The prevalence, molecular characterization, and antimicrobial resistance profiling of Streptococcus agalactiae isolated from clinical mastitis cases on large dairy farms in China

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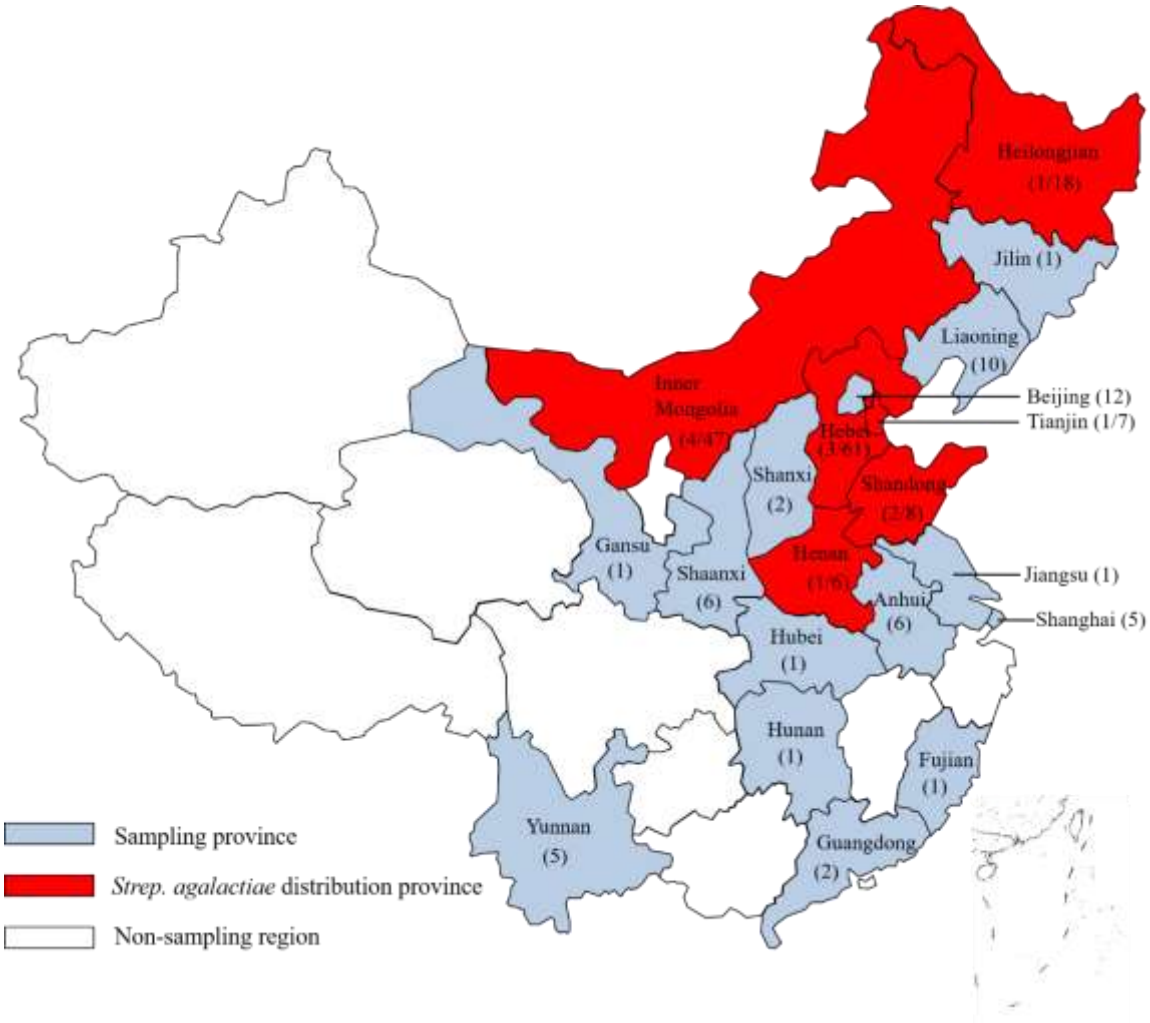
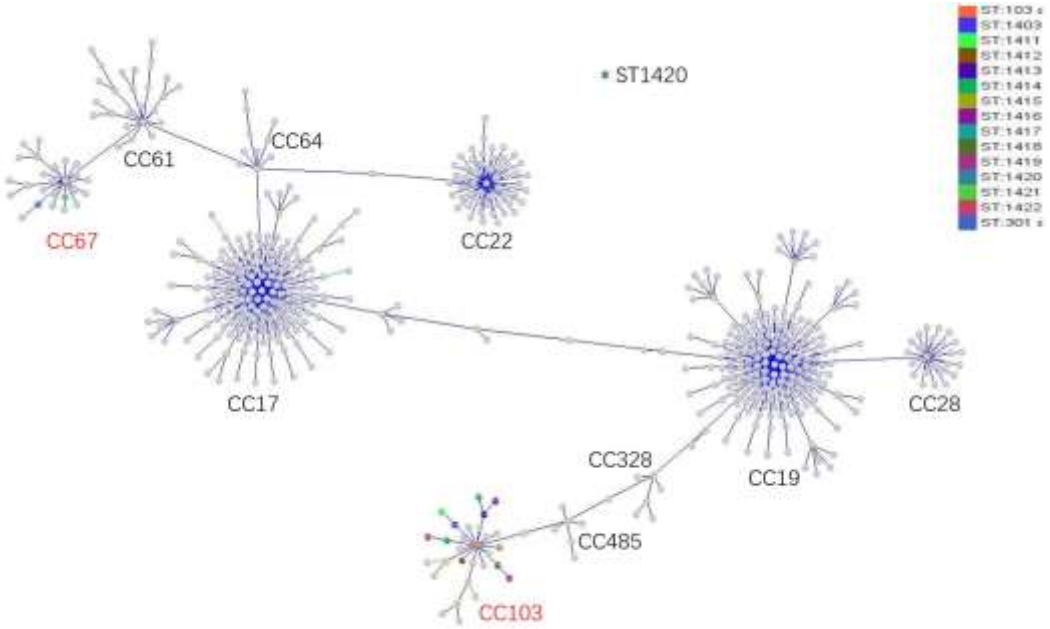
SUPPLEMENTARY FILE



Supplementary Figure 1. Geographic distribution of the 201 dairy herds and *Strep. agalactiae* isolated herds in each province of China.



Supplementary Figure 2: goeBURST dendrogram of the 140 *Strep. agalactiae* isolates

Supplementary Table 1. Distribution and prevalence of 140 *Streptococcus aglactiae* isolated from clinical mastitis in 201 large Chinese dairy herds

Province	Herds		Samples		*Streptococcus* spp.		*Streptococcus aglactiae*	
	No.	%	No.[1]	%	Isol	%	Isol	%
Anhui	6	3.0	159	3.9	37	23.3	0	0.0
Beijing	12	6.0	243	6.0	17	7.0	0	0.0
Fujian	1	0.5	12	0.3	0	0.0	0	0.0
Gansu	1	0.5	30	0.7	0	0.0	0	0.0
Guangdong	2	1.0	98	2.4	12	12.2	0	0.0
Hebei	61	30.3	867	21.5	129	14.9	26	3
Henan	6	3.0	86	2.1	6	7.0	1	1.2
Heilongjiang	18	9.0	434	10.7	45	10.4	7	1.6
Hubei	1	0.5	55	1.4	2	3.6	0	0.0
Hunan	1	0.5	21	0.5	3	14.3	0	0.0
Jilin	1	0.5	8	0.2	2	25.0	0	0.0
Jiangsu	1	0.5	9	0.2	3	33.3	0	0.0
Liaoning	10	5.0	156	3.9	19	12.2	0	0.0
Inner Mongolia	47	23.4	1334	33.0	232	17.4	19	1.4
Shandong	8	4.0	426	3.1	93	21.8	83	19.5
Shanxi	2	1.0	26	0.6	1	3.8	0	0.0
Shaanxi	6	3.0	68	1.7	3	4.4	0	0.0
Shanghai	5	2.5	108	2.7	24	22.2	0	0.0
Tianjin	7	3.5	92	2.3	21	22.8	4	4.3
Yunnan	5	2.5	109	2.7	36	33.0	0	0.0
Total	201	100	4341	100	685	15.8	140	3.2

[1]Number of clinical mastitis isolates

Supplementary Table 2. Distribution and isolated time of 140 *Streptococcus aglactiae*

Province	Herd	Date of sampling	Samples	Number of isolates	Prevalence (%)	95% CI[1] (%)
Shandong	A	2017.04.26	50	29	58	44.2_70.6
		2019.05.12	67	11	16.4	9.4_27.1
		2019.08	98	42	42.9	33.5_52.7
	J	2018.12.02	12	1	8.3	1.5_35.4
Hebei	C	2018.12.27	27	3	11.1	3.9_28.1
	K	2019.08.02	121	21	17.4	11.6_25.1
	F	2018.05.03	43	2	4.7	1.3_15.5
	E	2018.12.26	65	2	3.1	0.9_10.6
Inner Mongolia	G	2018.03.07	83	10	12.1	6.7_20.8
	H	2018.04.19	25	4	16	6.4_34.7
	I	2018.05.03	39	3	7.7	2.7_20.3
Heilongjiang	S	2018.10.29	58	7	12.1	6_22.9
Tianjin	D	2018.10.28	28	4	14.3	5.7_31.5
Henan	L	2019.08	36	1	2.8	0.5_14.2
Total	12	-	752	140	18.6	16_21.6

[1] 95% confidence interval

Supplementary Table 3. Information of colonal complex (CC) and sequence type (ST) of the 140 *Strep. agalactiae* isolates

CC	ST	Iso.	%	Overall %(95 %CI)	Allale						
					adhP	*pheS*	*atr*	*glnA*	*sdhA*	*glcK*	*tkt*
103	103	50	35.71	97.86 (93.89_99.27)	16	1	6	2	9	9	2
	1403	1	0.71		16	1	6	2	7	9	2
	1411	6	4.29		16	7	6	2	7	9	2
	1412	1	0.71		16	16	6	2	9	9	2
	1413	37	26.43		16	9	6	2	9	9	2
	1414	34	24.29		16	70	6	2	9	9	2
	1415	3	2.14		16	37	6	2	9	9	2
	1416	1	0.71		16	9	6	98	9	9	2
	1417	1	0.71		16	9	67	2	9	9	2
	1418	1	0.71		16	21	6	2	9	9	2
	1419	1	0.71		97	21	6	2	9	9	2
	1422	1	0.71		16	70	53	2	9	9	2
67	301	1	0.71	1.43 (0.39_5.06)	13	1	1	13	1	28	5
	1421	1	0.71		13	70	1	13	1	1	5
	1420	1	0.71	0.71 (0.12_3.93)	13	9	173	13	1	1	5

Supplementary Table 4. MIC of each antimicrobial against isolates collected in Farm A changes from 2017 to 2019. Minimum concentration of an antimicrobial that inhibited the visible growth of 50% (MIC_{50}) and 90% of microorganisms (MIC_{90}).

Antimicrobial	Year	NO.	Isolates at each indicated MIC (μg/mL)														*P*-value[1]	MIC50	MIC90
			<0.015	0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	>32			
Penicillin	2017	29	-	-	-	1	21	7	-	-	-	-	-	-	-	-	*<0.001*	0.125	0.125
	2019	53	1	3	17	19	11	2	-	-	-	-	-	-	-	-		0.03	0.06
Cefalexin	2017	29	-	-	-	-	-	-	-	-	5	24	-	-	-	-	*0.026*	4	4
	2019	53	-	-	-	-	-	-	2	1	19	30	1	-	-	-		4	4
Ceftiofor	2017	29	1	2	26	-	-	-	-	-	-	-	-	-	-	-	*0.009*	0.03	0.03
	2019	53	1	19	30	1	-	2	-	-	-	-	-	-	-	-		0.03	0.03
Cefquinome	2017	29	-	-	7	22	-	-	-	-	-	-	-	-	-	-	*<0.001*	0.06	0.06
	2019	53	-	3	39	11	-	-	-	-	-	-	-	-	-	-		0.03	0.06
Clindamycin	2017	29	-	-	5	22	-	-	-	-	-	-	-	-	2	-	*<0.001*	0.06	0.06
	2019	53	-	39	4	8	1	-	-	1	-	-	-	-	-	-		0.015	0.06
Tetracycline	2017	29	-	-	-	-	-	-	-	-	-	-	-	15	14	-	0.924	16	32
	2019	53	-	-	-	-	-	-	-	-	-	-	2	26	25	-		16	32
Enrofloxacin	2017	29	-	-	-	-	-	1	26	2	-	-	-	-	-	-	*<0.001*	0.5	0.5

	2019	53	-	-	-	-	-	22	30	1	-	-	-	-	-	-		0.5	0.5
Amoxi/clav[3]	2017	29	-	-	-	-	-	-	29	-	-	-	-	-	-	-	*0.006*	0.5	0.5
	2019	53	-	-	-	-	-	16	35	2	-	-	-	-	-	-		0.5	0.5
Daptomycin	2017	29	-	-	-	-	-	-	-	1	2	-	22	4	-	-	*<0.001*	8	16
	2019	53	-	-	-	-	-	-	-	-	-	30	21	2	-	-		4	8

1. Italic font represents significant difference ($P < 0.05$).

Supplementary Table 5. Difference in MIC of antimicrobials against isolates from major farms

Antimicrobials	Farms[1]	NO.	Isolates at each indicated MIC (μg/mL)														Overall *P*-value[2]
			<0.015	0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	>32	
Penicillin	A[a]	82	1	3	17	20	32	9	-	-	-	-	-	-	-	-	
	K[b]	21	-	-	5	15	-	-	1	-	-	-	-	-	-	-	*<0.001*
	G[c]	10	-	-	-	-	3	7	-	-	-	3	-	-	-	-	
Cefalexin	A[a]	82	-	-	-	-	-	-	2	1	24	54	1	-	-	-	
	K[b]	21	-	-	-	-	-	-	-	-	19	2	-	-	-	-	*<0.001*
	G[a]	10	-	-	-	-	-	-	-	-	4	6	-	-	-	-	
Ceftiofor	A[a]	82	2	21	56	1	-	2	-	-	-	-	-	-	-	-	
	K[b]	21	-	12	8	-	-	-	-	-	1	-	-	-	-	-	*0.042*
	G	10	-	4	6	-	-	-	-	-	-	-	-	-	-	-	
Tetracycline	A[a]	82	-	-	-	-	-	-	-	-	-	-	2	41	39	-	
	K[b]	21	-	-	-	-	-	-	-	2	19	-	-	-	-	-	*<0.001*
	G[a]	10	-	-	-	1	-	-	-	-	-	-	1	3	5	-	
Enrofloxacin	A[a]	82	-	-	-	-	-	23	56	3	-	-	-	-	-	-	*<0.001*

	K[b]	21	-	-	-	-	-	-	8	12	1	-	-	-	-	-	
	G[b]	10	-	-	-	-	-	-	7	3	-	-	-	-	-	-	
Amoxi/clav[3]	A[a]	82	-	-	-	-	-	16	64	2	-	-	-	-	-	-	
	K[b]	21	-	-	-	-	-	-	20	1	-	-	-	-	-	-	*0.035*
	G	10	-	-	-	-	-	1	7	1	1	-	-	-	-	-	
Daptomycin	A[a]	82	-	-	-	-	-	-	-	1	2	30	43	6	-	-	
	K[a]	21	-	-	-	-	-	-	-	-	-	3	16	2	-	-	*<0.001*
	G[c]	10	-	-	-	-	-	-	-	-	-	-	2	8	-	-	
Erythromycin	A[a]	82	45	-	28	2	2	1	-	1	-	-	-	-	-	3	
	K[b]	21	21	-	-	-	-	-	-	-	-	-	-	-	-	-	*<0.001*
	G[a]	10	2	-	6	1	-	-	-	-	-	-	-	-	-	-	

1. a–c indicate within three major farms with various superscript difference ($P < 0.05$).

Supplementary Table 6. Difference in MIC of antimicrobials against isolates of three major STs

Antimicrobials	ST[1]	NO.	Isolates at each indicated MIC (μg/mL)														Overall *P*-value[2]
			<0.015	0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	>32	
Penicillin	103[a]	49	-	-	-	1	31	17	-	-	-	-	-	-	-	-	
	1413[b]	37	-	3	7	16	3	5	1	-	-	2	-	-	-	-	*<0.001*
	1414[b]	34	-	-	12	13	-	8	-	-	-	1	-	-	-	-	
Cefalexin	103[a]	49	-	-	-	-	-	-	-	-	10	38	1	-	-	-	
	1413[b]	37	-	-	-	-	-	-	1	1	25	9	1	-	-	-	*<0.001*
	1414[a]	34	-	-	-	-	-	-	-	-	12	22	-	-	-	-	
Cefquinome	103[a]	49	-	-	15	34	-	-	-	-	-	-	-	-	-	-	
	1413[b]	37	-	4	22	8	-	2	-	-	-	1	-	-	-	-	*<0.001*
	1414[b]	34	-	-	26	6	2	-	-	-	-	-	-	-	-	-	
Clindamycin	103[a]	49	-	1	16	26	-	-	-	1	-	-	1	-	4	-	
	1413[b]	37	-	17	14	2	-	-	1	-	2	-	1	-	-	-	*<0.001*
	1414[b]	34	-	18	9	3	1	-	-	-	1	-	-	-	2	-	
Tetracycline	103[a]	49	-	-	-	-	2	-	-	-	-	-	2	22	23	-	*0.016*
	1413[b]	37	-	-	-	-	1	1	-	2	10	-	1	11	11	-	

	1414	34	-	-	-	1	-	-	-	-	6	-	-	12	15	-	
Daptomycin	103[a]	49	-	-	-	-	-	-	-	1	2	3	36	7	-	-	
	1413	37	-	-	-	-	-	-	-	-	-	11	17	9	-	-	*0.015*
	1414[b]	34	-	-	-	-	-	-	-	-	-	18	11	5	-	-	
Erythromycin	103[a]	49	18	-	22	-	-	1			-	-	-	-	-	3	
	1413[b]	37	24	-	7	1	1	2	1	1	-	-	-	-	-	2	*0.028*
	1414[b]	34	23	-	5	1	1	-	1	-	-	-	-	-	-	5	

1. a–c indicate within three major provinces with various superscript difference ($P < 0.05$).