLST sequence types (ST) have been described among 87 ORT strains that originated mainly from turkeys and chickens (Thieme et al., 2016). In the present study, existing MLST data from one representative of each ST (n=14) were included for comparison (Table 1). Six of those strains were isolated from chickens, five from turkeys as well as one from a pheasant, a guineafowl and a rook, respectively each. In this study, additional 11 ORT strains isolated from pigeons and ten strains isolated from different birds of prey were analyzed by MLST.

2.2. Gene amplification and sequencing

DNA extraction was performed by heat cell lysis for Gram-negative bacteria. PCR was conducted with published primer pairs for MLST according to Thieme et al. (2016) with a few modifications. Since the designed primer sets did not produce amplicons for ORT strains of pigeon origin new primers were designed for the genes *gdhA* (2), *pgi* (1) and *pmi* (1) (Table 2). New reverse primers pgiX-r and pmiX-r were combined with established forward primers (pgi-f and pmi-f, respectively). The annealing temperatures were optimized (Table 2).

2.3. MLST analysis

Sequences were visually checked using the Chromas Lite software (version 2.01; Technelysium Pty Ltd, South Brisbane, Australia) and were uploaded to BioNumerics (version 7.1.; Applied Maths, Sint-Martens-Latem, Belgium). Each new allele received a new number in order of its appearance beginning with the first cipher after already assigned numbers. Seven allele numbers formed the allelic profile and were assigned to a specific ST.

Polymorphic sites were determined with START2 (Jolley, Feil, Chan, & Maiden, 2001). First, sequences of strains isolated from pigeons and birds of prey were examined separately and the number of polymorphic sites was determined. Second, polymorphic sites were determined by involving existing MLST sequences of ORT serotype reference strain A (RefA) to include one representative of the main cluster of ST1 that represents the majority of strains from turkeys and chickens (Thieme et al., 2016). Finally, polymorphic sites of existing MLST sequences from galliform birds were compared to those from non-galliform birds. All ORT strains analyzed in the previous study (Thieme et al., 2016) were included for this calculation. The results of different analyses are provided in separate columns of Table 3.

The phylogenetic tree was built with concatenated sequences of the seven alleles with the maximum likelihood method in the MEGA 6 software (Tamura, Stecher, Peterson, Filipski, & Kumar, 2013). The discriminatory power (D) was determined with the formula of Simpson's index of discriminatory ability (Hunter & Gaston, 1988).

2.4. Nucleotide sequence accession numbers and MLST database

The DNA sequences of the distinct alleles at the seven loci of ORT strains from pigeons and birds of prey have been deposited in GenBank (www.ncbi.nlm.nih.gov/genbank) under accession nos. KX505747-KX505759 (*adk*), KX505760-KX505774 (*aroE*), KX505775-KX505790 (*fumC*), KX505791-KX505802 (*gdhA*), KX505803-KX505815 (*mdh*), KX505816-KX505828 (*pgi*) and KX505829-KX505841 (*pmi*).

Information about primers, PCR conditions, allele sequences, sequence types and isolates have been made publicly available in the ORT MLST database (http://pubmlst.org/orhinotracheale/) hosted at the University of Oxford (Jolley & Maiden, 2010). Users are encouraged to upload their MLST data together with background information of ORT strains for comparison and epidemiological studies.

3. Results and discussion

As part of the present study, a novel MLST scheme was established enabling the characterization of ORT strains under standardized conditions and providing a public database that can easily be extended to allow for worldwide comparison of sequence data (Thieme et al., 2016). First results indicated that ORT strains isolated from turkeys and chickens and from different geographic origins are phylogenetically closely related and were not assigned to distinct STs. This study provides further insights into the phylogenetic relationships of ORT based on more diverse avian host origins by comparing existing MLST results with new sequence data of strains isolated from non-galliform birds.

3.1. MLST analysis

Partial sequences of seven housekeeping genes were obtained from 11 ORT strains isolated from pigeons and ten ORT strains isolated from birds of prey and were combined with existing MLST data of 14 strains mainly of poultry origin (Thieme et al., 2016). ORT strains from pigeons generated seven to nine alleles at each locus with ten different STs, whereas strains isolated from birds of prey revealed five to seven alleles at each locus and seven different STs (Table 4). In total, 17 new STs were detected in the 21 strains giving a high discriminatory power of 0.9762. Together with the formerly described MLST data (Thieme et al., 2016), a total of 31 STs have been identified in 108 ORT strains from galliform and non-galliform birds causing an overall discriminatory power of the MLST scheme of 0.8046. Interestingly, 23 of all known ORT STs (74.2%) were only represented by one ORT strain, which included the majority of STs identified in strains collected from non-galliform birds (Table 1).

The number of polymorphic sites among ORT strains differed strongly between host species and between housekeeping genes. Within ORT strains isolated from pigeons, nine (*fumC*) to 31 (*aroE*) different polymorphic sites were observed. In strains from birds of prey, the number of polymorphisms was considerably higher in all gene loci reaching a maximum of 124 (*aroE*). The total number of

Table 1
Details of 35 *Ornithobacterium rhinotracheale* strains isolated from galliform and non-galliform birds.

Strain ID	Host	Geographic origin	Year of isolation	Serotype	ST	*adk*	*aroE*	*fumC*	*gdhA*	*mdh*	*pgi*	*pmi*	Reference
RefA	Chicken	South Africa	1991	A	1	1	1	1	1	1	1	1	Thieme et al., 2016
RefC	Chicken	USA	1991	C	2	2	2	2	2	2	2	2	Thieme et al., 2016
GK1112/96	Pheasant	Germany	1996	D	3	3	3	1	3	3	3	3	Thieme et al., 2016
RefF	Turkey	The Netherlands	1994	F	4	4	4	3	4	4	4	4	Thieme et al., 2016
RefG	Chicken	France	1995	G	5	1	5	1	1	1	1	5	Thieme et al., 2016
RefJ	Chicken	The Netherlands	1997	J	6	5	6	4	5	5	5	6	Thieme et al., 2016
RefN	Guineafowl	Belgium	1992	N	7	3	7	5	6	2	6	2	Thieme et al., 2016
RefO	Rook	Germany	1983	O	8	6	8	6	7	6	7	7	Thieme et al., 2016
GB 2221/11/2	Turkey	Germany	2011	A	9	1	5	1	1	1	1	1	Thieme et al., 2016
GB 137/10/2	Chicken	Germany	2010	n.t.	10	7	9	7	8	7	8	8	Thieme et al., 2016
GB 738/10/3	Turkey	Germany	2010	C	11	8	10	8	9	8	9	9	Thieme et al., 2016
GB 1573/11/17	Turkey	Germany	2011	n.t.	12	8	10	7	9	8	9	9	Thieme et al., 2016
GB 2399/13	Chicken	Germany	2013	A	13	9	11	9	10	1	10	10	Thieme et al., 2016
GB 978/14/1	Turkey	Germany	2008	F (H)[a]	14	10	12	7	11	9	11	11	Thieme et al., 2016
GV1	Turkey vulture	Germany	2011	H	15	11	1	10	12	10	1	12	this study
GV6	Harris's hawk	Germany	2011	I	16	12	13	11	13	11	12	13	this study
GV9	Common kestrel	Germany	2010	n.t.	17	13	14	12	5	12	13	14	this study
GV10	Peregrine falcon	Germany	2010	A	18	14	15	13	14	13	14	14	this study
GV11	Saker falcon	Germany	2010	n.t.	18	14	15	13	14	13	14	14	this study
GV12	Saker-gyrfalcon	Germany	2010	H	18	14	15	13	14	13	14	14	this study
GV13	Red kite	Germany	2010	F	16	12	13	11	13	11	12	13	this study
GV143	Common kestrel	Germany	2010	J	19	13	16	14	5	13	15	15	this study
GV149	Common kestrel	Germany	2010	H/J	20	13	17	15	5	13	16	14	this study
165-2/2015	Common buzzard	Germany	2015	n.t.	31	23	27	25	23	22	24	24	this study
T37	Pigeon	Germany	2011	n.t.	21	15	18	16	15	14	17	16	this study
T49	Pigeon	Germany	2011	n.t.	22	16	19	17	16	15	18	17	this study
T52	Pigeon	Germany	2011	K	23	17	20	18	17	16	19	18	this study
T66	Pigeon	Germany	2011	n.t.	24	18	21	19	18	17	20	19	this study
T85	Pigeon	Germany	2011	A (I)[a]	25	19	22	20	19	18	21	20	this study
T91	Pigeon	Germany	2011	L	26	20	23	21	20	19	22	21	this study
T92	Pigeon	Germany	2011	L	26	20	23	21	20	19	22	21	this study
T97	Pigeon	Germany	2011	A (I)[a]	27	21	24	22	21	20	22	20	this study
T102	Pigeon	Germany	2011	J	28	19	23	21	20	19	22	21	this study
T143	Pigeon	Germany	2011	n.t.	29	19	25	23	22	21	23	22	this study
T203	Pigeon	Germany	2012	n.t.	30	22	26	24	20	19	22	23	this study

n.t.: ORT strain that could not be typed with available antisera A to L.
[a] Slight serotype cross-reactions of ORT strains are given in parentheses.

polymorphic sites for all gene loci was more than three times less for ORT strains isolated from pigeons (130) compared to strains derived from birds of prey (446). By inclusion of MLST sequences from a representative of ST1 (RefA) which is the predominant ST for strains of poultry origin (Thieme et al., 2016), the number of polymorphic sites among strains derived from pigeons increased by at least 1.8 at all gene loci. The highest increase by a factor of 7.38 was noted for the *gdhA* gene. In contrast, within strains isolated from birds of prey only a very slight increase was observed for four out of seven genes (namely *aroE, fumC, gdhA* and *mdh*) reaching a total number of 452 polymorphic sites for all gene loci. This clearly illustrates the small genetic distance between ORT strains isolated from poultry and birds of prey compared to the remarkably high genetic variation towards of pigeon-derived strains. Overall, the 86 ORT strains isolated from galliform birds (i.e., 67 turkeys, 17 chickens, one pheasant and one guineafowl, previously analyzed by Thieme et al., 2016) reached a total number of 438 polymorphic sites for all gene loci. In comparison, the total number of polymorphic sites among the 22 ORT strains from non-galliform birds (i.e., 10 pigeons, 11 birds of prey and a rook) was 1.65 fold higher (721) and may reflect the more diverse avian host origin (Table 3).

The link between ORT strains of non-galliform bird origin and their hosts was even more evident in the phylogenetic analysis. The overall structure of the maximum likelihood tree was similar to that published by Thieme et al. (2016) but with an additional main cluster C (Fig. 1). Cluster A included six ORT strains, three strains of poultry origin (Thieme et al., 2016) and three strains from different species of the family Accipitridae (common buzzard, Harris's hawk and red kite). ORT strains isolated from the Accipitridae grouped closer together and formed a distinct lineage in cluster A. The strains from a Harris's hawk and a red kite shared ST16 (Fig. 1). Interestingly, the latter ORT strain (red kite) and two strains isolated from turkeys belonged to serotype F, a rare ORT serotype that was identified only in strains of cluster A. Cluster B contained the majority of strains (mainly from galliform birds) published by Thieme et al. (2016) as well as seven ORT strains isolated from birds of prey. Six of them (all from falcons) formed a separate subcluster Bb, only GV1 (from a turkey vulture) was integrated in subcluster Ba.

Table 2
Additional primers for multilocus sequence typing of *Ornithobacterium rhinotracheale* strains from pigeons.

Gene target	Protein product	Primer	Sequence	Product	Annealing temperature
gdhA	glutamate dehydrogenase/ leucine dehydrogenase	gdhAX-f	TCNGCAAAYATCCATGTAG	480 bp	54 °C
		gdhAX-r	ACCGTTACACAAAATRTCTG	480 bp	54 °C
pgi	glucose-6-phosphate isomerase	pgiX-r	TCRGATTTTCCAAARGCAAG	492 bp	52 °C
pmi	phosphomannose isomerase	pmiX-r	ATTCACTTTCGATGACAG	489 bp	50 °C

Table 3
Polymorphic sites per gene locus.

Gene locus	Pigeons	Pigeons+RefA	Birds of prey	Birds of prey+RefA	Galliform birds	Non-galliform birds	Total
	(n=11)	(n=12)	(n=10)	(n=11)	(n=86)	(n=22)	(n=108)
adk	20	83	23	23	51	89	106
aroE	31	62	124	124	73	153	165
fumC	9	39	100	101	56	109	116
gdhA	13	96	96	99	105	140	151
mdh	19	35	49	50	72	62	95
pgi	13	50	27	27	59	67	95
pmi	25	88	27	28	22	101	104
Total	**130**	**453**	**446**	**452**	**438**	**721**	**832**

Table 4
Maximum number of alleles per gene locus.

Gene locus	Protein product	Pigeons	Birds of prey
adk	*adenylate kinase-like kinase*	8	5
aroE	*shikimate 5-dehydrogenase*	9	7
fumC	*fumarase, class II*	9	7
gdhA	*glutamate dehydrogenase/ leucine dehydrogenase*	8	5
mdh	*malate dehydrogenase (NAD)*	8	5
pgi	*glucose-6-phosphate isomerase*	7	7
pmi	*phosphomannose isomerase*	8	5
STs in total		**10**	**7**

STs, sequence types

3.2. Pigeons

The pigeon-derived ORT strains were clearly separated from other ORT strains and formed cluster C. They did not share a single allele in all seven gene loci with strains from other avian hosts. They were internally of high genetic similarity but were clearly distant from strains of the other two main clusters A and B (Fig. 1). The phylogenetic distance to other strains also became obvious by extensive nucleotide differences in primer regions of their housekeeping gene sequences, with the effect that new primers had to be designed for three gene loci. Moreover, two deletions and one insertion of three bases were identified in the gene locus *aroE*. More precisely, ORT strains T37 and T49 had a deletion of one base triplet at position 256, whereas T85 had an insertion of a base triplet at position 365 each leading to a change of one amino acid in the protein Shikimate 5-dehydrogenase.

Two strains (T91 and T92) shared ST26. They originated from two

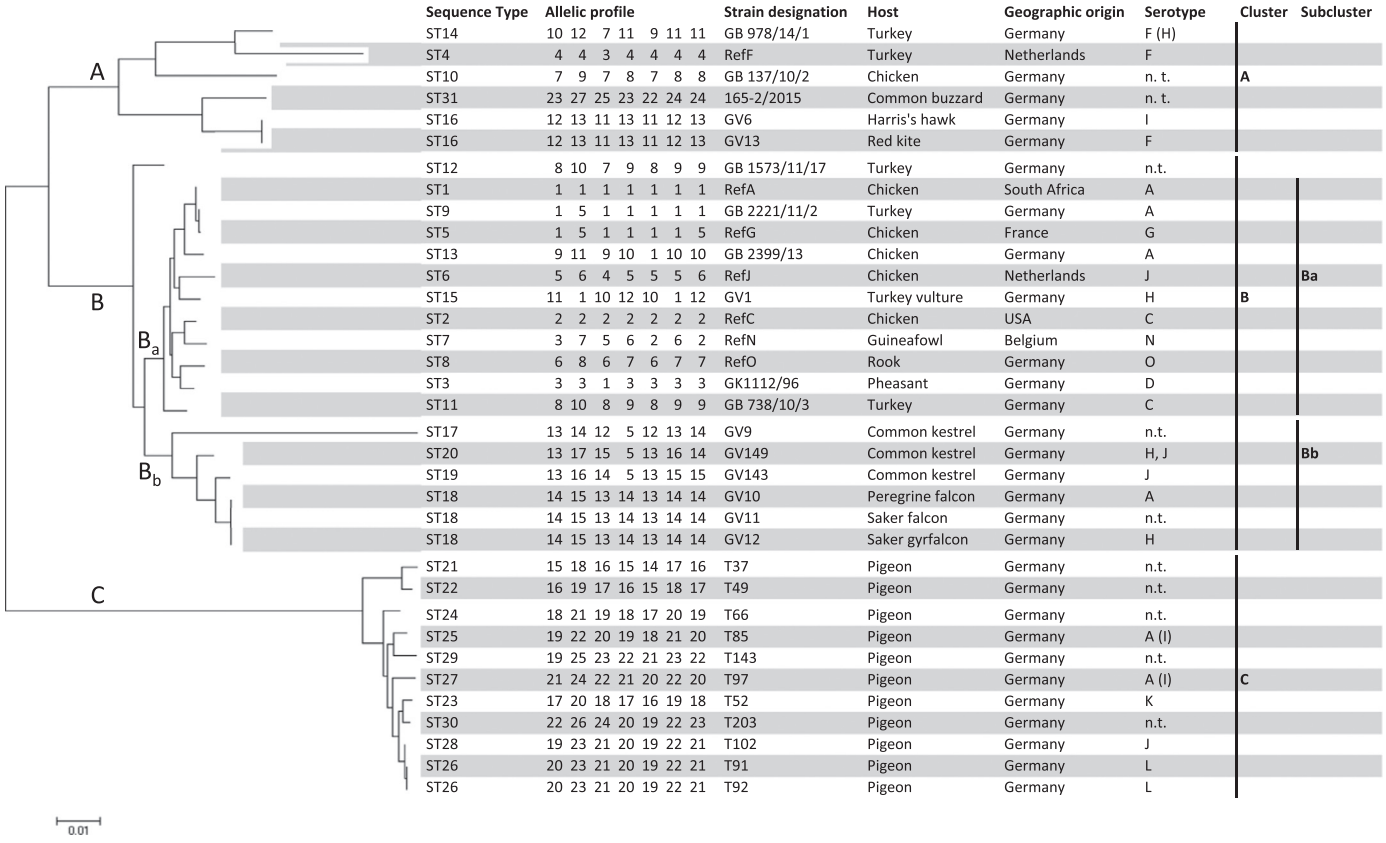

Fig. 1. Phylogenetic tree showing the relatedness of 35 representative ORT strains generated from MLST sequences by using the maximum likelihood method of MEGA 6 (Tamura et al., 2013). The ORT strains included in the phylogenetic analysis consist of 14 representative strains for ST1 to ST14, 11 strains isolated from pigeons and ten strains isolated from birds of prey. Three main clusters (A, B, C) and two subclusters (Ba, Bb) are shown. Details on sequence type (ST), allelic profile, strain designation, host, geographic origin and serotype were provided. Slight serotype cross-reactions in the agar gel precipitation test are given in parentheses. 'n.t.' stands for ORT strains that could not be typed with available antisera A to L.

birds within the same pigeon loft and had identical serotype profiles. Two more ORT strains (T97 and T102) were isolated from pigeons kept within one loft. Both strains differed completely in their serotype (A and J) and allelic profiles (ST27 and ST28). The ORT strains isolated from feral pigeons (T37 and T49) formed a distinct lineage within the main cluster C, whereas the remaining strains were isolated from pigeons kept within the same loft.

A limited number of ORT strains from pigeons in Asia have been investigated in previous studies (Chou et al., 2009; Mirzaie & Hassanzadeh, 2013; Mirzaie et al., 2011; Tsai & Huang, 2006). In accordance with our MLST results, these authors showed that strains derived from the order Columbiformes differed markedly in their phenotypic and genetic characteristics from other ORT isolates. Based on 16S rRNA gene analyses, strains from pigeons consistently had their own cluster distant from the majority of ORT strains of poultry origin as well as from strains isolated from other bird species (Chou et al., 2009; Mirzaie & Hassanzadeh, 2013; Tsai & Huang, 2006). In two studies, however, a single strain from a turkey (Mirzaie & Hassanzadeh, 2013, Iran) and a single strain from a chicken (Chou et al., 2009, Taiwan) were included in the pigeon cluster. In the Taiwanese study, 94 ORT strains from galliform birds and non-galliform birds were further analyzed by two DNA fingerprinting methods (Chou et al., 2009). Compared to results of 16S rRNA gene analysis, both methods produced more indistinct fingerprint patterns, where pigeon-derived strains grouped among ORT strains from poultry and other non-poultry host species. Still, all analyses consistently showed that pigeon-derived strains, even strains from different geographic origins, were more related to each other than to those from other avian hosts (Chou et al., 2009; Mirzaie & Hassanzadeh, 2013).

3.3. Birds of prey

Based on MLST results, strains isolated from birds of prey are genetically very heterogeneous yielding the highest number of polymorphic sites among their housekeeping gene sequences in relation to the small number of strains included in the analysis. With regard to the avian taxonomy, new insights into the systematics of birds and their family relationships showed that birds of prey represent a non-uniform phylogenetic group, where the family of Falconidae is closer related to the orders Passeriformes and Psittaciformes than to the family of Accipitridae (Hackett et al., 2008; Suh et al., 2011). Our MLST results reflected these host-associated phylogenetic relationships. Falconidae-derived ORT strains formed their own subcluster Bb within the main cluster B (Fig. 1), whereas ORT strains isolated from three different species of the Accipitridae (Harris's hawk, red kite and common buzzard) grouped more distant in cluster A. In contrast, the single ORT strain isolated from a turkey vulture (family Catharitidae) had a close relationship with poultry-derived strains and was assigned to subcluster Ba, which included the vast majority of strains isolated from galliform birds as well as a single strain isolated from a crow (order Passeriformes).

3.4. Non-galliform birds vs. galliform birds

In general, ORT strains isolated from non-galliform birds had a higher genetic heterogeneity based on MLST than strains derived from domesticated poultry species but at the same time showed close phylogenetic relationships to other strains isolated from their bird family. Interestingly, ORT strains isolated from pigeons or respective from birds of prey never shared an identical ST with strains isolated from other bird taxa. These results are even more interesting, as for example birds of prey like falcons frequently feed on other birds such as chickens and pigeons, increasing the risk of interspecies transmission. For ORT strains isolated from birds of prey compared to strains isolated from galliform birds, at least some identical alleles were found. A single strain isolated from a turkey vulture shared two identical alleles (*aroE*-1 and *pgi*-1) with strains from domesticated poultry. In addition, three strains that originated from common kestrels in Germany were in one gene locus (*gdhA*-5) identical to strains of ST6 isolated from a turkey and chickens of different geographic origins.

Only one ORT strain isolated from a passerine bird species (rook) was analyzed by MLST and alone represented ST8 within subcluster Ba. Earlier studies, which included up to three isolates from rooks, consistently showed that the isolates have their own branch phylogenetically closely related to isolates from poultry hosts (Amonsin et al., 1997; Mirzaie & Hassanzadeh, 2013; Tsai & Huang, 2006). So far, however, the general occurrence of ORT in rooks and in other passerine bird species is largely unknown.

The role of wild birds as reservoir hosts and asymptomatic carriers of ORT have been repeatedly discussed by different authors. Similar to MLST results, high genetic heterogeneity has been found among ORT strains isolated from wild birds compared to those of poultry origin (Amonsin et al., 1997; Chou et al., 2009). Amonsin et al. (1997) were first who assumed based on their genetic results that ORT was probably introduced from wild birds into domesticated poultry, although only few isolates of wild bird origin were included in the analyses. Similarly, Chou et al. (2009) found higher genetic variation among ORT strains isolated from a range of non-galliform birds compared to those from galliform birds, suggesting that the bacterium might have evolved in wild birds before its emergence as a novel pathogen in poultry. The importance of interspecies transmission from wild birds to domesticated poultry is well documented for other avian pathogens such as for avian influenza viruses (Alexander, 2007). Similar transmission events have been described for disease outbreaks of fowl cholera associated with *Pasteurella multocida* (Christensen, Dietz, & Bisgaard, 1998; Hansen, 2013; Snipes et al., 1989). Wild birds are usually asymptomatically infected and therefore considered as a natural reservoir for the respective infectious agent. In the present study, ORT strains of non-galliform bird origin were isolated from apparently healthy birds or from individual birds suffering from other diseases such as aspergillosis or pasteurellosis (data not shown). Similar to isolates from other studies (Amonsin et al., 1997; Chou et al., 2009; Tsai & Huang, 2006), it remains unclear whether ORT belongs to the normal mucosal bacterial flora of the respiratory tract of these birds and possibly provides a certain level of host adaptation as it is known for other bacteria like Pasteurellaceae (Hansen, 2013).

4. Conclusion

To gain insights into the origin of ORT and its sudden occurrence in domesticated poultry bacterial typing at population level may be considered an important mechanism to understand relevant phylogenetic relationships between bacteria and their hosts (Christensen & Bisgaard, 2010). The MLST results clearly showed that ORT strains from birds of prey had close genetic relationships to pathogenic strains circulating among turkeys and chickens. Our results further indicate that strains isolated from pigeons are genetically distant from all other ORT strains and may taxonomically represent their own ORT-like species.

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