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Original article

Antibiotic resistance and molecular characteristics of *Staphylococcus aureus* isolated from pigs in Hunan, China

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Abstract

Staphylococcus aureus (*S. aureus*) has been recognized as one of the important zoonotic pathogens. However, it was limited about the epidemiology and genetic characteristics of *S. aureus* isolated from pigs in Hunan province, china. The aim of this study was to determine the characteristics of 163 *S. aureus* isolated from 590 pigs in Hunan Province, China. All isolates were characterized by *agr* typing, detection of virulence genes and antibiotic resistance genes, lethal test of mice and antibiotic susceptibility tests. The results showed that 30 strains of the 163 isolates were divided into *agr*I (18.40%), *agr*II(36/163, 22.09%), *agr*III (20/163, 12.27%,), *agr* IV(20/163,12.27%) and the remaining 57 isolates were amplified negative by *agr* primers. In the 163 isolates, the detection rate of the virulence genes *hlb*, *hld*, *hla*, *icaA*, *seb*, *fnbA*, *eta*, *etb*, *sea*, *tst* and *pvl* ranged from 2.45% to 100%. The 43 isolates that were lethal to the mice, had β-hemolytic activity, the number of virulence genes of which was 7.8% higher than that of the remaining 120 non-fatal strains. The resistance rates of the 163 isolates to the 15 antibiotics were 0% (0/163) - 100% (163/163). All isolates were susceptible to Vancomycin and only 7 isolates were methicillin - resistant *S. aureus* (MRSA). The detection rates of the 11 resistance genes was 0% (0/163) - 100% (163/163). This study first to describes the epidemiology and characteristics of *S. aureus* from pigs in Hunan Province, which will help in tracking the evolution of epidemic strains and preventing pig-human transmission events.

Key words: antibiotic resistance, molecular characterization, pig-associated S. aureus, Hunan

Introduction

Staphylococcus aureus is a commensal opportunistic pathogen present in both healthy and diseased humans and animals (Argudin et al. 2011, Fluit et al. 2001). Due to the broad spectrum of virulence factors and its ability to develop antibiotic resistance, infection of *S. aureus* results in high morbidity and mortality worldwide (Friães et al. 2015, Katherine et al. 2015). Colonization of *S. aureus* in animals has been receiving comprehensive attention since animals may potentially act as a reservoir of human infection (Aires 2017, OdetokuN et al. 2018). The pig is one of the most important hosts of *S. aureus* (Dong et al. 2018). To our

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knowledge, *S. aureus* is widespread in pigs in China (Jun et al. 2017). Hunan Province is recognized as one of the key areas for pig breeding in China. However, little was known about the epidemiology and characteristics of *S. aureus* in the pig in Hunan Province. In this study, *agr* type, virulence gene detection and resistance gene detection and antibiotic susceptibility tests were used to study the isolates from pig lung and pig swabs in Hunan province.

Materials and Methods

Staphylococcus aureus strains

From October 2018 to September 2019, a total of 163 strains of *S. aureus* were isolated and identified from the lungs and swabs of infected pigs from Hunan province. These strains were stored at -80℃ for this study.

DNA extraction and Primers in the study

The nucleic acids of *S. aureus* isolates were extracted using methods provided by an Ezup Column Bacteria Genomic DNA Purification Kit purchased from Shanghai Bioengineering Co., Ltd. Molecular detection of the genes was carried out according to the following protocol: initial denaturation at 95℃ for 4 min followed by 30 cycles of denaturation at 94℃ for 1 min, annealing at a suitable temperature (Table 1) for 30 s and extension at 72℃ for 1 min, and a final extension step at 72℃ for 7 min. The primers used in the study are listed in Table 1.

Agr type

According to the methods described by Zhang (2018), multiplex PCR amplification was used for *agr* typing of 163 isolates with primers Pan - agr I (agr I type), Pan - agrII (agr IItype), Pan - agr III (agr III type), Pan - agr IV (agr IV type). Based on the sizes of PCR amplification products, the *agr* types were identified on the basis of the criteria in Table 1.

Detection of virulence genes by PCR, *β*-hemolysis test, detection of pathogenicity of isolates by animal test

The 11 virulence genes *sea, seb, tst, pvl, eta, etb, hla, hlb, hlb, icaA,* and *fnbA* of the 163 isolates were detected using the primers in Table 1. All 163 isolates were inoculated on blood Agar plates to verify their β-hemolytic activity. All 163 isolates were injected into Kunming mice to identify their pathogenicity following the requirements of the Animal Ethics Committee, College of Veterinary Medicine, Hunan Agricultural University. 2×10^9 CFU of the isolates were injected into each mouse and each strain was injected into three mice. Those isolates which caused the death of at least one mouse within 48 hours, were considered to be pathogenic strains.

Antibiotic susceptibility test and PCR detection of resistance genes

Antibiotic susceptibility of the 163 isolates to the 15 antibiotics was assessed using the disk diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI 2009). The 15 antibiotics were penicillin, ampicillin, cefoxitin, oxacillin, cefotaxime, norfloxacin, ciprofloxacin, levofloxacin, gentamicin, streptomycin, tetracycline, erythromycin, clindamycin, vancomycin and compound xinnuoming. In order to verify the antibiotic susceptibility tests, the 11 resistance genes *gyrA, aac(6') /aph (2'), tetk, blaz, tetM, LinA, ermA, sul1, MsrA* and *mecA* were detected from all 163 isolates by the primers in Table 1.

Results

Agr type

The results showed that the 163 isolates were divided into *agr*I (18.40%, 30/163), *agr*II (22.09%, 36/163), *agr*III (12.27%, 20/163), *agr* IV (12.27%, 20/163), with 57 isolates (34.97%, 57/163) negative by PCR with *agr* primers (Table 2). There was no significant difference in the distribution of each *agr* of the isolates between the lung and the swab except for type IV. In 20 isolates of *agr* IV, there were 5 and 15 isolates for lung samples and swab, respectively. The isolates with a negative coagulase test were only distributed in *agr* II (3 strains) and *agr* negative (10 strains).

PCR amplification of the virulence genes

The results showed that all 11 virulence genes were detected positive in different degrees by PCR in all 163 isolates, including *hlb* (100%), *hld* (100%), *icaA* (85.28%), *hla* (84.05%), *sea* (44.79%), *eta* (38.04%), *etb* (38.04%), *seb* (31.90%), *fnbA* (30.06%), *tst* (6.75%) and *pvl* (2.45%). The number of virulence genes for each of the isolates was different, ranging from 3 to 9 (Table 3). In all 163 isolates, the number of isolates with 4 virulence genes (48 strains) was the largest, accounting for 29.45%, while the number of isolates with 9 virulence genes (4 strains) was the least, accounting for 2.45%.

Table 1. Oligonucleotide primers used in the study.

Target gene	primers sequence(5' -3')	Size (bp)	annealing temperature (°C)	Reference
	Virulence genes (11)			
sea	F: GGATATTGTTGATAAATATAAAGGGAAAAAAG R: GTTAATCGTTTTATTATCTCTATATATTCTTAATAGT	439	50	Yanping et al 2011
seb	F: AGATTTAGCTGATAAATACAAAGATAAATACG R: TCGTAAGATAAACTTCAATCTTCACATCT	494	52	Yanping et al 2011
tst	F: TTTT TTAT CGTAAGCCCTTTGATTGC R: CACCCGTTTTATCGCTTGAA	550	51	Ote et al 2011
pvl	F: GTCGTTAGGAATAATCACTCC R: CCTGTTGATGGACCACTATTAA	423	48	Ote et al 2011
eta	F: TTGTAAAAGGACAAACAAGTGC R: TTCCCAATACCAACACCA	544	49.5	Ote et al 2011
etb	F: TTACAAGCAAAAGAATACAGCG R: GGAAGATTATGTTGTCCGCC	641	50	Ote et al 2011
hla	F: TGCCGCAGATTCTGATATTAA R: TGCCGCAGATTCTGATATTAA	845	51	Ote et al 2011
hlb	F:GCGGTTGTGGATTCGATAAT R: GGCTTTGATTGGGTAATGATC	524	50	Ote et al 2011
hld	F: GGGATGGCTTAATAACTCATACTT R: CAGAGATGTGATGGAAAATAGTTGA	236	48	Ote et al 2011
icaA	F: CTTGCTGGCGCAGTCAATAC R: CCAACATCCAACACATGGCA	178	55	Pereyra et al 2016
fnbA	F: GTGAAGTTTTAGAAGGTGGAAAGATTAG R: GCTCTTGTAAGACCATTTTTCTTCAC	643	55	Tritan A et al 2003
	Resistance genes (11)			
mec A	F: GTTGTAGTTGTCGGGTTTGG R: CTTCCACATACCATCTTCTTTAAC	336	55	Fluit et al 2001
blaz	F: TCAAACAGTTCACATTGCC R: TCAAACAGTTCACATTGCC	790	55	Argudinet et al 2011
LinA	F: GGTGGCTGGGGGGTAGATGTATTAACTGG R: GCTTCTTTTGAAATACATGGTATTTTTCGATC	323	57	Lina G et al 1999
ermA	F: GTTCAAGAACAATCAATACAGAG R: GGATCAGGAAAAGGACATTTTAC	410	52	Lina G et al 1999
MsrA	F: GGCACAATAAGAGTGTTTAAAGG R: AAGTTATATCATGAATAGATTGTCCTGTT	940	50	Lina G et al 1999
gyrA	F: AGTACATCGTCGTATACTATATGG R: ATCACGTAACAGTTCAAGTGTG	280	55	Lina G et al 1999
aac /aph	F: CCAAGAGCAATAAGGGCATA R: CACTATCATAAC-CACTACCG	220	55	Tai XY et al 2019
Sul1	F: AGGCTGGTGGTTATGCACTC R: CACCGAGACCAATAGCGGAA	260	60	Tai XY et al 2019
vanA	F: GGGAAAACGACAATTGC R: GTACAATGCGGCCGTTA	885	58	RamosT E et al 2003
tetk	F: GTAGCGACAATAGGTAATAGT R: GTAGCGACAATAGGTAATAGT	360	48	Strommenger et al 2003
tetm	F: GTGTGACCAACTTTACCGAA R: GCTTTGTATCTCCAAGAACAC	501	55	Strommenger et al 2003
	Agr type (5)			
pan	ATGCACATGGTGCACATGC			Zhang,2018
agr I	GTCACAAGTACTATAAGCTGCGAT	441	55	Zhang,2018
agrII	TATTACTAATTGAAAAGTGGCCATAGC	575	55	Zhang,2018
agr III	GTAATGTAATAGCTTGTATAATAATACCCAG	323	55	Zhang,2018
agr IV	CGATAATGCCGTAATAC CCG	659	55	Zhang,2018

Table 2. Statistical analysis of *agr* typing and biological characteristics of 65 isolates.

Agr type	Sample		Number of CET		Number of VGs	Hemolytic activity		Pathogenicity		Number of MAR	Number of ARGs
	Lung	Swab	+	-	Average	Yes	No	Yes	No	Average	Average
I (30)	17	13	30	0	5.92	27	3	17	13	9.42	7.5
II (36)	15	21	33	3	5.85	33	3	3	33	9.64	7.71
III (20)	10	10	20	0	5.75	17	3	13	7	8.63	7.5
IV (20)	5	15	20	0	5.75	20	0	10	10	10.5	7.75
- (57)	30	27	47	10	5.04	54	3	0	57	8.65	7.73
Total163	77	86	150	13		151	12	43	120		

Note: +: positive; -: negative; VGs:Virulence genes; CET: coagulation enzyme test; MAR: multiple antibiotic resistance; ARGs:Antibiotic resistance genes.

Table 3. Distribution of virulence genes of all 163 isolates.

virulence genes		*S. aureus* isolates		Rate (%)
Number	Name	Number	Total	
3	*hla, hlb, hld*	5	5	3.07
4	*hla, hlb, hld, icaA*	33	48	29.45
	hlb, hld, icaA, seb	11		
	hla, hlb, hld, sea	4		
5	*hla, hlb, hld, icaA, sea*	21	38	23.31
	eta, etb, hlb, hld, sea	15		
	fnbA, hla, hlb, hld, icaA	2		
6	*hla, hlb, hld, icaA, sea, seb*	19	25	13.34
	fnbA, hla, hlb, hld, icaA, seb	3		
	fnbA, hla, hlb, hld, icaA, sea	3		
7	*eta, etb, fnbA,hla, hlb, hld, icaA*	6	12	7.36
	eta, etb, hla, hlb, hld, icaA, seb,	6		
8	*eta, etb, fnbA,hla, hlb, hld, icaA, seb*	9	31	19.02
	eta, etb, fnbA,hla, hlb, hld, icaA, sea	9		
	eta, etb, fnbA, hla, hlb, hld, icaA, tst	9		
	eta, etb, fnbA, hla, hlb, hld, icaA, pvl	4		
9	*eta, etb, fnbA,hla, hlb, hld, icaA, seb, tst*	2	4	2.45
	eta, etb, fnbA,hla, hlb, hld, icaA, sea,seb	2		

β-hemolysis test and detection of pathogenicity of isolates by animal test

In all 163 isolates, 151 strains (92.64%) showed β-hemolytic activity (Table 4), while the remaining 12 isolates did not show β-hemolytic activity. There was no difference in the number of isolates with β-hemolytic activity between samples from lungs (74 strains) and from swabs (77 strains). In the 151 strains with β-hemolytic activity, the number (51strains) *agr*-negative was the most, accounting for 31.29%, while the number (18 strains) of *agr* III was the least, accounting for 11.04%. However, the average number (5.5) of virulence genes of the isolates with β-hemolytic activity was 13.87% higher than that (4.83) of the isolates without β-hemolytic activity. In the 151 isolates with β-hemolytic activity, 96.69% (146/151) were positive for the coagulation enzyme test, but only 37 strains were lethal to the mice. This indicates that the β-hemolytic activity of the isolates was not directly related to their pathogenicity. The average number of multiple antibiotic-resistant (9.14) and resistant genes (7.64) of the isolates with β-hemolytic activity was lower than that of the isolates without β-hemolytic activity. This indicates that the isolates with β-hemolytic activity were more weakly resistant to antibiotics.

Table 4. Comparative analysis of the biological characteristics of isolation with β-hemolytic activity and that with no hemolytic activity.

Hemolytic activity	Sample		Agr typing					Number of VG	Results of CET		Pathogen icity		Number of MAR	Number of ARGs
	Lung	Swab	I	II	III	IV	-	Average	+	-	Yes	No	Average	Average
β-hemolytic(151)	74	77	28	34	18	20	51	5.5	146	5	37	114	9.14	7.64
no hemolytic(12)	3	9	2	2	2	0	6	4.83	4	8	6	6	10.17	7.83
Total(163)	77	86	30	36	20	20	57		150	13	43	120		

Note: +: positive; -: negative; VGs:Virulence genes; CET: coagulation enzyme test; MAR: multiple antibiotic resistance; ARGs:Antibiotic resistance genes.

Table 5. Comparative analysis of biological characteristics of highly pathogenic and non-pathogenic *S. aureus isolation strains.*

Pathogenic strain	Results of CET		Agr typing					Virulence gene	β hemato -lysis		sample source		Number of MAR	Number of ARGs
	+	-	I	II	III	IV	-	Average	Yes	No	Lungs	Swab	Average	Average
Yes (43)	43	0	20	3	10	10	0	5.52	37	6	19	24	9.35	7.65
No (120)	107	13	10	33	10	10	57	5.12	104	6	58	62	8.38	7.33
Total(163)	150	13	30	36	20	20	57		151	12	77	86		

Note: +: positive; -: negative; CET: coagulation enzyme test; MAR: multiple antibiotic resistance; ARGs:Antibiotic resistance genes.

Table 6. Detection results of resistance genes from all of the 163 isolates.

Relation	Gene	Number	Rate(%)
quinolones	*gyrA*	163	100
aminoglycosides	*aac(6') /aph (2')*	163	100
tetracycline	*tetk*	163	100
β-lactam	*blaz*	158	96.93
tetracycline	*tetM*	153	93.87
Lincoamides	*LinA*	145	88.96
macrolides	*ermA*	135	82.82
sulfanilamide	*sul1*	118	72.39
macrolides	*MsrA*	28	17.18
β-lactam	*mecA*	7	4.29
glycopeptides	*vanA*	0	0

Detection of pathogenicity of isolates by animal test

Of the 163 isolates, 43 were fatal to the mice. Each of the 43 pathogenic isolates was able to kill one mouse at least within 48 hours. The dead mice showed swelling and congestion in the lung, the kidney and the spleen, and some of the spleen had necrosis and bleeding spots with the size of a needle tip. All 43 pathogenic isolates were positive for the coagulation enzyme test and 37 of them had β-hemolytic activity, mainly *agr* type I (46.51%, 20/43). The average number of virulence genes of the 43 pathogenic isolates (5.52) was 7.8% higher than that of the 120 non-pathogenic isolates (5.12). This indicates that the number of virulence genes of *S. aureus* is closely related to its pathogenicity. There was no significant difference in the distribution of the pathogenic isolates between lung (19 strains) and swabs (24 strains). The average number of multiple antibiotic resistance of pathogenic isolates (9.35) was 11.58% higher than that of non-pathogenic isolates (8.38), and the average number of antibiotic resistance genes (7.65) was 4.4% higher than that of non-pathogenic isolates (7.33) (Table 5).

PCR detection of resistance genes

The detection rate of 11 resistance genes was 0 (0/163) - 100% (163/163) (Table 6). In all isolates, the detection rate was 0% for gene *van* related to glyco-

Table 7. The result antibiotic susceptibility tests of 15 antibiotics to the 163*S.aureus* isolates.

Antibiotic	Number of isolates and resistance rate(%)					
	R	Rate	I	Rate	S	Rate
Streptomycin	163	100	0	0	0	0
Gentamicin	158	96.93	0	0	5	3.07
Erythromycin	155	95.09	2	1.23	6	3.68
Penicillin	155	95.09	2	1.23	6	3.68
Clindamycin	138	84.66	15	9.20	10	6.13
Levofloxacin	130	79.75	0	0	33	20.25
Tetracycline	118	72.39	40	24.54	5	3.07
Ampicillin	118	72.39	10	6.13	35	21.47
Cefotaxime	113	69.33	10	6.13	40	24.54
Ciprofloxacin	113	69.33	3	1.84	47	28.83
CX	103	63.19	5	3.07	55	33.72
Cefotaxime	15	9.20	50	30.67	98	60.12
Oxacillin	10	6.13	0	0	153	93.87
Cefoxitin	8	4.91	0	0	155	95.09
Vancomycin	0	0	0	0	163	100

Note: CX: Compound Xinnuoming; R: Number of the Resistance isolates; I: Number of the Intermediate isolates; S: Number of the Sensitivity isolates.

peptide such as vancomycin while that of genes *gyra*, *aac/aph* and *tetk*, related to Quinolones, Glucosamine and Tetracycline, was 100%. The resistance rate of the 7 remaining genes was 4.29% - 96.93%.

Each isolate had a variety of resistance genes, the number of which was 5 - 9. In all isolates, the number of isolates with 7 resistance genes was the most (36.81%, 60 /163), while those with 5 resistance genes was least (3.07%, 5 /163). In the 33 isolates with 9 resistance genes, 28 strains had the genes *aac (6') / aph (2')*, *blaz*, *ermA*, *gyrA*, *LinA*, *msrA*, *su1*, *tetk* and *tetM*, while 5 strains had the genes: *aac (6') / aph (2')*, *blaz*, *ermA*, *gyrA*, *LinA*, *mecA*, *su1*, *tetk* and *tetM*.

Antibiotic susceptibility tests

A total of 15 antibiotics were used to identify the resistance of the isolates. The resistance rates of the isolates to the antibiotics were 0% (0/163) - 100% (163/163) (Table 7). The resistance rates of the isolates were clearly polarized. The resistant rate of the isolates to the 4 antibiotics Cefotaxime, Oxacillin, Cefoxitin and Vancomycin was ≤ 9.2% (15/163), while that to the remaining 11 antibiotics was ≥ 63.19% (103/163). Moreover, seven isolates that were resistant to Cefoxitin, were detected as *mecA*- positive by PCR, which indicated that the 7 strains were MRSA. According to the results of antibiotic susceptibility tests, the following four antibiotics (Vancomycin, Cefoxitin, Oxacillin, and Cefotaxime) are recommended for clinical treatment of pig - associated *s. aureus* infections.

In this study, each of the isolates was found to be resistant to a variety of antibiotics. The number of antibiotic resistance of all isolates was 4-14. The number of isolates resistant to 11 antibiotics was ≥ 63.19%, while that to the remainder of the isolates resistant to 10 kinds of antibiotics was the highest, with 48 strains, followed by the number of isolates resistant to 9 kinds of antibiotics, with 40 strains. The total number of these two types of isolates was 88 strains, accounting for 53.99%. The number (4 strains) of isolates resistant to each of the 4, 12, 13, 14 antibiotics was the least. This indicates that the antibiotic resistance of the pig - associated *S. aureus* was not optimistic in clinical treatment. All 4 isolates resistant to 4 antibiotics were pathogenic methicillin- susceptible *S. aureus*, which had 6 virulence genes and 5 antibiotic resistance genes, while their coagulase test was positive and had β - hemolysis. Moreover, all 4 isolates resistant to 14 antibiotics were pathogenic MRSA, which had 6 virulence genes and 9 antibiotic resistance genes, while their coagulase test were positive and had β-hemolysis.

Discussion

This is the first study of the characteristics of *S. aureus* from pigs in Hunan Province in China. 106 of all 163 isolates in this study were divided into 4 *agr* types. Due to the negative amplification by PCR with *agr* typing primers, the other 57 isolates could

not be typed by *agr*. In all *agr* types, the type with the largest number (30) of isolates was *agr*II, but the type with both the average largest number (5.92) of virulence genes and the average largest number (17) of pathogenic isolates was *agr*I, perhaps indicating that the *agr* I *S. aureus* are the most harmful to pigs. In a previous study the highest proportion of *S. aureus* isolates in all 4 *agr* types was *agr* I, at 39.2%, while it was *agr*IIin this study, at 18.40%. All isolates from the pig farms were identified as *agr* IV (Zhang 2018), while *agr*I, *agr*II, *agr*III and *agr* IV were identified in this study. The different results of these studies are worth further study.

Generally, *S. aureus* has a combination of virulence factors, which are thought to contribute to the pathogenicity (Edwards et al. 2010). The pathogenicity and virulence of *S. aureus* are associated with the capacity of this organism to produce several virulence factors, including enterotoxin serotypes A - Q (SEA - SEQ), toxic shock syndrome toxin -1 (TSST - 1), cytolytic toxins, exfoliative toxins, Hemolysin (hl) Panton--Valentine leukocyte (PVL), protein A, and several enzymes (McCormick et al. 2001). Hemolysin is an important virulence factor of *S. aureus*, which can destroy the red blood cells, platelets and lysosomes of human and animal hosts (Zhang 2018). In this study all 163 isolates carried a panel of virulence genes *hlb* and *hld,* while *hla* was detected positive from 158 isolates, including 38 pathogenic isolates and 120 non- pathogenic isolates. This indicates that *hl* genes (*hla*, *hlb,hl*d) are not directly related to the pathogenicity of *S.aureus*. In addition to the *hl* gene, other virulence genes are required to be involved in the pathogenesis of *S.aureus*. Panton-Valentine leucocyte (*pvl*) is a staphylococcal exotoxin which destroys leukocytes and phagocytes in animal hosts (Fan et al. 2019). In this study, the *pvl* genes were only detected positive from four non-pathogenic isolates. This may indicate that *S. aureus* from pigs does little damage to leukocytes and phagocytes in pigs.

The average number of both antibiotic resistance genes and virulence genes of the pathogenic isolates was higher than that of non-pathogenic isolates (Table 5). This indicates that pathogenic *S. aureus* is more resistant to antibiotics than non-pathogenic *S. aureus*. *S. aureus* easily achieves antibiotic resistance, the most typical of which is methicillin resistant *S. aureus* (MRSA) (Ramos et al. 2003, Tai 2019). In this study, we found that the MRSA-positive rate in 163 isolates was 4.29% (7strains), which is lower than that in Korea (Dong et al. 2018). Vancomycin is the first choice for the treatment of severe MRSA infection (Heng et al. 2021, Strommenger et al. 2003). However, with the increase of clinical dosage, *S. aureus* with reduced Vancomycin susceptibility and Vancomycin resistant *S. aureus* began to appear (Gao 2019, Lewis et al. 2011). It is gratifying to note that all isolates were Vancomycin- sensitive *S. aureus*, indicating that Vancomycin can be used to treat *S. aureus* infection in pigs in Hunan Province. It should be noted that although the resistance rate in a panel of antibiotics (Cefotaxime, Oxacillin, Cefoxitin and vancomycin) was below 10%, the remaining 11 antibiotic - resistant rates were above 63%. This indicates that, with the increase of antibiotics in pig farms, it is difficult to be optimistic in the treatment of *S. aureus* infection. With the rapid development of pig- farming in Hunan Province, more studies are needed to understand the epidemiology and characteristics of *S. aureus* in pigs and to elucidate its relationship with the human.

Conclusions

MRSA was found for only a very small number (7) of the 163 strains of pig- associated *S. aureus* isolates in Hunan Province. No Vancomycin- intermediate *S. aureus* and Vancomycin- resistant *S. aureus* were found here. The pathogenic *S. aureus* isolates were only a few (about 25%).

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