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SHORT COMMUNICATION

Molecular Prevalence and Antimicrobial Susceptibility of *Mannheimia haemolytica* Isolated from Fatal Sheep and Goats Cases in Jiangsu, China

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ABSTRACT

The objective of present study was to examine the prevalence and antimicrobial resistance of *Mannheimia haemolytica* isolated from the dead goats and sheep with pneumonia in Jiangsu, China. These bacteria were isolated, identified, serotyped with multiplex PCR, characterized by MLST and evaluated for drug susceptibility. The overwhelming majority of isolates (19/21) from goats and 1 isolate from sheep were identified as serotype 2, while only 1 isolate (1/21) from goat characterised as serotype 1. MLST profiles of *Mannheimia haemolytica* isolates revealed that these were diversity and most of isolates belong to ST44 (7/21) and ST7 (6/21). There were high levels of resistance against streptomycin (48%) and mezlocillin (43%) among the isolates. In conclusion, *Mannheimia haemolytica* isolated from goats in Jiangsu exhibited diversity of STs in genetics and simplicity in serotype.

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INTRODUCTION

Mannheimia haemolytica (*M. haemolytica*) is the bacterial pathogen associated with pneumonia causing high morbidity and mortality in the ruminants including bovine, sheep, goats and chamois (McRae *et al*., 2016). *M. haemolytica* has been classified into a total of 12 capsular serovars as 1, 2, 5, 6, 7, 8, 9, 12, 13, 14, 16 and 17, which serotype 2 is adapted to sheep and goat (Klima *et al*., 2017). Moreover, multilocus sequence typing (MLST) profile of *M. haemolytica* revealed the considerable diversity in ST and evolutionary relationships of isolates obtained from some countries and various hosts (Petersen *et al*., 2009; Omaleki *et al*., 2016).

There is a large amount of goats and sheep raised in China. Accompanied with rapid increase of feedlot industries in Jiangsu since the beginning of 2011, there were vast goats and sheep transported or shipped from Shangdong, Henan, Anhui province of China. Morbidity and mortality in goats and sheep usually appears in 10 days after arrived at the feedlot, causing considerable economic losses. Some clinical cases in pneumonia were sent to Animal Hospital of Yangzhou University in Jiangsu, China and diagnosed. *M. haemolytica* strains were isolated from those fatal cases in recent years. To our knowledge, very few studies have been performed that describing the prevalence of *M. haemolytica* in this area. Therefore, the objectives of this study were to investigate STs, serotypes and antimicrobial resistance of *M. haemolytica* isolates in Jiangsu Province of China.

MATERIALS AND METHODS

Sample collection and *M. haemolytica* isolation: Samples were obtained from liver, spleen, kidney and lung of clinically ill or dead goats and sheep from Animal Hospital of Yangzhou University in Jiangsu, China, between 2012 to 2017. Samples were streak out on sheep blood agar and incubated for 24 h at 37°C. Single colony was picked out and plated on sheep blood agar and MacConkey's agar for purification, respectively.

***M. haemolytica* identification and serotyping:** The isolates were identified using PCR amplifying 16S rDNA. PCR products were sent to the Genscript Company and further confirmed by sequencing. The 16s rDNA sequences of these isolates were searched using the Nucleotide BLAST tool (BLASTn, http://www.ncbi.nlm.nih.gov/BLAST) and were submitted to GenBank.

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Serotyping of isolates were identified using multiplex PCR assay described previously (Klima *et al.*, 2017). Amplicon were detected by the agarose gel and sequenced to confirm.

MLST analysis: Housekeeping genes (adk, aroE, deoD, gapDH, gnd, mdh and zwf) of *M. haemolytica* isolates were amplified by PCR and sequenced using methods described previously (Petersen *et al*., 2009). The resultant sequences were searched and submitted in the *M. haemolytica* MLST website (http://pubmlst.org/mhaemolytica/), and named as the allele type and sequence type (ST). Identified STs were aligned with representative STs, which each ST complex downloaded from the *M. haemolytica* MLST website (http://pubmlst.org/mhaemolytica/). Phylogenetic and molecular evolutionary analyses were conducted on the basis of the concatenation of seven MLST loci using the MEGA software (Tamura *et al*., 2007).

Antimicrobial susceptibility testing: Antimicrobial susceptibility testing was performed by Kirby-Bauer Disk Diffusion using 12 categories of drug (Table 2) in accordance with the standard the Clinical and Laboratory Standards Institute (CLSI, 2009).

RESULTS AND DISCUSSION

***M. haemolytica* isolates and serotyping:** All isolates were demonstrated slightly β-haemolytic colonies on sheep blood agar, while colonies of these isolates not present in MacConkey's agar. A total of 21 *M. haemolytica* strains (Table 1) were isolated from goats (n=20) and sheep (n=1) in Jiangsu, China from May 2012 to July 2017, and further confirmed by sequencing of the 16S rRNA gene. 16S rRNA gene sequences were provided with GenBank accession numbers: MG725924-MG725943, MG725945. According to Multiplex PCR assay, 20 isolates from goats (n=19) and sheep (n=1) were capsular serotype 2, only one isolate from goat was serotype 1. Therefore, serotype 2 is most prevalent in goats of the Jiangsu province of China. More, our results are similar with the previous reports that *M. Haemolytic* serotype 2 is commonly associated with disease in goats and sheep (Petersen *et al*., 2009, Klima *et al*., 2017).

Multilocus sequence typing: All 21 *M. haemolytica* isolates were exhibited different MLST profiles with 8 ST types including ST7, 8, 14, 16, 43, 44, 45, 46 (Table 1). ST44 and ST7 circulated in Jiangsu, China, and were represented by 7 and 6 isolates, respectively. ST43, 44, 45, 46 were identified for the first time. Considerable diversity of STs in our study was also found in isolates from goats of French alps and sheep of Australian (Petersen *et al*., 2009; Omaleki *et al*., 2016). ST7, 8 and 14 were found in goats from Jiangsu and first reported in the Asia, belongs to CC8 which consisted of nine different STs, demonstrated among the French isolates, and not found in Australia. Moreover, CC8 is the most predominant clonal complexes (CC) found in this pneumonia outbreak. In addition, ST16, 43, 44, 45, and 46 are not currently categorized with a CC.

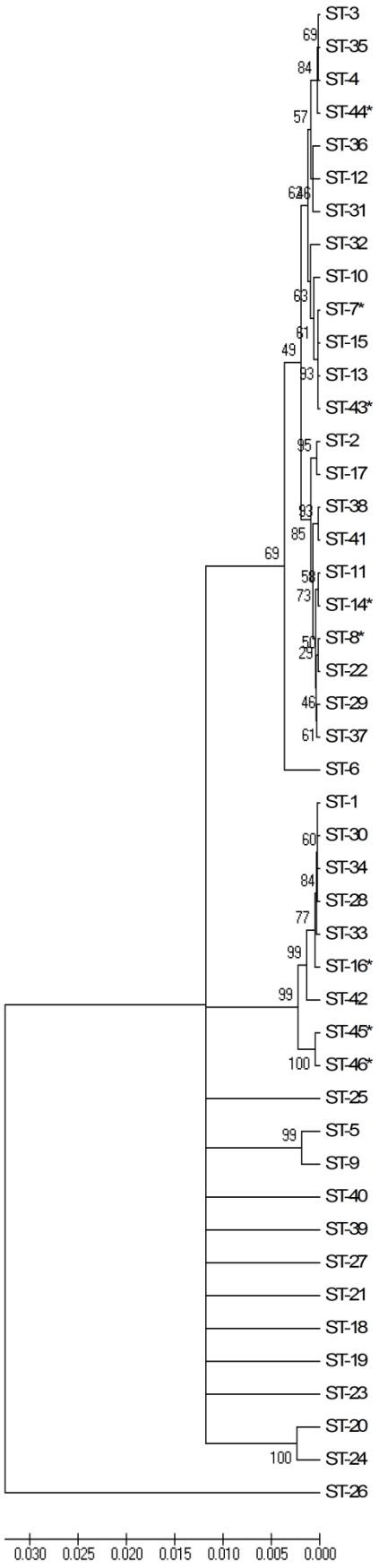

Fig. 1: Phylogenetic organization of *M. haemolytica* isolates of different MLST sequence types (STs) based on Neighbor-joining method. Numbers at the node are bootstrap values and the bar represents 0.005 nucleotide changes. *ST7, 8, 14, and 16 identified in this study and the previously study (Petersen *et al*., 2009) and *ST43, 44, 45, 46 identified in this study.

Table 1: Sequence types (ST) and serotypes of *Mannheimia haemolytic* isolates

Isolates	Host	Serotype	Region	Year	ST	CC
201205YF	goat	2	Taizhou,Jiangsu	2012	07	08
201206YS	goat	2	Taizhou,Jiangsu	2012	43	
201206YH	goat	2	Huaian, Jiangsu	2012	44	
201210YF1	goat	2	Huaian, Jiangsu	2012	44	
201210YF2	goat	2	Huaian, Jiangsu	2012	44	
201304YF1	goat	2	Yangzhou, Jiangsu	2013	07	08
201304YF2	goat	2	Yangzhou, Jiangsu	2013	07	08
201304YF3	goat	1	Zhenjiang, Jiangsu	2013	16	
201306YF	goat	2	Yangzhou, Jiangsu	2013	45	
201307YF1	goat	2	Yangzhou, Jiangsu	2013	44	
201307YF2	goat	2	Yangzhou, Jiangsu	2013	44	
201307YF3	goat	2	Yangzhou, Jiangsu	2013	07	08
201401YS1	goat	2	Nantong, Jiangsu	2014	14	08
201401YS2	goat	2	Yangzhou, Jiangsu	2014	07	08
201402YL	goat	2	Yangzhou, Jiangsu	2014	44	
201404YF	goat	2	Yangzhou, Jiangsu	2014	08	08
201412YF1	sheep	2	Nantong, Jiangsu	2014	46	
201412YF2	goat	2	Huaian, Jiangsu	2014	08	08
201504YF	goat	2	Yangzhou, Jiangsu	2015	44	
201505YF	goat	2	Zhenjiang, Jiangsu	2015	07	
201707YF	goat	2	Nantong, Jiangsu	2017	46	

There was single isolate obtained from goat belonging to serotype 1 and ST types 16. ST16 were occurred in isolates obtained from two animal species including sheep (n=5, serotype 6 and 16), goat (n=1, serotype 2) (Petersen *et al*., 2009). The others 7 STs types were found in 20 isolates from goats (n=19) and sheep (n=1) belonged to serotype 2.

The phylogenetic organization of *M. haemolytica* isolates is belonged to different group due to a high diversity of ST (Fig. 1). The phylogentic analysis suggested that there have a close relationship between ST7, 8, 14, 43, and 44, isolated from France and China, suggesting their origin from a common ancestor and limitation to some area (Fig. 1). Meanwhile, ST16, isolated from UK, Norway, France, China and Australia, is closely related to 45, and 46, suggesting their origin from another common ancestor and world-wide distribution (Fig. 1). Therefore, integrated with the previous study (Petersen *et al*., 2009; Omaleki *et al*., 2016), the result suggested that STs of *M. haemolytica* may be presented geographic features.

Antimicrobial susceptibility testing: The 21 isolates were tested for antimicrobial susceptibility (Table 2). The resistance rates for STR, MEZ, KAN, TMSZ, NOR, GEN, AMK, DOX, LVX, and CRO were 48, 43, 42, 38, 33, 24, 10, 10, 10 and 10%, respectively. The high prevalence of antimicrobial resistances against STR, MEZ, KAN, TMSZ, NOR and GEN may be associated with this common usage of these drug for the disease control and treatment since it has been proved that there are relationships between frequent usage and drug resistance (Cameron and McAllister, 2016). In the veterinary clinic of China, SXT and NOR were used to inject the sick goat constantly, which led to high resistance of this bacterium. In contrast, reports in Australia show susceptibility to STR and NOR in that those two kinds of agent are not accessible to food producing animals (Omaleki *et al*., 2016). Moreover, in some reports, *M. Haemolytica* isolates showed strong resistant to tetracyclines and macrolide (Timist *et al*., 2017), especially for isolates collected from feedlot at any production stage for prophylaxis or metaphylaxis.

No isolates were resistant to FLO in this study, which is consistent with the previous reports (Timist *et al*., 2017). Compared with other animals such as pigs and chickens, florfenicol should be employed to ruminants by intramuscular and intravenous administration (Ali *et al*., 2003) rather than oral administration (Voorspoels *et al*., 1999) since they have special gastrointestinal tract. Furthermore, florfenicol is used to treat infected animals following intramuscular administration, instead of

Table 2: Antimicrobial susceptibility of isolates

Antimicrobial drug[a]		NOR	LVX	GEN	STR	KAN	AMK	POL	TMSZ	DOX	FLO	MEZ	CRO
Drug content/μg		10	5	10	10	30	30	300	23.75/1.25	30	30	75	30
Breakpoints	Resistant	≤12	≤13	≤12	≤12	≤13	≤14	≤11	≤24	≤10	≤14	≤17	≤13
	Intermediate	13-16	14-16	13-14	13-14	14-17	15-16	/	25-31	11-13	15-18	18-20	14-20
	Susceptible	≥17	≥17	≥15	≥15	≥18	≥17	≥12	≥32	≥14	≥19	≥21	≥21
Isolates	201205YF	25	**0**[b]	0	0	11	0	11	24	0	20	15	30
	201206YS	22	25	10	0	12	0	0	20	12	24	0	20
	201206YH	10	16	0	12	0	16	14	16	20	20	14	20
	201210YF1	0	26	14	0	0	18	13	0	19	27	10	0
	201210YF2	0	26	14	0	0	18	13	0	19	27	10	0
	201304YF1	29	28	20	22	21	29	17	29	17	30	29	39
	201304YF2	29	29	22	22	21	23	16	27	27	31	36	40
	201304YF3	30	22	20	17	12	22	17	32	20	30	36	38
	201306YF	36	28	17	11	24	16	12	30	17	36	22	36
	201307YF1	0	38	18	10	0	22	16	0	12	22	0	22
	201307YF2	0	30	18	10	0	22	16	0	12	22	0	22
	201307YF3	0	38	0	0	34	20	24	0	0	36	0	40
	201401YS1	26	30	22	16	25	18	15	34	24	38	34	32
	201401YS2	32	30	20	16	28	21	18	42	24	32	40	36
	201402YL	32	36	20	18	24	21	20	35	20	20	16	34
	201404YF	35	33	31	30	30	30	30	42	32	37	41	41
	201412YF1	36	40	28	18	24	26	15	32	16	36	46	48
	201412YF2	28	30	38	32	12	20	22	32	26	30	28	36
	201504YF	6	6	12	30	40	40	20	18	20	20	20	14
	201505YF	35	34	20	17	27	17	20	34	30	35	36	32
	201707YF	30	31	20	10	20	21	13	30	15	30	40	42
Resistant isolates number		07	02	05	10	9	02	02	08	02	00	09	02
	rate	0.33	0.10	0.24	0.48	0.42	0.10	0.10	0.38	0.10	0.00	0.43	0.10

a NOR, norfloxacin; LVX, levofloxacin; GEN, gentamicin; STR, streptomycin;KAN,kanamycin; AMK, amikacin; POL, polymyxin B;TMSZ, Sulfamethoxazole/trimethoprim; DOX,doxycycline; FLO,florfenicol; MEZ, mezlocillin; CRO, ceftriaxone. b bold represents resistant.

prophylaxis or metaphylaxis for the entire herds dosing with in-feed antimicrobial over extended duration for practices. Therefore, none or few isolates from ruminants resistant to FLO will be explained for limited usage for administration route in practices.

All the 5 isolates collected in 2012 showed resistance to more than three kinds of antimicrobial agent. Although most of isolates (13/21) were resistant to one or more antimicrobial agent, 8 isolates were susceptible to the detected antimicrobial agent.

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Authors contribution: YW and YY wrote the article, ZZ & SG executed the experiment, XZ & DC analyzed the data.

REFERENCES

Ali BH, Al-Qarawi AA and Hashaad M, 2003. Comparative plasma pharmacokinetics and tolerance of florfenicol following intramuscular and intravenous administration to camels, sheep and goats. Vet Res Commun 27:475-83.

Cameron A and McAllister TA, 2016. Antimicrobial usage and resistance in beef production. J Anim Sci Biotechnol 7:68.

Klima CL, Zaheer R, Briggs RE, *et al*., 2017. A multiplex PCR assay for molecular capsular serotyping of *Mannheimia haemolytica* serotypes 1, 2, and 6. J Microbiol Meth 139:155-60.

Mcrae KM, Baird HJ, Dodds KG, *et al*., 2016. Incidence and heritability of ovine pneumonia and the relationship with production traits in New Zealand sheep. Small Rumin Res 145:136-41.

Omaleki L, Browning GF, Allen JL, *et al*., 2016. Molecular epidemiology of an outbreak of clinical mastitis in sheep caused by *Mannheimia haemolytica*. Vet Microbiol 191:82-7.

Petersen A, Christensen H, Kodjo A, *et al*., 2009. Development of a multilocus sequence typing (MLST) scheme for *Mannheimia haemolytica* and assessment of the population structure of isolates obtained from cattle and sheep. Infect Genet Evol 9:626-32.

Tamura K, Dudley J, Nei M, *et al*., 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) Software Version 4.0. Mol Biol Evol 24:1596-9.

Timist E, Hallewell J, Booker C, *et al*., 2017. Prevalence and antimicrobial susceptibility of *Mannheimia haemolytica*, *Pasteurella multocida*, and *Histophilus somni* isolated from the lower respiratory tract of healthy feedlot cattle and those diagnosed with bovine respiratory disease. Vet Microbiol 208:118-25.

Voorspoels J, D'Haese E, Craene BAD, *et al*., 1999. Pharmacokinetics of florfenicol after treatment of pigs with single oral or intramuscular doses or with medicated feed for three days. Vet Rec 145:397-9.