Genetic variability and limited clonality of *Mycoplasma hyorhinis* in pig herds

Bettina Trüeb[a,1], Elena Catelli[b,1], Adrian Luehrs[c], Heiko Nathues[d], Peter Kuhnert[a,*]

[a]*Institute of Veterinary Bacteriology, Vetsuisse Faculty, University of Bern, Laenggassstr. 122, 3001 Bern, Switzerland*

[b]*Department of Veterinary Science, University of Parma, Via del Taglio 8, 43126 Parma, Italy*

[c]*Field Station for Epidemiology, University of Veterinary Medicine Hannover, Foundation, Bueschelerstr. 9, 49456 Bakum, Germany*

[d]*Clinic for Swine, Department of Clinical Veterinary Medicine, Vetsuisse Faculty, University of Bern, Bremgartenstr. 109a, 3001 Bern, Switzerland*

[1]equal contribution

*Corresponding author: Peter Kuhnert, Institute of Veterinary Bacteriology, University of Bern, Laenggassstr. 122, 3001 Bern, Switzerland. peter.kuhnert@vetsuisse.unibe.ch

Tel +41 31 6312485

Fax +41 31 6312634

Abstract

Mycoplasma hyorhinis is a common inhabitant of the upper respiratory tract and tonsils of pigs. Its role as a possible pathogen remains controversial. In order to gain more insight into the epidemiology and population structure of *Mycoplasma hyorhinis* we genetically characterized 60 isolates by multi locus sequence typing (MLST). The *M. hyorhinis* strains originated from Swiss and German pig herds with knowledge on the clinical background. The MLST scheme of Tocqueville *et al.* (J.Clin.Microbiol. 2014) was optimized, primers for the six MLST gene fragments were newly designed to allow amplification and sequencing with a single protocol. A total of 27 ST were observed with the 60 strains, 26 of those were previously unknown types. Generally identical genotypes were observed within a farm but they differed between farms. The identical genotype was also observed in three different Swiss farms. On the other hand different genotypes within a farm were found with three German farms. The Swiss isolates formed a distinct cluster but otherwise there was no geographical nor a clinical association with specific clusters observed. Data shows a high variability of *M. hyorhinis* comparable to what is observed for *Mycoplasma hyopneumoniae*. Similar to this pathogen the population structure of *M. hyorhinis* also shows some limited clonality with predominant genotypes within an animal and a single farm but different ones between farms. The comparable population structure of *M. hyopneumoniae* and *M. hyorhinis* could indicate a similar evolution of the two species in the common pig host.

Keywords: MLST, genotyping, pig disease, enzootic pneumonia, coughing

1. Introduction

Mycoplasma hyorhinis, a common inhabitant of the upper respiratory tract and tonsils of pigs, was first described in the fifties by Switzer (1955). It is generally regarded as a commensal, however, a role of *M. hyorhinis* as a potential pathogen has also been discussed. Infections with this organism are subclinical, even though arthritis, polyserositis, conjunctivitis and otitis as well as abortions are clinical disorders that may be observed in an infected pig (Friis and Feenstra, 1994; Kobisch and Friis, 1996; Shin et al., 2003). *Mycoplasma hyorhinis* has also been found associated with pneumonia, however, rather as a secondary pathogen (Falk et al., 1991; Kawashima et al., 1996; Kobayashi et al., 1996; Lin et al., 2006). Nevertheless, an increase in the detection of *M. hyorhinis* in recent years has been observed and the relevance of this pathogen as an emerging issue causing significant economic losses is suspected (Dos Santos et al., 2015).

Genotyping allows epidemiological investigation and population studies. This can help to clarify the role of a possible pathogen, identify specifically involved strains and their transmission. Multilocus sequence typing (MLST) is a highly selective and specific method which has been utilized for the characterization of many bacterial species including *Mycoplasma hyopneumoniae* and *M. hyorhinis* (Mayor et al., 2008; Tocqueville et al., 2014). Genotyping of *M. hyorhinis* using MLST is based on sequences of internal fragments from six housekeeping genes, i.e. the chromosomal replication initiation protein (*dnaA*), the RNA polymerase β subunit (*rpoB*), the DNA gyrase subunit B (*gyrB*), the glutamyl-tRNA synthetase (*gltX*), the adenylate kinase (*adk*) and the guanylate kinase (*gmk*).

In this study, we optimized the MLST scheme of six loci by defining completely new primer sets. They were used to genetically characterize 60 *M. hyorhinis* isolates belonging to twenty-five Swiss and German herds. Information about the herd health status, i.e. clinical respiratory disease, of the fattening units from where strains came from were gathered as well. The MLST data of the *M. hyorhinis* isolates was used to elucidate genetic variability of the species and was related to the clinical presentation of the pig in order to define possible virulent strains.

2. Materials and methods

2.1. Strains and template preparation

A total of 60 *M. hyorhinis* isolates with 47 from Germany and 13 from Switzerland previously included in a transnational cross sectional study determining the prevalence of *M. hyorhinis* were analyzed (Luehrs, 2015). For every source of strains, relevant pathological conditions in the corresponding animals were available from databases. This includes presence of (enzootic) pneumonia varying from mild, moderate to severe assessed during lung scoring after dissection, as well as arthritis and/or polyserositis. Moreover, the simultaneous infection with *M. hyopneumoniae* was examined in every sample using real-time PCR (Dubosson et al., 2004).

Strains of *M. hyorhinis* were kept at -80°C and cultured fresh in 1 ml liquid medium (Mycoplasma Experience, Bletchingley, Great Britain) for 1-2 days at 37°C. Once medium color change from red to yellow occurred the culture was centrifuged for 10 min. at 5000xg. The pellet was dissolved in 70µl ddH_2O and boiled for 15 min. at 95°C. This crude lysate was then kept at -20°C until being used for PCR.

2.2. Optimization of MLST

The MLST scheme of Tocqueville et al. (2014) using the six housekeeping genes *dnaA, rpoB, gyrB, gltX, adk* and *gmk* served as a basis to optimize the protocol. Primers with identical annealing temperatures were defined, allowing amplification of all targets with a single PCR protocol instead of 3 different ones as described by Tocqueville et al. (2014). For this purpose target genes were extracted from available genome sequences of *M. hyorhinis* strains DBS 1050, HUB-1, GDL-1, MCLD, SK76 as well as the type strain ATCC17981^T (=BTS7^T) and sequences were aligned. Consensus sequence stretches as close as possible to the starting sequence used for typing were defined and primers with similar annealing temperature selected (Table 1). Each PCR was performed in a 30-µl total volume containing 12 pmol of each primer, 1 x FIREPol® Master Mix Ready to Load (Solis BioDyne, Tartu, Estonia) and 1 µl lysate at the following cycling conditions:

3 min denaturation at 95°C, followed by 35 cycles at 95°C for 30 s, 55°C for 30 s and 72°C for 45 s. A final extension step for 7 min at 72°C was included. Subsequently, the amplification products of the six genes from one strain were pooled and then purified using the "High pure PCR purification Kit" (Roche Diagnostics, Rotkreuz, Switzerland). Finally the purified pooled PCR products was used for sequencing with the BigDye® Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher, Reinach, Switzerland), using the same primers as for PCR (Table 1). For this purpose 96-well plates (Thermo Fisher) were prepared with corresponding primers (5 pmol/well) using a multichannel pipette. The plates allowed batch-wise analysis of 8 strains and could be kept at -20°C until being used for sequencing (Korczak et al., 2009).

2.3. Data analysis

Sequences were edited using the Sequencher program v5.1 (GeneCodes, Ann Arbor, MI, USA) and imported into Bionumerics v7.5 (AppliedMaths, Sint Marten, Belgium). Sequence types (ST) were determined using the MLST plugin allowing direct query of the PubMLST database (www.pubmlst.org). New alleles and allele combinations were submitted to the curator of the database to assign allele and ST numbers. The MLST sequences of the 33 strains from the study of Tocqueville et al. (2014) as well as the ones of the type strain BTS7^T (GenBank accession ARTL00000000) were also imported to the database and included in the analyses. The resulting 94 entries were used for creating a minimal spanning tree based on allele numbers and a UPGMA tree based on allele sequences in Bionumerics.

The START2 program (pubmlst.org) was used to calculate the ratio of synonymous and nonsynonymous substitutions as well as the index of association (I_A). The index of discrimination (D) was calculated using the formula of Hunter and Gaston (1988). LIAN (guanine.evolbio.mpg.de/cgi-bin/lian/lian.cgi.pl) was used to calculate the genetic diversity (H).

3. Results

3.1. Genetic diversity of M. hyorhinis

All 60 isolates could be analyzed with the optimized MLST scheme. There were two new alleles observed for *dnaA* (totaling 12) and *gyrB* (totaling 8), as well as three new alleles for the targets *rpoB* (totaling 10), *gltX* (totaling 10), *adk* (totaling 7) and *gmk* (totaling 8). With the additional new alleles the genetic diversity (*H*) of the six loci varies from 0.4155 (*gltX*) to 0.7596 (*gyrB*). The ratios of nonsynonymous and synonymous substitutions varied from 0.1303 (*dnaA*) to 0.6785 (*gmk*). The index of association (I_A) was 0.026, indication no linkage disequilibrium. A total of 27 ST were defined, 26 of them were new resulting in a total of 55 different *M. hyorhinis* genotypes based on MLST. Including all strains, i.e. also those from the same herd and same animals, the index of discrimination (*D*) was 0.982. When only single representatives of such repetitive isolations were included in the analysis the index of discrimination (*D*) was 0.995.

All the isolates from our study belong to the clonal complex 1 as defined by at least 4 common alleles (Tocqueville et al., 2014). The clonal relationship of STs is graphically shown by the minimal spanning tree in Fig. 1. Six of the seven previously observed singletons remained while ST-28 was integrated into complex 1 (Tocqueville et al., 2014). There is only one common ST (ST-13) between our study and the latter one as shown in Fig.2.

3.2. Limited clonality of M. hyorhinis

There are no clear and discrete geographic clusters present, even though some grouping can be observed. The type strain BTS7^T originating from the USA was determined as ST-16 based on *in silico* analysis of its genome sequence. This ST was also observed in France. The Chinese isolate represents an out-group while most of the branches consist of isolates from more than one country. Noteworthy, all Swiss isolates can be found on a single cluster containing only isolates from one German farm where polyserositis was diagnosed in piglets. Another discernable cluster is formed by only German isolates. This cluster contains isolates from farm DE_03 where two slightly

different strains based on a single base-pair change in *gmk* could be detected. Clearly different strains were observed in farm DE_16. In all other cases isolates from a single farm had the same genotype, while isolates from different farms were also of different genotype. Identical genotypes were also found when multiple isolates from single animals were analyzed (indicated by C2, C3). Identical genotypes were observed in three Swiss farms. In German farms, *M. hyorhinis* was isolated from animals that were in most cases (32 of 44) affected by *M. hyopneumoniae* as was diagnosed by real-time PCR indicated by ABC/REP in Fig. 2. In one case with a clear clinical status of enzootic pneumonia (strain numbers 10003) a follow up was done a few months later (strain numbers. 10025). While at both stages *M. hyorhinis* could be isolated from broncho-alveolar lavage fluid (BALF) and showed identical genotypes in different animals, the isolates from the two different time points differed in genotypes, indicating a switch of *M. hyorhinis* population over time.

There were no clusters or genotypes associated with certain clinical manifestations.

4. Discussion

This study analyzed the genetic variability of *M. hyorhinis* from pig farms in Germany and Switzerland. Genotypes were put in relation to available clinical data and were also compared to isolates contained in the PubMLST database. For this purpose the MLST scheme of Tocqueville et al. (2014) was optimized to allow a uniform PCR amplification and sequencing protocol. The new protocol, based on a completely new set of primers for the six target genes, proved to be robust. All 60 isolates could be genotyped. The genetic diversity of *M. hyorhinis* increased by 26 genotypes to a total of 55 different STs based on MLST. While in the previous study by Tocqueville et al. (2014) mainly isolates from France were genotyped, we analyzed strains from Germany and Switzerland. Except from one farm, where the previously identified ST-13 was found, all other were new STs. Generally the same ST was observed within a farm and also within a single animal. The same ST

could be observed even within three different Swiss farms. On the other side different ST were observed in three German farms (DE_16, DE_03 and DE_05). From farm DE_05 one sample resulted in an ambiguous base call at two positions what made a genotyping impossible but showed that it contained a mix of at least two different strains (data not shown). This was an indirect indication that indeed more than one strain can be present in a single animal, even though it might not be the rule. Nevertheless, the observation that within a farm and individual animal the same genotype is generally found is a fact that would allow for direct genotyping of clinical material. This approach is successfully used with *M. hyopneumoniae* (Kuhnert and Overesch, 2014; Kuhnert et al., 2011; Mayor et al., 2008) and could therefore also be applied for *M. hyorhinis*. This allows a rapid straight forward genotyping eliminating the culture step.

The high variability but still limited clonality of *M. hyorhinis* is very similar to what can be observed with *M. hyopneumoniae* (Kuhnert and Overesch, 2014; Mayor et al., 2008; Mayor et al., 2007; Nathues et al., 2011). Given this observation and fact that *M. hyorhinis* is mainly a commensal inhabiting the same niche as the more pathogenic *M. hyopneumoniae* could point to a similar selection pressure if not co-evolution of the two species. Nevertheless, while whole genome comparisons between these two species showed that rearrangements are common, clear differences in gene composition indicate that a direct interaction and dependency of the two is rather unlikely.

Clinical information was gathered from all cases including diagnostic results on enzootic pneumonia (EP) caused by *M. hyopneumoniae*. While *M. hyopneumoniae* was detected in more than 70% of German cases, all Swiss cases were negative for this pathogen (Fig.2). This reflects the different situation in these two countries. While in Switzerland only a few sporadic cases of EP are observed per year due to a successful eradication program, EP is still endemic in Germany (Nathues et al., 2014; Stark et al., 2007). Furthermore, clinical data is also available for strains contained in the PubMLST database (Tocqueville et al., 2014). No correlation or association of specific genotypes or clusters with clinical manifestations could be discerned. Similar observations were made by Dos Santos et al. (2015) using a multi-locus variable-number tandem-repeat analysis

(MLVA) that is based on more variable genes than the house-keeping genes used for MLST. These authors also could not find an association between isolates and clinical manifestations. The MLVA approach was less discriminating than MLST with a diversity index of 0.814 compared to 0.982, respectively. However, the MLVA is based on only 2 genes compared to the six genes used for MLST and is therefore less work intensive and less expensive than the MLST. Nevertheless, the MLST is highly reproducible with all six targets successfully analyzable in all strains whereas with MLVA one of the targets could not be amplified in about 2% of strains and more than 40% of clinical specimens (Dos Santos et al., 2015). Moreover, we could improve the MLST by designing new primers that allow a single PCR amplification and sequencing protocol. The PCR purification step using pooled samples as well as the pre-preparing of 96-well microtiter plates containing all sequencing primers for 8 strains further reduce hands on time and costs. This makes MLST the method of choice if comparing efforts and outcome.

In conclusion the MLST scheme for *M. hyorhinis* was optimized and the number of isolates analyzed by this method was extended. Thereby the variability of *M. hyorhinis* in farms and related clinical data of the host to genetic data of the potential pathogen was systematically investigated. A limited clonality of *M. hyorhinis* was observed, i.e. a high variability of genotypes was accompanied by identical genotypes within a farm and individual animal. No specifically virulent genotypes could be discerned, indicating the commensal nature of *M. hyorhinis* in the pig lung.

5. Acknowledgements

This project was partly funded by the European Union's Horizon 2020 Programme under the Grant Agreement n°633184 and Swiss Government.

6. References

Dos Santos, L.F., Clavijo, M.J., Sreevatsan, S., Rovira, A., Moreira, M.A., Pieters, M., 2015. Genotyping of Mycoplasma hyorhinis using multiple-locus variable number tandem repeat analysis. J. Microbiol. Methods 111, 87-92.

Dubosson, C.R., Conzelmann, C., Miserez, R., Boerlin, P., Frey, J., Zimmermann, W., Häni, H., Kuhnert, P., 2004. Develpment of two real-time PCR assays for the detection of *Mycoplasma hyopneumoniae* in clinical samples. Vet.Microbiol. 102, 55-65.

Falk, K., Hoie, S., Lium, B.M., 1991. An abattoir survey of pneumonia and pleuritis in slaughter weight swine from 9 selected herds. II. Enzootic pneumonia of pigs: microbiological findings and their relationship to pathomorphology. Acta Vet. Scand. 32, 67-77.

Friis, N.F., Feenstra, A.A., 1994. Mycoplasma hyorhinis in the etiology of serositis among piglets. Acta Vet. Scand. 35, 93-98.

Hunter, P.R., Gaston, M.A., 1988. Numerical index of the discriminatory ability of typing systems: an application of Simpson's index of diversity. J.Clin.Microbiol. 26, 2465-2466.

Kawashima, K., Yamada, S., Kobayashi, H., Narita, M., 1996. Detection of porcine reproductive and respiratory syndrome virus and Mycoplasma hyorhinis antigens in pulmonary lesions of pigs suffering from respiratory distress. J. Comp. Pathol. 114, 315-323.

Kobayashi, H., Morozumi, T., Miyamoto, C., Shimizu, M., Yamada, S., Ohashi, S., Kubo, M., Kimura, K., Mitani, K., Ito, N., Yamamoto, K., 1996. Mycoplasma hyorhinis infection levels in lungs of piglets with porcine reproductive and respiratory syndrome (PRRS). J. Vet. Med. Sci. 58, 109-113.

Kobisch, M., Friis, N.F., 1996. Swine mycoplasmoses. Rev. Sci. Tech. 15, 1569-1605.

Korczak, B.M., Zurfluh, M., Emler, S., Kuhn-Oertli, J., Kuhnert, P., 2009. Multiplex strategy for MLST, *fla*-typing and genetic determination of antimicrobial resistance of Swiss *Campylobacter jejuni* and *Campylobacter coli* isolates. J.Clin.Microbiol. 47, 1996-2007.

248 Kuhnert, P., Overesch, G., 2014. Molecular epidemiology of *Mycoplasma hyopneumoniae* from
249 outbreaks of enzootic pneumonia in domestic pig and the role of wild boar. Vet. Microbiol.
250 174, 261-266.
251 Kuhnert, P., Overesch, G., Belloy, L., 2011. Genotyping of *Mycoplasma hyopneumoniae* in wild
252 boar lung samples. Vet.Microbiol. 152, 191-195.
253 Lin, J.H., Chen, S.P., Yeh, K.S., Weng, C.N., 2006. Mycoplasma hyorhinis in Taiwan: diagnosis
254 and isolation of swine pneumonia pathogen. Vet. Microbiol. 115, 111-116.
255 Luehrs, A., 2015. Occurrence of *Mycoplasma hyorhinis* infections in fattening pigs and association
256 with clinical symptoms and pathological lesions of Enzootic Pneumoniae. Thesis of the
257 Vetsuisse Faculty, University of Bern, Bern, Switzerland.
258 Mayor, D., Jores, J., Korczak, B.M., Kuhnert, P., 2008. Multilocus sequence typing (MLST) of
259 *Mycoplasma hyopneumoniae*: A diverse pathogen with limited clonality. Vet.Microbiol.
260 127, 63-72.
261 Mayor, D., Zeeh, F., Frey, J., Kuhnert, P., 2007. Diversity of *Mycoplasma hyopneumoniae* in pig
262 farms revealed by direct molecular typing of clinical material. Vet.Res. 38, 391-398.
263 Nathues, H., Beilage, E.G., Kreienbrock, L., Rosengarten, R., Spergser, J., 2011. RAPD and VNTR
264 analyses demonstrate genotypic heterogeneity of *Mycoplasma hyopneumoniae* isolates from
265 pigs housed in a region with high pig density. Vet.Microbiol. 152, 338-345.
266 Nathues, H., Chang, Y.M., Wieland, B., Rechter, G., Spergser, J., Rosengarten, R., Kreienbrock, L.,
267 Grosse Beilage, E., 2014. Herd-level risk factors for the seropositivity to Mycoplasma
268 hyopneumoniae and the occurrence of enzootic pneumonia among fattening pigs in areas of
269 endemic infection and high pig density. Transbound. Emerg. Dis. 61, 316-328.
270 Shin, J.H., Joo, H.S., Lee, W.H., Seok, H.B., Calsamig, M., Pijoan, C., Molitor, T.W., 2003.
271 Identification and characterization of cytopathogenic Mycoplasma hyorhinis from swine
272 farms with a history of abortions. J. Vet. Med. Sci. 65, 501-509.

Stark, K.D., Miserez, R., Siegmann, S., Ochs, H., Infanger, P., Schmidt, J., 2007. A successful national control programme for enzootic respiratory diseases in pigs in Switzerland. Rev.Sci.Tech. 26, 595-606.

Switzer, W.P., 1955. Studies on infectious atrophic rhinitis. IV. Characterization of a pleuropneumonia-like organism isolated from the nasal cavities of swine. Am. J. Vet. Res. 16, 540-544.

Tocqueville, V., Ferre, S., Nguyen, N.H., Kempf, I., Marois-Crehan, C., 2014. Multilocus sequence typing of *Mycoplasma hyorhinis* strains identified by a real-time TaqMan PCR assay. J. Clin. Microbiol. 52, 1664-1671.

Figure legends

Fig. 1 Minimal spanning tree of *M. hyorhinis* sequence types (ST) based on allele numbers built in Bionumerics v7.6. A clonal complex relation is defined as a minimum of 4 identical alleles out of the six. Branch length and intensity reflects number of different alleles. The circle size represents the number of isolates belonging to the specific ST which is indicated by the corresponding number.

Fig. 2 Cluster analysis and strain information of *M. hyorhinis* isolates. A UPGMA tree was built in Bionumerics v7.6 based on the six partial gene sequences used for MLST. The isolate name, sequence type (ST), country and year of isolation, clinic and source are indicated. For the strains analyzed in this study the real-time PCR results for *M. hyopneumoniae* (ABC/REP) are included as well. The bar indicates the genetic distance.

Table 1. Primers used for uniform MLST of *Mycoplasma hyorhinis*

Gene	Primer	Sequence	seq. (bp)[a]
dnaA	dnaA-F2	CAGAAGTCTTAGGTGGTTTTG	459
	dnaA-R2	TGTGGAATGATCCTTGCCTC	
rpoB	rpoB-F2	TCAAGCTGTTCCATTAATTACTAC	509
	rpoB-R2	GCACTAACTTCTGATCCAATAC	
gyrB	gyrB-F2	GATTCTGATGGTTCACATATTAG	358
	gyrB-R2	GTCTAGGTTTTTTGCATATTTTGC	
gltX	gltX-F2	CTGAAAGACTCTCAAAATCACC	448
	gltX-R2	TTACAAGCCTTTTTGAAATTAGTTC	
adk	adk-F2	CGATGGCATCTAATTCTTTTAAAG	437
	adk-R2	TACTCAGGCAAAGTTTTTAGAAC	
gmk	gmk-F2	GCGCCTGTTTCTGTTAATATTG	452
	gmk-R2	AAGAGACAAAAGACCTAATGAAG	

[a] length of the informative sequence, i.e. of the PCR product without primer sequences

Table 1. Primers used for uniform MLST of *Mycoplasma hyorhinis*

Gene	Primer	Sequence	seq. (bp)[a]
dnaA	dnaA-F2	CAGAAGTCTTAGGTGGTTTTG	459
	dnaA-R2	TGTGGAATGATCCTTGCCTC	
rpoB	rpoB-F2	TCAAGCTGTTCCATTAATTACTAC	509
	rpoB-R2	GCACTAACTTCTGATCCAATAC	
gyrB	gyrB-F2	GATTCTGATGGTTCACATATTAG	358
	gyrB-R2	GTCTAGGTTTTTTGCATATTTTGC	
gltX	gltX-F2	CTGAAAGACTCTCAAAATCACC	448
	gltX-R2	TTACAAGCCTTTTTGAAATTAGTTC	
adk	adk-F2	CGATGGCATCTAATTCTTTTAAAG	437
	adk-R2	TACTCAGGCAAAGTTTTTAGAAC	
gmk	gmk-F2	GCGCCTGTTTCTGTTAATATTG	452
	gmk-R2	AAGAGACAAAAGACCTAATGAAG	

[a] length of the informative sequence, i.e. of the PCR product without primer sequences

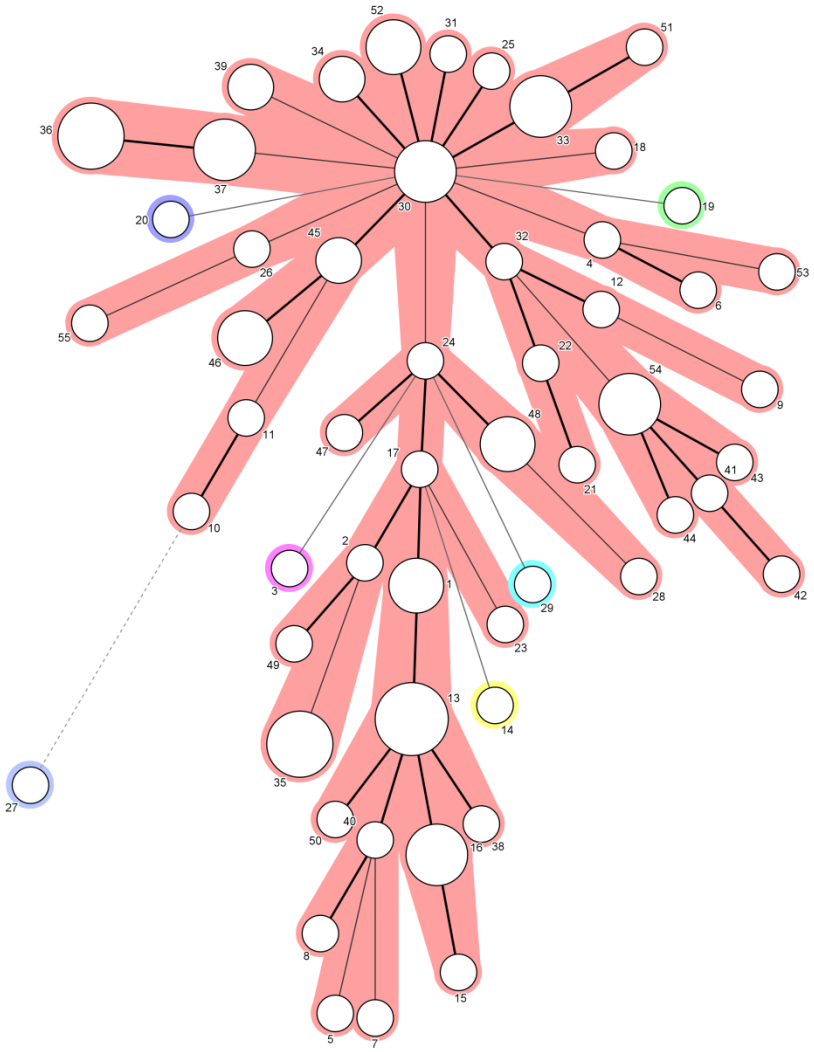
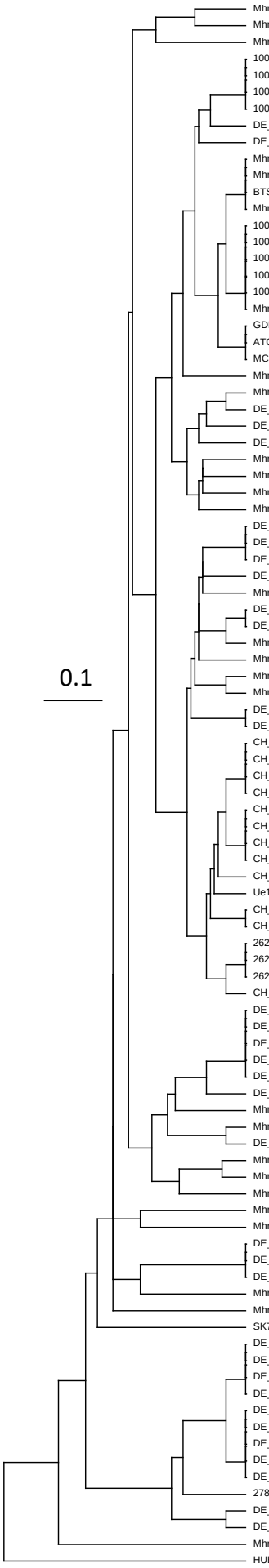


Fig. 1 Minimal spanning tree.



Strain	ST	country	year	clinic	source	ABC/REP
Mhr52	21	France	2004	carrier	nasal swab	
Mhr66	22	France	2004	pneumonia	lung	
Mhr17	23	France	2009	pericarditis	heart	
10003-61	54	Germany	2014	enzootic pneumonia	BALF	+
10003-41	54	Germany	2014	enzootic pneumonia	BALF	+
10003-11	54	Germany	2014	enzootic pneumonia	BALF	+
10003-31	54	Germany	2014	enzootic pneumonia	BALF	+
DE_13_30	43	Germany	2014	moderate pneumonia/pleurisy	lung	-
DE_14_31	44	Germany	2014	moderate pneumonia	lung	+
Mhr142	16	France	1999	arthritis	joint fluid	
Mhr211	16	France	1981	carrier	nasal swab	
BTS7	16	USA	1953	atrophic rhinitis	nasal swab	
Mhr143	16	France	1999	arthritis	joint fluid	
10025-10	13	Germany	2014	enzootic pneumonia follow up	BALF	-
10025-1	13	Germany	2014	enzootic pneumonia follow up	BALF	-
10025-2	13	Germany	2014	enzootic pneumonia follow up	BALF	-
10025-5	13	Germany	2014	enzootic pneumonia follow up	BALF	-
10025-3	13	Germany	2014	enzootic pneumonia follow up	BALF	-
Mhr208	13	France	1998	arthritis	joint fluid	
GDL-1	1	USA	?	other	cell line	
ATCC 25021	1	USA	1955	pneumonia	lung	-
MCLD	1	Israel	2004	other	cell line	
Mhr141	15	France	1998	arthritis	joint fluid	
Mhr123	8	France	2007	carrier	tracheal mucus	
DE_05_48	40	Germany	2014	moderate pneumonia	lung	+
DE_04_16	38	Germany	2014	mild pneumonia/pleurisy	lung	-
DE_19_08	50	Germany	2015	severe pneumonia	lung	+
Mhr120	4	France	2007	pneumonia	lung	
Mhr180	7	France	2007	pneumonia	lung	
Mhr160	6	France	2007	carrier	tracheal mucus	
Mhr100	5	France	2006	carrier	tracheal mucus	
DE_17_13	48	Germany	2015	mild pneumonia	lung	-
DE_17_47	48	Germany	2015	mild pneumonia	lung	-
DE_17_16	48	Germany	2015	moderate pneumonia	lung	+
DE_16_40	47	Germany	2015	moderate pneumonia	lung	+
Mhr164	25	France	2006	carrier	tracheal mucus	
DE_15_12	45	Germany	2014	moderate pneumonia	lung	+
DE_15_12_C3	45	Germany	2014	moderate pneumonia	lung	+
Mhr168	12	France	2006	pneumonia	lung	
Mhr47	26	France	2007	pneumonia	lung	
Mhr60	17	France	2004	pneumonia	lung	
Mhr113	24	France	2007	carrier	tracheal mucus	
DE_04_31	39	Germany	2014	moderate pneumonia	lung	+
DE_04_32	39	Germany	2014	moderate pneumonia	lung	-
CH_08_20	33	Switzerland	2015	moderate pneumonia	lung	-
CH_08_31	33	Switzerland	2015	mild pneumonia	lung	-
CH_08_13	33	Switzerland	2015	mild pneumonia	lung	-
CH_08_22	33	Switzerland	2015	mild pneumonia	lung	-
CH_01_05	30	Switzerland	2015	moderate pneumonia	lung	-
CH_02_20	30	Switzerland	2015	moderate pneumonia/pleurisy	lung	-
CH_03_07	30	Switzerland	2015	carrier	lung	-
CH_03_03	30	Switzerland	2015	moderate pneumonia	lung	-
CH_04_09	31	Switzerland	2015	moderate pneumonia	lung	-
Ue1435_15_1	51	Switzerland	2015	other	BALF	-
CH_09_01	34	Switzerland	2015	mild pneumonia	lung	-
CH_09_07	34	Switzerland	2015	moderate pneumonia	lung	-
2627	52	Germany	2014	polyserositis	lung	-
2628	52	Germany	2014	polyserositis	lung	-
2625	52	Germany	2014	polyserositis	lung	-
CH_07_23	32	Switzerland	2015	moderate pneumonia	lung	-
DE_02_20	35	Germany	2014	moderate pneumonia	lung	+
DE_02_06	35	Germany	2014	moderate pneumonia/pleurisy	lung	-
DE_02_05	35	Germany	2014	severe pneumonia	lung	+
DE_02_36	35	Germany	2014	severe pneumonia	lung	+
DE_02_48	35	Germany	2014	moderate pneumonia	lung	+
DE_20_03	55	Germany	2015	moderate pneumonia	lung	+
Mhr30	28	France	2006	carrier	tracheal mucus	
Mhr39	2	France	2007	pneumonia	lung	
DE_18_19	49	Germany	2015	moderate pneumonia	lung	+
Mhr43	10	France	2007	pneumonia	lung	
Mhr48	11	France	2007	carrier	tracheal mucus	
Mhr9	9	France	2009	pericarditis	heart	
Mhr21	19	France	2009	arthritis	joint fluid	
Mhr204	20	France	1978	carrier	nasal swab	
DE_16_34	46	Germany	2015	mild pneumonia	lung	+
DE_16_34_C3	46	Germany	2015	mild pneumonia	lung	+
DE_16_34_C2	46	Germany	2015	mild pneumonia	lung	+
Mhr97	29	France	2006	carrier	tracheal mucus	
Mhr104	18	France	2006	carrier	tracheal mucus	
SK76	14	USA	1973	arthritis	joint fluid	
DE_03_45	37	Germany	2014	moderate pneumonia	lung	+
DE_03_23	37	Germany	2014	moderate pneumonia	lung	+
DE_03_16	37	Germany	2014	moderate pneumonia	lung	+
DE_03_08	37	Germany	2014	severe pneumonia	lung	+
DE_03_31	36	Germany	2014	severe pneumonia	lung	+
DE_03_12	36	Germany	2014	moderate pneumonia/pleurisy	lung	+
DE_03_11	36	Germany	2014	severe pneumonia	lung	+
DE_03_06	36	Germany	2014	severe pneumonia/pleurisy	lung	+
DE_03_02	36	Germany	2014	mild pneumonia	lung	+
2783	53	Germany	2014	pneumonia	lung	-
DE_07_13	41	Germany	2014	moderate pneumonia	lung	+
DE_10_05	42	Germany	2014	severe pneumonia	lung	+
Mhr55	3	France	2004	pneumonia	lung	
HUB-1	27	China	?	other	other	