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Epidemiological investigations and multilocus sequence typing of *Mycoplasma gallisepticum* collected in China

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ABSTRACT *Mycoplasma gallisepticum* (**MG**) is one of the important pathogens in poultry industry and has led to major economic losses. Understanding the epidemiology is crucial to improve the control and eradication program of MG. This study collected 1,250 chicken samples, including trachea and lung, from China in 2022 to investigate the epidemiology of MG. Among the collected samples, 938 samples were positive for MG infection, resulting in an average positive rate of 75.04%. Additionally, 570 samples were positive for both MG and *Mycoplasma synoviae* (**MS**) coinfection, with an average positive rate of 45.60%. A total of 183 MG infection positive samples in this study were selected for genotyping, and the multilocus sequence typing (**MLST**) method based on 7 housekeeping genes was used. As a result, 183 samples belonged to 11 sequence types (**STs**), with ST-78 being the most prevalent. After BURST analysis, all 183 sequences were divided into group 3. Besides, 119 reference sequences from database and 183 sequences of this study were selected to construct the phylogenetic tree using the neighbor-joining method. The results revealed that the sequences from China, total 196 sequences, were classified into 4 branches. The findings suggest that the MG strains in China exhibit diverse genotypes, which may be related to international trade and the use of live vaccines. Furthermore, we detected the drug susceptibility of 10 isolated strains randomly, which may be helpful to guide the clinical use of drugs to control MG infection.

Key words: *Mycoplasma gallisepticum*, epidemiology, MLST genotype, drug susceptibility

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INTRODUCTION

Mycoplasma gallisepticum (**MG**) as one of the virulent avian *Mycoplasma* species affects chickens and turkeys worldwide and is listed and notifiable to the World Organization for Animal Health (OIE) (Malik et al., 2021). MG infection causes chronic respiratory disease in chickens and turkeys, characterized by nasal discharge, tracheal rales, coughing, and dyspnea, resulting major economic losses in terms of reduced weight gain, egg production and hatchability, downgrading carcass quality, and the infected birds become susceptible to other diseases (Sawicka et al., 2020; Yadav et al., 2021). MG transmits horizontally by direct or indirect contact and vertically through the egg (Kleven, 2008; Matucci et al., 2020).

According to molecular analysis of reported cases worldwide, a molecular epidemiological map of the MG infection has been established. The results show that MG infections occur in chicken coops in different regions and of varying scales. Specifically, the United States (Staley et al., 2018), Europe (Michiels et al., 2016; Felice et al., 2020), and Asia (Norouzian et al., 2019; Limsatanun et al., 2022) are high-incidence areas. In addition, there is also seasonal variation in incidence of this disease (Feberwee et al., 2022), with autumn and winter being the high-risk seasons.

With the development of molecular biology technology, DNA fingerprinting techniques were used to genotype of MG strain frequently (Charlton et al., 1999, Marois et al., 2001). However, these techniques are time-consuming, laborious, and poorly repeatable. To improve the reproducibility, reliability and applicability on clinical samples, and reduce labor intensity, sequence-based genotyping methods have been described, such as sequencing variable surface proteins (mgc2, pvpA, gapA and MGA_0319) or variable intergenic spacer region (**IGSR**) between 23S rRNA and 16S rRNA (Jiang et al., 2009; Sprygin et al., 2010). However,

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these methods showed insufficient discriminatory power to differentiate related strains (Delaney et al. 2012, Staley et al. 2018).

Among sequence-based genotyping methods, multilocus sequence typing (**MLST**) (Beko et al., 2019) and core genome multilocus sequence typing (**cgMLST**) (Ghanem et al., 2018) provide several advantages for genotyping bacterial species (Larsen et al., 2012; Kwong et al., 2016). Compared to MLST, whole genome sequencing is more expensive and time-consuming. MLST is based on the nucleotide sequences of internal fragments of housekeeping genes, in which mutations are assumed to be largely neutral (Jolley et al., 2018). Until now, 2 MLST typing method of MG were established based on 6 loci (atpG, dnaA, fusA, rpoB, ruvB, uvrA) (Beko et al., 2019) and 7 loci (ugpA, atpG, DUF3196, mraW, plsC, dppC, lgT) (Ghanem and El-Gazzar, 2019) respectively.

In this study, we collected 1,250 chicken samples, including trachea and lung, from 15 provinces in China in 2022 to detect MG infection. In order to investigate the dominant genotypes of MG, MLST genotype based on 7 housekeeping genes was carried out. This study will promote to understanding of the prevalence and evolutionary relationship of MG in China, and support the effective prevention and control of it.

MATERIALS AND METHODS

Sample Collection, Genomic DNA Extraction, and MG Isolation

The samples used in this study were collected from commercial broiler chicken farms in Jiangsu, Hubei, Shandong, Anhui, Guangxi, Guangdong, Yunnan, Henan, Hebei, Zhejiang, Hunan, Chongqing, Sichuan, Fujian, and Guizhou provinces of China in 2022, detail information showed in Table 1. Five chickens were collected from one flock. All tissues collected were trachea and lung of chickens that looks healthy. Tissues from one chicken were seemed as one sample, so 5 samples were collected from one flock. Tissues from each flock were collected under aseptic conditions, and placed into grinding tubes for nucleic acid extraction. After ground, tissue samples were centrifuged and the supernatant was added to the MagaBio Plus Genomic DNA Extraction Kit (Bioer Technology, Hangzhou, China) according to the manufacturer's instructions. For MG isolation, cut the trachea and lung tissue and add it to the modified mycoplasma medium, in a sterile environment (Bradbury and Howell, 1974), and shaken at 37°C for 3 to 4 h, then filtered into new sterile tubes using a 0.45 μm filter. The same volume of fresh medium was added and cultured at 37°C until the color changed from red to orange-yellow. The 200 μL of culture was plated on MG solid media, containing 10% porcine serum, 3.0 g/L glucose, 100 mg/L L-cysteine, and 100 mg/L NAD at 37°C for 3 to 7 days and constantly observed. Fried-egg-like single MG clones were selected and cultured in liquid medium until the color changes. Then, 200 μL of the culture was plated on MG solid media at 37°C again. Purified MG colonies were obtained after repeating these operations 3 times.

RT-qPCR Detection

The genomic DNA extracted from tissue samples was used for MG-specific and MS-specific RT-qPCR detection respectively (Raviv and Kleven, 2009). Briefly, THUNDERBIRD probe qPCR Mix (TOYOBO, Shanghai, China) 10 μL, 300 nM of each forward primer, 300 nM of each reverse primer, 150 nM of each probe, 0.4 μL of 50 × ROX reference, 2 μL of template, ddH_2O were added up to 20 μL. The qPCR amplification program consisted of 95°C for 5 min, followed by 40 cycles of 95°C for 15 s and 60°C for 30 s. The primer sequences used for detecting MG and *Mycoplasma synoviae* (**MS**) in this assay are as follows: MG forward primer: 5′-TTGGGTTTAGGGATTGGGATT-3′; MG reverse primer: 5′-CCAAGGGATTCAACCATCTT-3′; MG probe: 5′-FAM-TGATGATCCAAGAACGTGAAGAACACC-BHQ1-3′; MS forward primer: 5′-CTAAATACAATAGCCCAAGGCAA-3′; MS reverse primer: 5′-

Table 1. The results of MG and MS infection among 1,250 samples collected in China in 2022.

		MG infection		MG and MS coinfection	
Region	Sample numbers	Positive number	Positive rate	Positive number	Positive rate
Henan	25	20	80%	9	36%
Hebei	50	46	92%	7	14%
Shandong	50	21	42%	20	40%
Jiangsu	345	237	68.70%	149	43.19%
Anhui	100	96	96%	86	86%
Zhejiang	15	10	66.67%	9	60%
Hunan	70	41	58.57%	17	24.29%
Hunbei	75	50	66.67%	21	28%
Chongqing	55	52	94.55%	15	27.27%
Yunnan	55	42	76.36%	25	45.45%
Sichuan	100	88	88%	35	35%
Guizhou	45	43	95.56%	33	73.33%
Guangdong	75	50	66.67%	48	64%
Guangxi	140	101	72.14%	59	42.14%
Fujian	50	41	82%	37	74%
Total	1250	938	75.04%	570	45.60%

CCTCCTTTCTTACGGAGTACA-3′; MS probe: 5′-CY5-AGCGATACACAACCGCTTTTAGAAT-BHQ1-3′.

MLST Gene Amplification

In this study, MG positive samples with Ct values less than 28 were selected for genotyping. The 7 housekeeping genes used for MLST were ugpA, atpG, DUF3196, mraW, plsC, dppC and lgT, the primer sequences are available on the PubMLST website (https://pubmlst.org/static/organisms/mycoplasma-gallisepticum/primers.pdf) (Ghanem and El-Gazzar, 2019). The PCR reaction mixture contained 25 μL Premix Taq (LA Taq Version 2.0 Plus dye), 0.5 μL of each 10 μM primer and 2 μL of genomic DNA template in a total volume of 50 μL. The PCR amplification was performed with an initial denaturation step of 95°C for 3 min, followed by 40 cycles of 95°C for 30 s, 54°C for 30 s, and 72°C for 90 s. The PCR products were then subjected to Sanger sequencing (Sangon Biotech, Shanghai, China).

The DNA sequences of the 7 housekeeping genes were compared and assembled using Lasergene 7.1, and then submitted to the PubMLST database. For each new sequence, an allele ID was assigned. Each MG sample generated an allele profile consisting of 7 loci. Based on the obtained allele patterns, sequence types (**STs**) were determined and compared with information from 119 reference sequences in the PubMLST database. These reference sequences from the United States, Australia, the United Kingdom, Israel, Jordan, Japan, and China. Based on the STs, BURST analysis was applied (Feil et al., 2004) to group strains into clonal complexes with 4 or more matching alleles.

In Vitro Antimicrobial Sensitivity Test

The drug sensitivity of the isolated MG strains was tested using antibiotics including enrofloxacin (**EN**), doxycycline (**DO**), chlortetracycline (**CH**), tiamulin (**TIA**), valnemulin (**VAL**), tylosin (**TY**), tylvalosin (**TYL**), Timicosin (**TIM**), spectinomycin (**SPE**), and lincomycin (**LIN**). The concentration of antibiotic solution was diluted to 128 μg/mL. Then the solutions were sterilized with a 0.22-μm millipore filter membrane. The MIC (minimum inhibitory concentration) test was carried out in 96-well microdilution plates (Zhang et al., 2022). Briefly, the MG culture was diluted in mycoplasma broth medium to 10^4 ccu/mL. The 128 μg/mL diluted antibiotic solution was usually diluted 2-fold continuous gradient with 100 μL Mycoplasma broth medium. After dilution, the 100 μL diluted MG culture was inoculated into each well. MG culture and antibiotics were included in all tests as negative controls and antibiotic controls, respectively. Plates were incubated at 37°C. The lowest concentration of antibiotic to show a color change denoted MIC. The MIC was read when the phenol red indicator in the negative control had just turned orange-yellow.

RESULTS

Epidemiological Information of Clinical Samples

Total of 1,250 samples were collected from Henan, Hebei, Shandong, Jiangsu, Anhui, Zhejiang, Hunan, Hubei, Chongqing, Yunnan, Sichuan, Guizhou, Guangdong, Guangxi and Fujian provinces in China in 2022. Considering that MS and MG co-infection is common in poultry farms (Sid et al., 2015; Giram et al., 2022), we also detected for MS infection. After RT-qPCR detection, 938 samples were positive for MG infection with an average positivity rate of 75.04%, while 570 samples were positive for co-infections of MG and MS with an average positivity rate of 45.60% (Table 1). The positivity rates varied across different regions (Figure 1). The largest number of samples was 345 collected from Jiangsu province, among which 237 were positive for MG infection and 149 were positive for co-infection of MG and MS, with positivity rates of 68.7% and 43.19%, respectively.

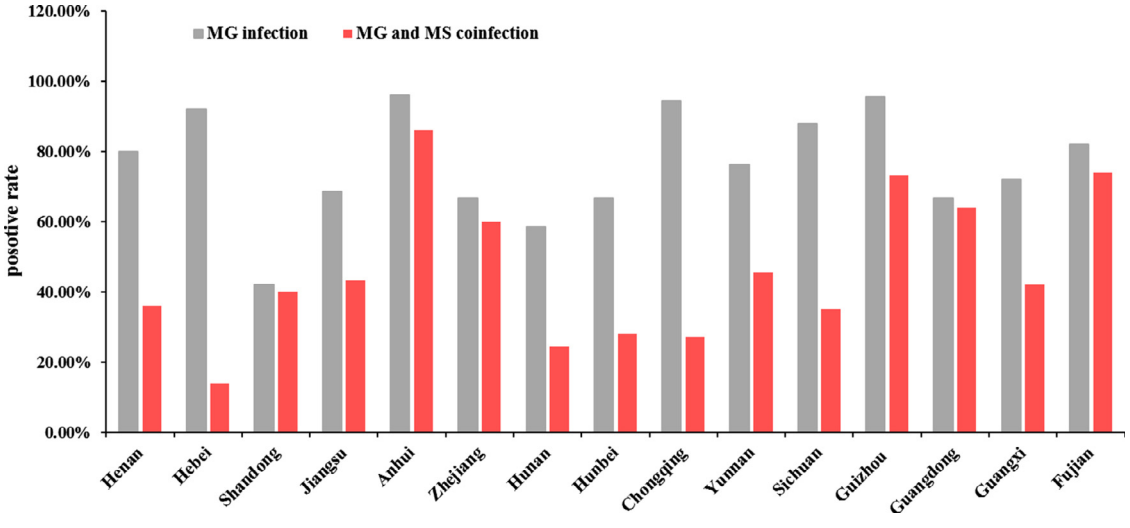

Figure 1. MG and MS infection rate in different regions in China.

Among the 100 samples collected from Anhui province, the positivity rate of MG infection was the highest, reaching 96%, while the positivity rate of MG and MS co-infection was also the highest at 86%; in contrast, among the 50 samples collected from Shandong province, the positivity rate of MG infection was the lowest, about 42%. These data indicate that MG infection is very common and severe in China, especially when combined with MS infection, which should receive more attention. Among the 938 MG-positive samples, 183 samples were selected for sequencing and gene typing. The detail information about sequenced samples were listed in Table 2.

Table 2. Information of 183 sequenced samples.

Number	IDs	Sample	Region	atpG	dppC	DUF3196	lgT	mraW	plsC	ugpA	ST
1	296	Guangxi/2022/HLG	Guangxi	17	13	20	20	19	18	18	36
2	297	Guangxi/2022/LGF-1	Guangxi	17	13	20	20	19	18	18	36
3	298	Guangxi/2022/LGF-2	Guangxi	3	13	23	44	21	9	1	81*
4	299	Guangxi/2022/FHC-1	Guangxi	2	36	23	34	21	18	23	82*
5	300	Guangxi/2022/FHC-2	Guangxi	3	13	29	34	21	28	1	73
6	301	Guangxi/2022/T2-1	Guangxi	3	13	4	44	28	9	1	78
7	302	Guangxi/2022/T2-2	Guangxi	3	13	23	44	21	9	1	81*
8	303	Guangxi/2022/H4-1	Guangxi	17	13	20	20	19	18	18	36
9	304	Guangxi/2022/H4-2	Guangxi	17	13	20	20	19	18	18	36
10	305	Guangxi/2022/H5-1	Guangxi	3	13	4	44	28	9	1	78
11	306	Guangxi/2022/H5-2	Guangxi	3	13	4	44	28	9	1	78
12	307	Guangxi/2022/LH-1	Guangxi	3	13	4	44	28	9	1	78
13	308	Guangxi/2022/LH-2	Guangxi	3	13	4	44	28	9	1	78
14	309	Guangxi/2022/CTX	Guangxi	3	13	29	34	21	28	1	73
15	310	Guangxi/2022/MQL	Guangxi	3	13	29	34	21	28	1	73
16	311	Guangxi/2022/WYP	Guangxi	3	13	23	44	21	9	1	81*
17	312	Guangxi/2022/WMD	Guangxi	17	13	20	20	19	18	18	36
18	313	Guangxi/2022/LZH	Guangxi	17	13	20	20	19	18	18	36
19	314	Guangxi/2022/HXX	Guangxi	3	13	4	44	28	9	1	78
20	315	Guangxi/2022/ZXY	Guangxi	3	13	4	44	28	9	1	78
21	316	Guangxi/2022/LS	Guangxi	17	13	20	20	19	18	18	36
22	317	Guangxi/2022/DRS1	Guangxi	3	13	23	44	21	9	1	81*
23	318	Guangxi/2022/TXL	Guangxi	3	13	23	44	21	9	1	81*
24	319	Guangxi/2022/WKX	Guangxi	3	13	23	44	21	9	1	81*
25	320	Guangxi/2022/OTS	Guangxi	3	13	4	44	28	9	1	78
26	321	Guangxi/2022/DCM	Guangxi	3	13	4	44	28	9	1	78
27	322	Guangxi/2022/LRC	Guangxi	3	13	4	44	28	9	1	78
28	323	Guangxi/2022/YZL	Guangxi	3	13	4	44	28	9	1	78
29	324	Guangxi/2022/LYZ	Guangxi	3	13	4	44	28	9	1	78
30	325	Fujian/2022/ZW	Fujian	17	13	20	20	19	18	18	36
31	326	Fujian/2022/XWF	Fujian	17	13	20	20	19	18	18	36
32	327	Fujian/2022/ZXQ	Fujian	17	13	20	20	19	18	18	36
33	328	Fujian/2022/GQY	Fujian	3	13	4	44	28	9	1	78
34	329	Fujian/2022/XLN	Fujian	3	13	4	44	28	9	1	78
35	330	Fujian/2022/ZXP	Fujian	3	13	4	44	28	9	1	78
36	331	Fujian/2022/HYS2983	Fujian	3	13	23	44	21	9	1	81*
37	332	Fujian/2022/HYS2986	Fujian	3	13	23	44	21	9	1	81*
38	333	Hebei/2022/WZP	Hebei	3	13	4	44	28	9	1	78
39	334	Hebei/2022/WST	Hebei	2	36	23	42	21	18	1	75
40	335	Hebei/2022/LCX	Hebei	3	13	23	44	21	9	1	81*
41	336	Hebei/2022/XFQ	Hebei	3	13	23	44	21	9	1	81*
42	337	Hebei/2022/WHZ	Hebei	3	13	23	44	21	9	1	81*
43	338	Hebei/2022/TT	Hebei	3	13	23	44	21	9	1	81*
44	339	Shandong/2022/HQH	Shandong	17	13	20	20	19	18	18	36
45	340	Zhejiang/2022/WAP	Zhejiang	2	36	23	34	21	18	23	82*
46	341	Zhejiang/2022/ZAQ	Zhejiang	3	13	4	44	28	9	1	78
47	342	Anhui/2022/CJH	Anhui	17	13	20	20	19	18	18	36
48	343	Anhui/2022/LFC	Anhui	17	13	20	20	19	18	18	36
49	344	Anhui/2022/WSY	Anhui	17	13	20	20	19	18	18	36
50	345	Anhui/2022/LJX	Anhui	3	13	23	44	21	9	1	81*
51	346	Anhui/2022/ZCH	Anhui	3	13	29	43	21	28	1	76
52	347	Anhui/2022/LP	Anhui	3	13	29	34	21	28	1	73
53	348	Anhui/2022/LB	Anhui	3	13	29	34	21	28	1	73
54	349	Anhui/2022/WLZ	Anhui	3	13	29	34	21	28	1	73
55	350	Anhui/2022/XWF	Anhui	3	13	29	34	21	28	1	73
56	351	Anhui/2022/ZYS	Anhui	3	13	29	34	21	28	1	73
57	352	Anhui/2022/GGL	Anhui	3	13	29	34	21	28	1	73
58	353	Anhui/2022/ZYF	Anhui	3	13	23	44	21	9	1	81*
59	354	Anhui/2022/CHL	Anhui	3	13	23	44	21	9	1	81*
60	355	Anhui/2022/FGS	Anhui	2	36	23	42	21	18	1	75
61	356	Anhui/2022/WXG	Anhui	2	36	23	42	21	18	1	75
62	357	Anhui/2022/ZHJ	Anhui	3	13	4	44	28	9	1	78
63	358	Anhui/2022/ZZB	Anhui	3	13	4	44	28	9	1	78

(*continued*)

Table 2 (*Continued*)

Number	IDs	Sample	Region	atpG	dppC	DUF3196	lgT	mraW	plsC	ugpA	ST
64	359	Anhui/2022/GHY	Anhui	3	13	29	34	21	28	1	73
65	360	Hunan/2022/DMY	Hunan	2	36	23	44	21	18	23	77
66	361	Hunan/2022/CDC	Hunan	2	36	23	44	21	18	23	77
67	362	Hunan/2022/XSY	Hunan	2	36	23	44	21	18	23	77
68	363	Hunan/2022/LHJ	Hunan	3	13	23	44	21	9	1	81*
69	364	Hunan/2022/LSB	Hunan	17	13	20	20	19	18	18	36
70	365	Hunan/2022/ZGB	Hunan	17	13	20	20	19	18	18	36
71	366	Hunan/2022/LZL	Hunan	3	13	4	44	28	9	1	78
72	367	Hunan/2022/PXN	Hunan	3	13	4	44	28	9	1	78
73	368	Hunan/2022/MMZ	Hunan	2	36	23	42	21	18	1	75
74	369	Jiangsu/2022/TWX	Jiangsu	3	13	23	44	21	9	1	81*
75	370	Jiangsu/2022/TYH	Jiangsu	3	13	4	44	28	9	1	78
76	371	Jiangsu/2022/SJS	Jiangsu	17	13	20	20	19	18	18	36
77	372	Jiangsu/2022/ZJG	Jiangsu	3	13	29	34	21	28	1	73
78	373	Jiangsu/2022/SWX	Jiangsu	3	13	4	44	28	9	1	78
79	374	Jiangsu/2022/ZZB	Jiangsu	3	13	29	34	21	28	1	73
80	375	Jiangsu/2022/LP	Jiangsu	3	13	4	44	28	9	1	78
81	376	Jiangsu/2022/HJZ	Jiangsu	3	13	23	44	21	9	1	81*
82	377	Jiangsu/2022/XFY	Jiangsu	3	13	23	44	21	9	1	81*
83	378	Jiangsu/2022/LFJ	Jiangsu	3	13	23	44	21	9	1	81*
84	379	Jiangsu/2022/ZQX	Jiangsu	3	13	23	44	21	9	1	81*
85	380	Jiangsu/2022/YHC	Jiangsu	2	36	23	42	21	18	1	75
86	381	Jiangsu/2022/ZXXQ-1	Jiangsu	3	13	29	34	21	28	1	73
87	382	Jiangsu/2022/ZXXQ-2	Jiangsu	2	36	23	34	21	18	23	82*
88	383	Jiangsu/2022/ZXXQ-3	Jiangsu	2	36	23	34	21	18	23	82*
89	384	Jiangsu/2022/ZXXQ-4	Jiangsu	3	13	29	43	21	28	1	76
90	385	Jiangsu/2022/ZGC	Jiangsu	2	36	23	42	21	18	1	75
91	386	Jiangsu/2022/LXJ	Jiangsu	3	13	4	20	19	18	18	83*
92	387	Jiangsu/2022/XQG	Jiangsu	3	13	4	44	28	9	1	78
93	388	Jiangsu/2022/XHJ	Jiangsu	17	13	20	20	19	18	18	36
94	389	Jiangsu/2022/JXG	Jiangsu	3	13	23	44	21	9	1	81*
95	390	Jiangsu/2022/SXZ	Jiangsu	3	13	29	34	21	28	1	73
96	391	Jiangsu/2022/MGM	Jiangsu	2	36	23	42	21	18	1	75
97	392	Jiangsu/2022/JH	Jiangsu	2	36	23	42	21	18	1	75
98	393	Jiangsu/2022/CJT	Jiangsu	2	36	23	42	21	18	1	75
99	394	Jiangsu/2022/DCM	Jiangsu	3	13	4	44	28	9	1	78
100	395	Jiangsu/2022/LL	Jiangsu	3	13	4	44	28	9	1	78
101	396	Jiangsu/2022/LSJ	Jiangsu	3	13	4	44	28	9	1	78
102	397	Jiangsu/2022/ZZM	Jiangsu	3	13	23	44	21	9	1	81*
103	398	Jiangsu/2022/XXS	Jiangsu	3	13	29	34	21	28	1	73
104	399	Jiangsu/2022/CL	Jiangsu	17	13	20	20	19	18	18	36
105	400	Jiangsu/2022/XKF	Jiangsu	3	13	29	34	21	28	1	73
106	401	Jiangsu/2022/ZTC	Jiangsu	3	13	29	34	21	28	1	73
107	402	Jiangsu/2022/HZZ	Jiangsu	17	13	20	20	19	18	18	36
108	403	Jiangsu/2022/HXM	Jiangsu	2	36	23	42	21	18	1	75
109	404	Jiangsu/2022/XHZ	Jiangsu	3	13	4	44	28	9	1	78
110	405	Jiangsu/2022/GKQ	Jiangsu	3	13	29	34	21	28	1	73
111	406	Jiangsu/2022/WLB	Jiangsu	2	36	23	42	21	18	1	75
112	407	Jiangsu/2022/LZH	Jiangsu	17	13	20	20	19	18	18	36
113	408	Sichuan/2022/YG	Sichuan	17	13	20	20	19	18	18	36
114	409	Sichuan/2022/LYB	Sichuan	17	13	20	20	19	18	18	36
115	410	Sichuan/2022/WKW	Sichuan	3	13	4	44	28	9	1	78
116	411	Sichuan/2022/CTW	Sichuan	3	13	23	44	21	9	1	81*
117	412	Sichuan/2022/HY	Sichuan	3	13	23	44	21	9	1	81*
118	413	Sichuan/2022/WGYU	Sichuan	2	36	23	34	21	18	23	82*
119	414	Sichuan/2022/YXH	Sichuan	17	13	20	20	19	18	18	36
120	415	Sichuan/2022/LYM	Sichuan	3	13	29	43	21	28	1	76
121	416	Sichuan/2022/WGYN	Sichuan	3	13	23	44	21	9	1	81*
122	417	Sichuan/2022/WXG	Sichuan	3	13	23	44	21	9	1	81*
123	418	Sichuan/2022/ZXY	Sichuan	3	13	4	44	28	9	1	78
124	419	Sichuan/2022/LJ	Sichuan	3	13	4	44	28	9	1	78
125	420	Sichuan/2022/ZYH	Sichuan	3	13	4	44	28	9	1	78
126	421	Sichuan/2022/HYH	Sichuan	3	13	4	44	28	9	1	78
127	422	Sichuan/2022/LYL	Sichuan	3	13	4	44	28	9	1	78
128	423	Sichuan/2022/ZGQ	Sichuan	3	13	4	44	28	9	1	78
129	424	Yunnan/2022/OLN	Yunnan	17	13	20	20	19	18	18	36
130	425	Yunnan/2022/HZX	Yunnan	2	36	23	42	21	18	1	75
131	426	Yunnan/2022/DHQ	Yunnan	2	36	23	42	21	18	1	75
132	427	Yunnan/2022/WHC	Yunnan	3	13	29	43	21	28	1	76
133	428	Yunnan/2022/PJD	Yunnan	3	13	29	43	21	28	1	76
134	429	Yunnan/2022/JWD	Yunnan	3	13	4	44	28	9	1	78
135	430	Yunnan/2022/BQY	Yunnan	3	13	4	44	28	9	1	78
136	431	Yunnan/2022/GZY	Yunnan	2	36	23	34	21	18	23	82*
137	432	Guangdong/2022/Q3	Guangdong	3	13	23	44	21	9	1	81*
138	433	Guangdong/2022/3-3	Guangdong	3	13	23	44	21	9	1	81*

(*continued*)

Table 2 (*Continued*)

Number	IDs	Sample	Region	atpG	dppC	DUF3196	lgT	mraW	plsC	ugpA	ST
139	434	Guangdong/2022/CG6-1	Guangdong	3	13	4	44	28	9	1	78
140	435	Guangdong/2022/QYF	Guangdong	3	13	4	44	28	9	1	78
141	436	Guangdong/2022/YL	Guangdong	3	13	4	44	28	9	1	78
142	437	Guangdong/2022/SWX	Guangdong	2	36	23	42	21	18	1	75
143	438	Guangdong/2022/OXZ	Guangdong	3	13	29	34	21	28	1	73
144	439	Guangdong/2022/HRQ	Guangdong	3	36	20	21	21	28	10	84*
145	440	Guangdong/2022/CG5-5	Guangdong	3	36	20	21	21	28	10	84*
146	441	Guangdong/2022/LRD5	Guangdong	3	13	23	44	21	21	1	85*
147	442	Guangdong/2022/LAH	Guangdong	3	13	23	44	21	21	1	85*
148	443	Guangdong/2022/XML	Guangdong	3	13	29	34	21	28	1	73
149	444	Guangdong/2022/XYX	Guangdong	3	13	23	44	21	9	1	81*
150	445	Guangdong/2022/ZXK	Guangdong	3	13	4	44	28	9	1	78
151	446	Guangdong/2022/ZX	Guangdong	17	13	20	20	19	18	18	36
152	447	Guizhou/2022/LM	Guizhou	3	36	20	21	21	28	10	84*
153	448	Guizhou/2022/WPS	Guizhou	3	36	20	21	21	28	10	84*
154	449	Guizhou/2022/DDY	Guizhou	3	36	20	21	21	28	10	84*
155	450	Guizhou/2022/ZWX	Guizhou	3	13	4	44	28	9	1	78
156	451	Guizhou/2022/ZWH	Guizhou	3	13	4	44	28	9	1	78
157	452	Guizhou/2022/WSJ	Guizhou	3	13	4	44	28	9	1	78
158	453	Guizhou/2022/ZQH	Guizhou	17	13	20	20	19	18	18	36
159	454	Guizhou/2022/QCS	Guizhou	3	13	29	43	21	28	1	76
160	455	Guizhou/2022/YY	Guizhou	2	36	23	42	21	18	1	75
161	456	Henan/2022/ZKK	Henan	3	13	23	44	21	21	1	85*
162	457	Henan/2022/ZZZ	Henan	17	13	20	20	19	18	18	36
163	458	Henan/2022/WYZ	Henan	17	13	20	20	19	18	18	36
164	459	Henan/2022/GAX	Henan	3	36	20	21	21	28	10	84*
165	460	Hubei/2022/MYM	Hubei	2	36	23	42	21	18	1	75
166	461	Hubei/2022/HXP	Hubei	3	13	4	44	28	9	1	78
167	462	Hubei/2022/WL	Hubei	2	36	23	34	21	18	23	82*
168	463	Hubei/2022/LYQ	Hubei	3	13	4	44	28	9	1	78
169	464	Hubei/2022/QJH	Hubei	3	13	23	44	21	21	1	85*
170	465	Hubei/2022/PAY	Hubei	3	13	29	34	21	28	1	73
171	466	Hubei/2022/GWB	Hubei	3	13	4	44	28	9	1	78
172	467	Hubei/2022/LBX	Hubei	3	36	20	21	21	28	10	84*
173	468	Hubei/2022/WF	Hubei	3	13	23	44	21	21	1	85*
174	469	Hubei/2022/WSC	Hubei	3	13	23	44	21	9	1	78
175	470	Chogqing/2022/ZDQ	Chogqing	17	13	20	20	19	18	18	36
176	471	Chogqing/2022/LZF	Chogqing	17	13	20	20	19	18	18	36
177	472	Chogqing/2022/ZZQ	Chogqing	17	13	20	20	19	18	18	36
178	473	Chogqing/2022/DRC	Chogqing	17	13	20	20	19	18	18	36
179	474	Chogqing/2022/LSW	Chogqing	2	36	23	42	21	18	1	75
180	475	Chogqing/2022/ZGQ	Chogqing	3	13	4	44	28	9	1	78
181	476	Chogqing/2022/WYX	Chogqing	3	36	20	21	21	28	10	84*
182	477	Chogqing/2022/HDG	Chogqing	3	13	23	44	21	21	1	85*
183	478	Chogqing/2022/LHW	Chogqing	2	36	23	34	21	18	23	82*

*Represents new STs.

Multilocus Sequence Typing Genotyping

After submitting the sample sequences to the PubMLST database to comparison, it was found that 130 sample sequences belonged to ST-36, ST-73, ST-75, ST-76, ST-77, and ST-78, while 53 sequences did not match any ST in the database, indicating that these 53 sequences belonged to new STs. After re-annotation by the database administrators, these 53 sequences were divided into ST-81, ST-82, ST-83, ST-84, and ST-85. Among all these genotypes, ST-78 had the highest proportion of 27.32% (50/183), followed by ST-36 with 18.03% (33/183), and then ST-81 with 16.39% (30/183). The detailed information was listed in Table 3.

Allelic Variations of Genotypes

As shown in Table 4, there were 3 allelic variants of the atpG gene, among which atpG-3 was the most common, accounting for 66.67% (122/183). Two allelic variants of the dppC gene, among which dppC-13 was the most common, accounting for 80.33% (147/183). Four allelic variants of the DUF3196 gene, among which DUF3196-23 was the most common, accounting for 35.52% (65/183). Six allelic variants of the lgT gene, among which lgT-44 was the most common, accounting

Table 3. The number of STs for 183 samples in our study.

ST	Number	Percentage
ST-36	33	18.03%
ST-73	21	11.48%
ST-75	17	9.29%
ST-76	6	3.28%
ST-77	3	1.64%
ST-78	50	27.32%
ST-81*	30	16.39%
ST-82*	8	4.37%
ST-83*	1	0.55%
ST-84*	8	4.37%
ST-85*	6	3.28%

*Represent new STs.

Table 4. The alleles of 7 loci and the distribution of these alleles.

Gene	Allele	Frequency	Percentage	Number of alleles
atpG	2	28	15.30%	3
	3	122	66.67%	
	17	33	18.03%	
dppC	13	147	80.33%	2
	36	36	19.67%	
DUF3196	4	50	27.32%	4
	20	41	22.40%	
	23	65	35.52%	
	29	27	14.75%	
lgT	20	34	18.58%	6
	21	8	4.37%	
	34	29	15.85%	
	42	17	9.29%	
	43	6	3.28%	
	44	89	48.63%	
mraW	19	34	18.58%	3
	21	100	54.64%	
	28	49	26.78%	
plsC	9	80	43.72%	4
	18	62	33.88%	
	21	6	3.28%	
	28	35	19.13%	
ugpA	1	130	71.04%	4
	10	8	4.37%	
	18	34	18.58%	
	23	11	6.01%	

for 48.63% (89/183). Three allelic variants of the mraW gene, among which mraW-21 was the most common, accounting for 54.64% (100/183). Four allelic variants of the plsC gene, among which plsC-9 was the most common, accounting for 43.72% (80/183). Four allelic variants of the ugpA gene, among which ugpA-1 was the most common, accounting for 71.04% (130/183). No new allelic variant sequences were found in our samples.

BURST Analysis

BURST analysis was used for genetic clustering analysis by setting allelic profiles matching at 4 or more loci to any other member of the group. A total of 302 sequences were analyzed, including 183 sequences from this study and 119 reference sequences from domestic and foreign sources obtained through the database. These 302 sequences belonged to 78 STs and were divided into 6 groups and one singleton group (Supplemental Table 1). The number of STs in different groups varied greatly (Supplemental Table 2). Group 3 was the largest group, containing 202 sequences and 21 STs, accounting for 66.89% (202/302) of the total sequences. Group 1 was the second-largest group, containing 39 sequences and 17 STs, accounting for 12.91% (39/302) of the total sequences. The singleton group contained 17 sequences and 15 STs, accounting for 5.63% (17/302) of the total sequences. As shown in Supplemental Table 2, all 183 sequences from this study belonged to Group 3.

Furthermore, a phylogenetic tree was generated using the concatenated nucleotide sequences of 7 housekeeping genes, and neighbor-joining method was used for clustering analysis of the 302 sequences, among which 196 sequences were originated from China (Figure 2), the 183 sequences from our study were marked with red stars.

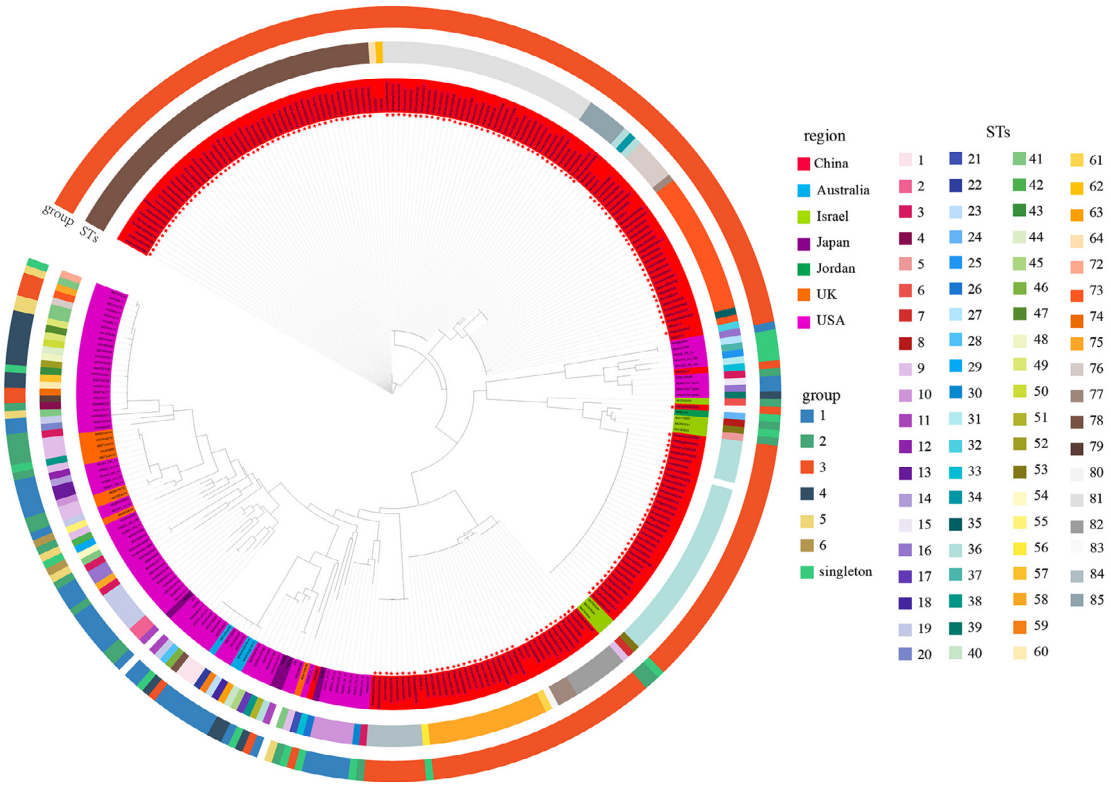

Figure 2. MLST neighbor joining tree of 302 MG samples. The rings (starting from the innermost) contain information on isolate identifiers, the red stars mean the samples collected in this study, the different background colors of isolates represent different groups. The outer rings from inside to outside are ST type and group.

Table 5. MICs of MG strains.

Strains	MICs (μg/mL)									
	EN	DO	CH	TIA	VAL	TY	TYL	TIM	SPE	LIN
AH-LJX	4	1	32	0.015625	0.015625	1	0.25	8	2	128
CQ-LHW	4	0.25	16	0.03125	0.015625	2	0.5	32	4	32
SC-ZGQ	4	0.125	8	0.03125	0.015625	4	4	128	4	32
JS-TYH	2	0.25	16	0.03125	0.0625	2	2	32	2	16
GD-Q3	4	0.0625	16	0.03125	0.015625	1	1	8	0.5	16
GD-3-3	4	0.5	32	0.03125	0.015625	32	1	128	2	128
GZ-LM	4	1	16	0.03125	0.03125	4	2	32	2	32
HB-TT	1	0.0625	16	0.015625	0.015625	2	1	32	0.25	16
GX-DRS	2	0.25	16	0.0625	0.015625	4	2	16	2	16
FJ-XLN	4	0.125	16	0.03125	0.015625	4	1	32	2	16

Abbreviations: CH, chlortetracycline; DO, doxycycline; EN, enrofloxacin; LIN, lincomycin; SPE, spectinomycin; TIA, tiamulin; TIM, Timicosin; TY, tylosin; TYL, tylvalosin; VAL, valnemulin.

From this phylogenetic tree, it can be seen that 196 sequences from China were more similar to each other but divided into 4 clades, 39 sequences from China were relatively close to most foreign samples from the United States, the United Kingdom, Japan, and Australia, and 33 sequences from China, 7 from Israel, and 1 from Jordan clustered together and were related to F strain.

Minimal Inhibitory Concentration

Ten isolated MG strains were randomly selected for drug susceptibility determination. The results are shown in Table 5. All isolates had low MIC values for pleuromutilin, the MICs for tiamulin ranging from 0.015625 to 0.0625 μg/mL and for valnemulin ranging from 0.015625 to 0.03125 μg/mL. The MG isolates displayed variance in MICs for tetracyclines, MIC values for doxycycline ranging from 0.0625 to 1μg/mL, while for chlortetracycline ranging from 8 to 32 μg/mL. Besides, The MG isolates also displayed variant MIC values for macrolides, MICs for tylosin and tylvalosin was similar, 9/10 MG strains for tylosin and 8/10 srtains for tylvalosin ranged from 1 to 4 μg/mL, while the lowest MICs for tilmicosin was 8 μg/mL. The MICs for enrofloxacin ranging from 1 to 4 μg/mL, for spectinomycin ranging from 0.25 to 4 μg/mL. High MIC value was detected in all MG strains for lincomycin ranging from 16 to 128 μg/mL.

DISCUSSION

MG and MS are the most important Mycoplasma species in global poultry industry, causing significant economic losses (Felice et al., 2020). This study collected 1,250 samples from 15 provinces in China in 2022 to detect the infection rate of MG and MS. Among the 1,250 samples, 938 were positive for MG, with an average positivity rate of 75.04%, and 570 were positive for both MG and MS, with an average positivity rate of 45.60% (Table 1). The situation of MG and MS infection in China is not optimistic and should be given more attention.

MG has become one of the most important pathogens threatening the global poultry industry, causing respiratory and reproductive disorders. Efficient monitoring and epidemiological investigations are crucial. This study used MLST method developed by Ghanem and El-Gazzar (2019) to genotype 183 MG samples. MLST is a validated molecular typing method that has been successfully applied to many bacterial species, including Mycoplasma spp., such as *Mycoplasma bovis* (Menghwar et al., 2017), *Mycoplasma iowae* (Ghanem and El-Gazzar, 2016), *M. gallisepticum* (Ghanem and El-Gazzar, 2019), *Mycoplasma hyopneumoniae* (Zhang et al., 2021), *Mycoplasma agalactiae* (McAuliffe et al., 2011), and *Mycoplasma hyorhinis* (Foldi et al., 2020). We found 5 new STs relative to the database. Among the 183 samples collected in 2022, ST-78 had the highest proportion, followed by ST-36 and ST-81. This study selected 7 housekeeping genes atpG, dppC, DUF3196, lgT, mraW, plsC, and ugpA to classify these 183 samples. All 7 genes had at least 2 alleles, with the highest genetic variability detected in the lgT gene, which had 6 alleles. No new allele sequences were found. By analyzing STs and alleles, we found that the genotype of MG is very diverse in China.

The MLST database (PubMLST) can be accessed for free on the internet and contains reference data provided by other researchers for molecular epidemiology and molecular evolutionary analysis. After BURST analysis, 302 sequences belonging to 78 STs were identified, divided into 6 groups and a singleton group (Supplemental Table 1), of which 196 Chinese sequences belonged to Group 3. Phylogenetic tree analysis revealed that some sequences from China were closely related to foreign sequences, and some sequences had high homology with vaccine strain F. We speculate that the diversity of Chinese MG genotypes is related to international trade and the use of live vaccines.

By measuring the drug resistance of different strains, it is possible to effectively guide the use of medication for treatment. After test, all strains are sensitive to pleuromutilin; there are differences in resistance to fluoroquinolones, tetracyclines, and macrolides drugs. All strains have developed resistance to tiamulin, valnemulin, and doxycycline. This results here are consistent with other research conclusions. However, the representativeness of the 10 strains is relatively poor, and more drug sensitivity tests are needed.

Taken together, this study detected the prevalence of MG infection in 1,250 samples collected from China in 2022, and genotyped 183 MG samples using MLST method based on 7 housekeeping genes, and analyzed the genetic evolutionary relationship between these 183 samples and 119 reference sequences, the genotype of MG in China is diverse. All MG isolated strains are sensitive to pleuromutilin. Through the analysis of epidemiological and molecular epidemiological data continuously, as well as the understanding of the pathogen transmission routes, the use of vaccines and antibiotics, will help to improve the control and eradication plan of MG in China.

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Author Contributions: WXN, ZQ and CF conceived and designed the experiments, carried out the experiments, analyzed the data, and wrote the manuscript. ZQ and LHZ participated in performing experiments. ZQY participated in revising the manuscript. WDA and YZQ collected the tissue samples. All the authors have read and approved the final version of the manuscript.

DISCLOSURES

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.psj.2023.102930.

REFERENCES

Beko, K., Z. Kreizinger, K. M. Sulyok, A. B. Kovacs, D. Grozner, S. Catania, J. Bradbury, I. Lysnyansky, O. M. Olaogun, B. Czanik, H. Ellakany, and M. Gyuranecz. 2019. Genotyping *Mycoplasma gallisepticum* by multilocus sequence typing. Vet. Microbiol. 231:191–196.

Bradbury, J. M., and L. J. Howell. 1974. Delayed growth and atypical colony formation of *Mycoplasma synoviae*. Res. Vet. Sci. 17:408–410.

Charlton, B. R., A. A. Bickford, R. L. Walker, and R. Yamamoto. 1999. Complementary randomly amplified polymorphic DNA (RAPD) analysis patterns and primer sets to differentiate *Mycoplasma gallisepticum* strains. J. Vet. Diagn. Invest. 11:158–161.

Delaney, N. F., S. Balenger, C. Bonneaud, C. J. Marx, G. E. Hill, N. Ferguson-Noel, P. Tsai, A. Rodrigo, and S. V. Edwards. 2012. Ultrafast evolution and loss of CRISPRs following a host shift in a novel wildlife pathogen, *Mycoplasma gallisepticum*. PLoS Genet. 8:e1002511.

Feberwee, A., S. de Wit, and R. Dijkman. 2022. Clinical expression, epidemiology, and monitoring of *Mycoplasma gallisepticum* and *Mycoplasma synoviae*: an update. Avian Pathol. 51:2–18.

Feil, E. J., B. C. Li, D. M. Aanensen, W. P. Hanage, and B. G. Spratt. 2004. eBURST: inferring patterns of evolutionary descent among clusters of related bacterial genotypes from multilocus sequence typing data. J. Bacteriol. 186:1518–1530.

Felice, V., C. Lupini, G. Mescolini, F. Silveira, A. Guerrini, E. Catelli, and A. Di Francesco. 2020. Molecular detection and characterization of *Mycoplasma gallisepticum* and *Mycoplasma synoviae* strains in backyard poultry in Italy. Poult. Sci. 99:719–724.

Foldi, D., K. Beko, O. Felde, Z. Kreizinger, A. B. Kovacs, F. Toth, K. Banyai, K. Kiss, I. Biksi, and M. Gyuranecz. 2020. Genotyping *Mycoplasma hyorhinis* by multi-locus sequence typing and multiple-locus variable-number tandem-repeat analysis. Vet. Microbiol. 249:108836.

Ghanem, M., and M. El-Gazzar. 2016. Development of multilocus sequence typing (MLST) assay for *Mycoplasma iowae*. Vet. Microbiol. 195:2–8.

Ghanem, M., and M. El-Gazzar. 2019. Development of a multilocus sequence typing assay for *Mycoplasma gallisepticum*. Avian Dis. 63(4):693–702.

Ghanem, M., L. Wang, Y. Zhang, S. Edwards, A. Lu, D. Ley, and M. El-Gazzar. 2018. Core genome multilocus sequence typing: A standardized approach for molecular typing of mycoplasma gallisepticum. J. Clin. Microbiol. 56:e01145-17.

Giram, P., P. Bhutada, C. Prajapati, S. S. Koratkar, S. Patil, D. Hooda, V. Rale, and S. S. Tongaonkar. 2022. Percent positivity and phylogenetic analysis of *Mycoplasma gallisepticum* and *Mycoplasma synoviae* in commercial poultry from the different states of India. Vet. World 15:1843–1851.

Jiang, H. X., J. R. Chen, H. L. Yan, X. N. Li, Z. L. Chen, and Z. L. Zeng. 2009. Molecular variability of DR-1 and DR-2 within the pvpA gene in *Mycoplasma gallisepticum* isolates. Avian Dis. 53:124–128.

Jolley, K. A., J. E. Bray, and M. C. J. Maiden. 2018. Open-access bacterial population genomics: BIGSdb software, the PubMLST.org website and their applications. Wellcome Open Res. 3:124.

Kleven, S. H. 2008. Control of avian mycoplasma infections in commercial poultry. Avian Dis. 52:367–374.

Kwong, J. C., K. Mercoulia, T. Tomita, M. Easton, H. Y. Li, D. M. Bulach, T. P. Stinear, T. Seemann, and B. P. Howden. 2016. Prospective whole-genome sequencing enhances national surveillance of *Listeria monocytogenes*. J. Clin. Microbiol. 54:333–342.

Larsen, M. V., S. Cosentino, S. Rasmussen, C. Friis, H. Hasman, R. L. Marvig, L. Jelsbak, T. Sicheritz-Ponten, D. W. Ussery, F. M. Aarestrup, and O. Lund. 2012. Multilocus sequence typing of total-genome-sequenced bacteria. J. Clin. Microbiol. 50:1355–1361.

Limsatanun, A., S. Pakpinyo, K. Limpavithayakul, and T. Prasertsee. 2022. Targeted sequencing analysis of *Mycoplasma gallisepticum* isolates in chicken layer and breeder flocks in Thailand. Sci. Rep. 12:9900.

Malik, Y. S., A. Arun Prince Milton, S. Ghatak, and S. Ghosh. 2021. Avian mycoplasmosis. Pages 171−181 in Role of Birds in Transmitting Zoonotic Pathogens. Springer Singapore, Singapore.

Marois, C., F. Dufour-Gesbert, and I. Kempf. 2001. Molecular differentiation of *Mycoplasma gallisepticum* and *Mycoplasma imitans* strains by pulsed-field gel electrophoresis and random amplified polymorphic DNA. J. Vet. Med. B Infect. Dis. Vet. Public Health 48:695–703.

Matucci, A., E. Stefani, M. Gastaldelli, I. Rossi, G. De Grandi, M. Gyuranecz, and S. Catania. 2020. Molecular differentiation of *Mycoplasma gallisepticum* outbreaks: a last decade study on Italian farms using GTS and MLST. Vaccines (Basel) 8:665.

McAuliffe, L., F. Gosney, M. Hlusek, M. L. de Garnica, J. Spergser, M. Kargl, R. Rosengarten, R. D. Ayling, R. A. J. Nicholas, and R. J. Ellis. 2011. Multilocus sequence typing of *Mycoplasma agalactiae*. J. Med. Microbiol. 60(Pt 6):803–811.

Menghwar, H., C. He, H. Zhang, G. Zhao, X. Zhu, F. A. Khan, M. Faisal, M. A. Rasheed, M. Zubair, A. M. Memon, A. Ridley, I. D. Robertson, Y. Chen, and A. Guo. 2017. Genotype distribution of Chinese *Mycoplasma bovis* isolates and their evolutionary relationship to strains from other countries. Microb. Pathog. 111:108–117.

Michiels, T., S. Welby, M. Vanrobaeys, C. Quinet, L. Rouffaer, L. Lens, A. Martel, and P. Butaye. 2016. Prevalence of *Mycoplasma gallisepticum* and *Mycoplasma synoviae* in commercial

poultry, racing pigeons and wild birds in Belgium. Avian Pathol. 45:244–252.
Norouzian, H., G. Farjanikish, and H. Hosseini. 2019. Molecular characterisation of *Mycoplasma gallisepticum* isolates from Iran in the period 2012-2017. Acta Vet. Hung. 67:347–359.
Raviv, Z., and S. H. Kleven. 2009. The development of diagnostic real-time TaqMan PCRs for the four pathogenic avian mycoplasmas. Avian Dis. 53:103–107.
Sawicka, A., M. Durkalec, G. Tomczyk, and O. Kursa. 2020. Occurrence of *Mycoplasma gallisepticum* in wild birds: a systematic review and meta-analysis. PLoS One 15:e0231545.
Sid, H., K. Benachour, and S. Rautenschlein. 2015. Co-infection with multiple respiratory pathogens contributes to increased mortality rates in Algerian poultry flocks. Avian Dis. 59:440–446.
Sprygin, A. V., D. B. Andreychuk, N. P. Elatkin, N. G. Zinyakov, S. N. Kolosov, N. S. Mudrak, V. N. Irza, V. V. Drygin, A. V. Borisov, and N. A. Perevozchikova. 2010. Genetic diversity of *Mycoplasma gallisepticum* field isolates using partial sequencing of the pvpA gene fragment in Russia. Avian Dis. 54:899–904.
Staley, M., C. Bonneaud, K. J. McGraw, C. M. Vleck, and G. E. Hill. 2018. Detection of *Mycoplasma gallisepticum* in house finches (Haemorhous mexicanus) from Arizona. Avian Dis. 62:14–17.
Yadav, J. P., P. Tomar, Y. Singh, and S. K. Khurana. 2021. Insights on *Mycoplasma gallisepticum* and *Mycoplasma synoviae* infection in poultry: a systematic review. Anim. Biotechnol. 33:1–10.
Zhang, H., Y. Wang, L. Gao, Y. Wang, and R. Wei. 2021. Genotype diversity of *Mycoplasma hyopneumoniae* in Chinese swine herds based on multilocus sequence typing. BMC Vet. Res. 17:347.
Zhang, X., M. Guo, D. Xie, Y. Chen, C. Zhang, Y. Cao, and Y. Wu. 2022. Antibiotic resistance of *Mycoplasma synoviae* strains isolated in China from 2016 to 2019. BMC Vet. Res. 18:1.