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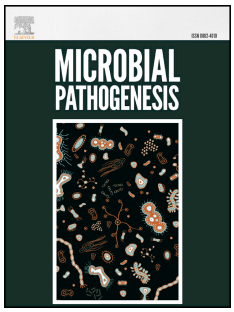

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Population structure and antimicrobial profile of *Staphylococcus aureus* strains associated with bovine mastitis in China

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Abstract

Staphylococcus aureus is a significant bacterial pathogen associated with bovine mastitis. The aim of the present study was to investigate and characterize of *S. aureus* strains isolated from the milk of cows suffering from mastitis in the mid-east of China. Among the 200 milk samples analyzed, 58 were positive for *S. aureus*, of these isolates, 11 isolates were methicillin-resistant *Staphylococcus* aureus (MRSA). All of the 58 *S. aureus* strains were classified in *agr* group I, while seven different sequence type (ST) patterns were identified and among them the most common was ST630 followed by ST188. All of the *S. aureus* isolates belonging to ST630 were resistant to more than four antimicrobials, and 22.2% of isolates belonging to ST188 were resistant to eight antimicrobials. Interestingly, while strong biofilm producers demonstrated higher resistance to multiple antimicrobials, they exhibited lower intracellular survival rates. The results of this study illustrated the distribution, antimicrobial susceptibility profiles, genotype, and the ability of biofilm production and mammary epithelial cells invasion of these *S. aureus* isolates. This study can provide the basis for the development of a disease prevention program in dairy farms to reduce the potential risk in both animal and human health.

Key words: *Staphylococcus aureus*; bovine mastitis; MLST typing; biofilm production; invasion assay.

1. Introduction

Milk production is an important branch of the Chinese agribusiness and China has become the third largest milk producing country in the world, even though most Asians are lactose intolerant. As the most important disease in the dairy industry worldwide, bovine mastitis can cause heavy economic burdens in dairy herds [1]. In China, several studies have reported the isolation of *S. aureus* in milk samples from cows with mastitis [2-5]; however, data related to molecular typing, biofilm production ability and the invasion of mammary epithelial cells by *S. aureus* from milk samples are scarce.

Because of the acquisition of the *mecA* gene, many *S. aureus* can be methicillin resistance (MRSA), which can cause important therapeutic problems when implicated in human or animal infections. However, interests in methicillin-susceptible *S. aureus* (MSSA) have also increased in recent years, as published reports have proven that MSSA can also be implicated in important infections and may help to explain the appearance and evolution of the different MRSA lineages [6]. Many different molecular typing methods have been developed for MSSA and MRSA genetic relevance study, such as phage typing [7], pulsed field gel electrophoresis (PFGE) [8]; multilocus sequence typing (MLST) [9], polymorphism of protein A gene (spa typing) [8] and accessory gene regulator gene (*agr* typing) [10].

The pathogenesis of *S. aureus* infection is very complex and worthy of making further research. Biofilm formation can impair the action of both the host immune system and the antimicrobial agent, which represents one of the most important survival mechanisms of bacteria persistently colonizing the extracellular niche [11]. *S. aureus* can produce a wide range of extracellular toxins, virulence factors, several exfoliatins and enterotoxins, which represent risks for humans and animals, as they are associated with severe infections.

Nevertheless, *S. aureus* is also known to penetrate, survive, and even multiply within a large variety of eukaryotic cells, such as the epithelial cells of the mammary gland or the immune cells [12]. This survival within the intracellular niche protects the bacteria from the antibiotics commonly used in mastitis treatment, and also enables them to persist in the host for a long time without causing apparent inflammation [13, 14].

The aim of the present work was thus to identify *S. aureus* strains gathered in 2014 from bovine mastitis milk in Jiangsu, China. *S. aureus* strains were studied further through *agr*-typing, MLST, antimicrobial susceptibilities, biofilm production and an invasion assay.

2. Materials and methods

2.1. Sample collection

In our study, samples were collected from three dairy farms, located in the north, center, and south areas, respectively, of Jiangsu Province in the year 2014. The three farms represent typical dairy production practices in each region. Milk samples were taken from cows with clinical mastitis, which manifested as decreased milk production, color change of the milk, and inflammation of the udder. Cotton swabs soaked in 70% ethanol were used to disinfect the surfaces of teats. The first few streams of milk were discarded. Then a milk sample was collected into a 10-ml sterile plastic tube. The collected samples were kept in a cooler with ice and transported to the laboratory within 8 hours.

2.2. Bacterial isolation and identification

Isolation and identification of *S. aureus* were performed according to China's National Technical Standard GB4789.10-2010. After incubation at 37°C for 24 h on Baird-Parker agar plates with 5% egg yolk and tellurite (BPA, Beijing Land Bridge Technology Ltd., Beijing, China), up to two presumptive colonies (black colonies surrounded by 2–5-mm clear zones) were selected from

a *S. aureus*-positive milk sample. Putative *S. aureus* isolates were further tested for hemolytic and coagulase activities, followed by PCR to identify the *S. aureus* specific gene *nuc1* [15]. All bacteria were routinely confirmed by the API STAPH® identification system (bioMe´rieux, Marcy l'Etoile, France).

S. aureus strains were tested for methicillin resistance using the disc diffusion method outlined in the Clinical Laboratory Institute Standards guidelines CLSI (M100-S25, 2015). Moreover, the *mecA* gene, which has been shown to confer methicillin resistance to *S. aureus* (MRSA), was also detected by PCR using primers described previously [16]. Only when the isolates were both *mecA* positive and cefoxitin resistant, which can be classified as MRSA.

2.3. Antimicrobial susceptibility testing

Antimicrobial resistance was determined by agar disk diffusion tests on Müeller-Hinton agar plates according to CLSI (M100-S25, 2015). In the agar disk diffusion tests, the isolates were tested against a total of 13 antimicrobials as follows: penicillin (P, 15 μg), ampicillin (AMP, 10 μg), azithromycin (AZM, 15 μg), cephalothin (KF, 30 μg), cefotaxime (CTX, 30 μg), ciprofloxacin (CIP, 5 μg), clindamycin (DA, 2μg), gentamicin (CN, 10 μg), kanamycin (KAN, 5 μg), ofloxacin (OFX, 5 μg), streptomycin (STR, 10 μg), tetracycline (TET, 30 μg) and sulfamethoxazole (SXT, 25 μg). In addition, susceptibility tests against vancomycin were performed using an MIC method according to CLSI guidelines. The standard reference strains *S. aureus* ATCC29213 and ATCC25923 served as quality control strains in every test run.

2.4. Genomic DNA extraction

A single colony of *S. aureus* was inoculated into LB culture medium, and the culture was shaken overnight at 37°C. Then the culture was used for preparation of genomic DNA using the TIANamp Bacteria DNA Kit (Tiangen Biotech Co., Ltd, Beijing, China) according to the manufacturer's instructions.

2.5. agr genotyping

The *agr* allele types (Ⅰ-Ⅳ) were determined by multiplex PCR as described [17]. In brief, multiplex PCRs were performed on 2 μl of DNA using Taq DNA polymerase (Yeasen Biotechnoligy Co. Ltd., Shanghai, China) and 1 μM of each of the following primers: Pan (5′-ATG CAC ATG GTG CAC ATG C-3′), agr1 (5′-GTC ACA AGT ACT ATA AGC TGC GAT-3′), agr2 (5′-TAT TAC TAA TTG AAA AGT GGC CAT AGC-3′), agr3 (5′-GTA ATG TAA TAG CTT GTA TAA TAA TAC CCA G-3′), and agr4 (5′-CGA TAA TGC CGT AAT ACC CG-3′). Amplifications were performed with the following PCR programme: 1 cycle at 94°C for 1 min; 26 cycles at 94°C for 30 s, 55°C for 30 s, and 72°C for 1 min; and finally 1 cycle at 72°C for 10 min. All PCR products were separated using gel electrophoresis on 1.5% agarose gels, stained with ethidium bromide and visualized under UV light.

2.6. Multilocus sequence typing (MLST)

MLST was carried out as described previously [9]. Specifically, the MLST analysis was conducted by sequencing fragments of seven house-keeping genes (*arcC*, *aroE*, *glpF*, *gmk*, *pta*, *tpi* and *yqiL*). Allele number and sequence types (STs) were assigned by using the *S. aureus* MLST website (http://saureus.mlst.net).

2.7. Biofilm production

Biofilm formation was evaluated by spectrophotometry in microplates using safranin staining [18]. The quantitative classification of biofilm production was based on cut-off values (ODc) and average OD values [19], leading to four categories of strains: OD ≤ ODc = not a biofilm producer; ODc < OD ≤ 2ODc = weak biofilm producer; 2ODc ≤ OD ≤ 4ODc = moderate biofilm producer; 4 OD < OD = strong biofilm producer.

2.8. Invasion assay

Bovine mammary epithelial cells (MAC-T) were used for in vitro bacterial internalization assays as previously described [18, 20, 21].

2.9. Statistical analysis

The antimicrobial resistance of *S. aureus* was analyzed using GraphPad PRIM software (version 5.02 for Windows; GraphPad software Inc.). To illustrate the evolutionary relatedness of *S. aureus* isolates, a population snapshot of the comparison of the MLST results of our sequence types (STs) with published STs was made using eBURST software, version 3.0 (Feil et al., 2004). A maximum likelihood tree was constructed with these STs to determine phylogenies. The best-fit substitution model was determined using MEGA 4.0 [22], and the likelihood of each model was measured with the Bayesian information criterion and corrected with the Akaike information criterion. The TrN93 model with a discrete gamma distribution (zG) allowing for invariant sites (zI) was used in the complete genome analysis.

3. Results

3.1. Isolation and identification of S. aureus

Among the 200 milk samples analyzed, 58 (29.0%) were positive for *S. aureus*, including 26 from Nanjing, 5 from Suzhou and 27 from Yangzhou. Of these 58 isolates, 11 isolates (19.0%, 11/58) harbored the *mecA* gene and were resistant to cefoxitin, thus were MRSA. All of these were isolated from a dairy farm in Yangzhou. Besides this, 6 *S. aureus* isolates (10.3%, 6/58) showed resistance to cefoxitin but did not carry the *mecA* gene.

3.2. Antimicrobial resistance phenotypes

Figure 1 shows the susceptibility of 58 *S. aureus* isolates to 21 antimicrobial agents. The most of the isolates were resistant to ampicillin (53/58, 91.4%) and penicillin (53/58, 91.4%), followed by ciprofloxacin (31/58, 53.4%), kanamycin (24/58, 34.5%) and streptomycin (13/58, 22.4%), and to a lesser

extent tetracycline (11/58, 19.0%), azithromycin (10/58, 17.2%), clindamycin (6/58, 10.3%) and ofloxacin (6/58, 10.3%) (Fig.1). However, no isolates were resistant to cephalothin and vancomycin, and the *S. aureus* isolates showed low resistance to cefotaxime and sulfamethoxazole (resistance rate ranged from 1.7% to 3.5%) (Fig.1). Fifty-five (94.8%) *S. aureus* isolates were resistant to at least one antimicrobial and the percentage of resistance to 1–3 antimicrobials was 72.4% (Table 1). In particular, approximately 22.4% of isolates was resistant to ≥ 5 antimicrobials (Table 1).

Fifty-five resistant *S. aureus* isolates belonging to 20 drug-resistance patterns were identified, with the most frequent being resistant to AMP CIP KAN P (15/55, 17.3%), and 9 MRSA isolates belonged to this drug-resistance pattern (Table 1). In addition, other resistance patterns such as AMP P (11/55, 19.0%) and AMP CIP OFX P (5/55, 9.1%) were also wide spread. Though various resistance patterns were identified, certain drugs resistance (such as: AMP, CIP, KAN, P) were found in different resistance patterns. In addition, four strains isolated from Suzhou were resistant to eight antibiotics (AMP AZM CIP CN DA P S TET) (Table 1).

3.3. agr-typing and MLST

Through multiplex PCR, the *agr* alleles were successfully identified in 58 isolates. All of the isolates were classified as *agr* I (Table 2). From the MLST results, seven different ST patterns were identified among the 58 *S. aureus* isolates (Table 1). ST630 and ST188 were the prominent STs in this study, represented by 24 and 18 *S. aureus* isolates respectively, followed by ST97, ST6 and ST71, while one strain displayed ST1 and one strain displayed ST5 (Table 1).

With the less stringent group definition of 5/7 shared alleles, five clusters of linked STs CC (clonal complex) were displayed in the snapshot, along with

two unlinked STs (Fig.2). Through cluster analysis, four clonal complexes CC1, CC5, CC97 and CC188 were found. ST97 was the primary founder of CC-97 and ST-71 was one of the SLVs (single-loucus) of it. CC-97 was the largest CC with 21 SLVs. However, we also found an uneven distribution of STs in the three cities and each city had its own particular STs. For example, ST97 and ST6 were only found in Nanjing, while ST630 and ST71 were identified in Yangzhou (Fig.2).

Phylogenetic trees revealed the relationship between our ST types with other ST types identified in mastitis milk in China (Fig.2). By cluster analysis, all unique STs were separated into two big clusters and our ST types (isolates from Jiangsu) all belonged to A clusters, including most of the mastitis-related ST types in China (Fig.3). ST-97 was closely related to ST-71 in A clusters, which were widely distributed in China, except Shandong province (Fig.3). It also had the nearer evolutionary distance with the clinic and subclinical mastitis -related ST types in Xinjiang, Zhejiang, Jiangsu and Neimeng. The genetic relationships among other STs in both clusters were far, especially ST-2166, which had the farthest genetic distance with other ST types in B cluster (Fig.3).

3.4. Biofilm production

In TSB_{glc}, only three strains (5.2%) did not produce any biofilm, while the majority were biofilm producers, respectively weak (n = 1, 1.7%), moderate (n = 7, 12.1%) or strong (n = 47, 81.0%) producers (Table 2).

3.5 Invasion assay

We observed that 54 strains (93.1%) showed an invasion rate lower than 1% of the initial inoculum, while only four strains (6.9%) showed an invasion rate greater than 1% (Table 2). We found that these four strains showed a high invasion rate in MAC-T cells, formed no or a weak biofilm in TSB_{glc}. In contrast, most strains showed a low invasion rate in MAC-T cells and formed a strong

biofilm in TSB_{glc} (Table 2).

4. Discussion

Many previous studies showed that members of the genus *Staphylococcus* were the major causative agent of bovine mastitis in China (Cao et al., 2010; Liu et al., 2007). In this study, we characterized 58 *S. aureus* isolates from bovine mastitis milk samples collected from Jiangsu province in China. The overall prevalence of *S. aureus* contamination was approximately 29.0%. This result is higher than that of a previous study conducted in China [4] and other developing countries, such as Turkey and Brazil [6, 23]. Moreover, the *S. aureus* contamination rate was higher than that in developed European countries and the United States [24, 25]. Different sampling seasons, sampling procedures, and isolation methods could affect the *S. aureus* prevalence results. Better sanitation in cow fairy, use of preharvest surveillance and control of *S. aureus* may account for the lower contamination rates in developed countries.

Antimicrobial resistance in *S. aureus* has become a significant public health concern. The antimicrobial resistance level in our study was relatively high. In the present study, the highest rates of antimicrobial resistance were against ampicillin and penicillin (both 91.4%), which are the most widely used antimicrobials in the treatment of bovine mastitis. This result was somewhat expected and agreed with previous reports from China and Brazil [3, 26]. However, the high rate of ciprofloxacin, streptomycin and ofloxacin resistance observed in this study is of concern. These antimicrobial agents are still widely used in human therapy in China because of their low cost and ready availability. Therefore, resistance to these antimicrobials by foodborne pathogens may generate problems for human disease treatment.

Our results indicated that 94.8% of the *S. aureus* isolates were resistant to at least one antimicrobial agent. Thirteen out of 58 staphylococcal isolates were

resistant to five or more antimicrobial classes including most of the commonly used antimicrobials in the region (Table 1). These results were similar to those previously reported in China [4, 5]. However, several clinical studies have reported lower levels of antimicrobial resistance among mastitic *S. aureus* in the United States and in European countries [27]. The uncontrolled use of antibiotic in the region could be a reason for the spread of multidrug-resistant isolates. In chronic and localized infections like mastitis, this may largely be dependent on the bacterium's ability to form biofilms in which bacterial cells can evade the antibiotic and host defenses employed to inhibit colonization and subsequent infections [28]. In the present study, most of the *S. aureus* isolates (47/58, 81.0%) were strong biofilm producers. This may be one of the reasons why the antimicrobial resistance level was relatively high in our study. Moreover, 90.0% of MRSA isolates could form biofilms in TSB_{glc}. Importantly, MRSA isolates that form biofilms also develop resistance to all the commonly used antibiotics to which the planktonic bacteria are susceptible .

MLST results revealed that ST630 was the most common genotype in isolates recovered from milk in this study. According to the *S. aureus* MLST database (http://saureus.mlst.net), strains belonging to ST630 have appeared only in bulk milk in Japan. ST1 and ST5, reported previously in major human *S. aureus* infections in Asia, Africa and Europe, were found in mastitis milk for the first time in our study. However, ST97, which were represented by seven isolates in our study, has been reported extensively in clinical and subclinical mastitis milk in multiple regions in China. ST97 can form various ST types through active locus mutation. For example, ST71 may have originated from ST97, and both ST71 and ST97 belong to CC-97, which includes mainly strains of clinical and subclinical bovine mastitis all over the world (Fig. 2). ST71 found in this study was reported for the first time in Asia. It was only found in

Europe and may appear from the single-locus mutation of ST97. Phylogenetic analyses revealed that the majority of STs in our study all belonged to cluster A and showed a close genetic relationship (Fig. 3). This supports the hypothesis that *S. aureus* is a clonal organism and spreads from cow to cow. In our study, isolates belonging to ST630 were frequently associated with the ACKP type (resistance to AMP, CIP, Kan, and Penicillin) and all MRSA isolates belonged to the ST type. This indicated the frequent emergence of ST630 *S. aureus* with high resistance rate in China [2]. The increasing prevalence of the MDR *S. aureus* ST630 clone will pose a considerable threat to the control of clinical *S. aureus* infections.

Investigation of MRSA from farm animals has intensified all over the world during recent years. However, limited information is available for the genetic characterization of MRSA from dairy cows in China [5, 29]. In the present study, using phenotypic and genotypic methods, 11 MRSA were identified from 58 *S. aureus* isolates. All these MRSA were found in Yangzhou city. We have learned that β-lactams were widely used for the treatment and prevention of bovine mastitis in this farm. Although effective, the massive use of these antimicrobials may exert a selection pressure favoring the emergence of methicillin-resistant *S. aureus* strains. Meanwhile, we also found five phenotype-positive MRSA isolates, which were *mecA* negative. The *mecA*-negative but phenotype-positive MRSA have been previously reported in Turkey and India [30, 31]. Recently, the presence of *mecA*-negative MRSA strains in bovine milk samples has also been reported in China [32]. In our study the *mecA*-negative MRSA strains showed high levels of antimicrobial resistance, and possessed a multi-drugs resistant phenotype AMP CIP OFX P (Table 1). However, most of the previous studies were based on genotype tests for identifying MRSA, which may misidentify these isolates as methicillin-sensitive

320 *S. aureus* (MSSA) and underestimate the true prevalence of MRSA. Thus, a
321 combination of genotypic and phenotypic tests is necessary to avoid false
322 positive or false negative results in identifying MRSA.

323 In the present study, the 58 isolates were classified as *agr* I by multiplex
324 PCR (Table 2). This finding agrees with a previous observation, where 88% of
325 the bovine *S. aureus* strains were classified in *agr* group I [33]. It was revealed
326 that the bovine *S. aureus* strains in Jiangsu area mainly belonged to *agr* group I.
327 Similarly, a study of human *S. aureus* showed that *agr* group I was prevalent
328 among clinical strains [34]. The *agr* group has been frequently associated with
329 MAC-T cell invasion and *in vivo* persistence. In fact, *agr* group I *S. aureus*
330 strains show a higher invasion rate than other *agr* groups [18]. Moreover, *agr*
331 group I *S. aureus* strains, which were *cap*5-positive but tested negative *in vitro*
332 for CP5 ELISA, showed a high invasion rate in MAC-T cells and formed a weak
333 biofilm in TSB_{glc} [18]. However, previous studies have obtained different results.
334 They showed that there was no association of the capsular phenotype with
335 biofilm formation of *S. aureus* strains from clinical bovine mastitis [35].
336 Oliveira *et al.* (2011) suggested that *S. aureus* invasion levels were not related to
337 biofilm formation. In the present study, four strains showed a high invasion rate
338 in MAC-T cells and formed no or weak biofilms in TSB_{glc}. In contrast, most
339 strains showed a low invasion rate in MAC-T cells and formed strong biofilms
340 in TSB_{glc}.

341 The present study describes the epidemiology and characteristic of *S. aureus*
342 from milk samples in Jiangsu located in the mid-east of China. However, the
343 study design and in particular the less number of sampling area and samples
344 limit the conclusions regarding correlate properties of which may be associated
345 with persistent mastitis. Further research is essential to using a larger strain
346 collection with isolates associated cow mastitis from more farms and more

diverse geographical locations in China, which can provide the basis for the development of a disease prevention program in dairy farms to reduce the potential risk in both animal and human health.

Conflict of interest

There are no conflicts of interest.

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Figure legends

Fig. 1. Distribution of antimicrobial resistance towards different classes of antibiotics among the 58 *Staphylococcus aureus* isolates in 2014.

Fig. 2. Population snapshot of all STs related to bovine mastitis included in the MLST database for *Staphylococcus aureus*. The snapshot displays the evolutionary relationships among STs found in this study and those in the database (all ST associated with bovine mastitis worldwide). The STs identified in this study are marked in red. The STs grouped based on the less stringent definition of five of seven shared alleles are connected by lines.

Fig. 3. Maximum composite likelihood tree showing the genetic relationships among the merged sequences of seven housekeeping gene fragments from *Staphylococcus aureus* recovered in this study and the ST type reported in China.

Table 1. Profile diversity of *Staphylococcus aureus* isolates based on MLST, serotyping, antimicrobial resistance, biofilm formation and intracellular survival

ST (n=58)	Allele profiles	MecA	FOX	City	Antimicrobial resistance[a]	No. of isolates	Biofilm formation				Intracellular survival	
							No n=3	Weak n=1	Moderate n=7	Strong n=47	<1%	>1%
ST1 (n=1)	(1,1,1,1,1,1,1)			Nj	AMP P	1	0	0	0	1	1	0
ST5 (n=1)	(1,4,1,4,12,1,10)			Nj	AMP KAN P	1	0	0	0	1	1	0
ST6 (n=4)	(12,4,1,4,12,1,3)			Nj	AMP P	3	0	0	0	3	3	0
				Nj	AMP P	1	0	0	1	0	1	0
ST71 (n=3)	(18,1,1,1,1,5,3)			Yz	AMP CIP CN OFX P S	1	0	0	1	0	1	0
				Yz	AMP CN P S	1	0	0	1	0	1	0
				Yz	AMP P S	1	0	0	0	1	1	0
ST97 (n=7)	(3,1,1,1,1,5,3)			Nj	AMP CN P TET	2	2	0	0	0	1	1
				Nj	AMP AZM CN P TET	1	0	0	0	1	1	0
			+	Nj	AMP AZM KAN CN P S TET	1	0	0	1	0	1	0
				Nj	AMP P S TET	1	1	0	0	0	0	1
				Nj	AMP P TET	1	0	0	0	1	1	0
				Nj	SXT	1	0	0	0	1	1	0
ST188 (n=18)	(3,1,1,8,1,1,1)			Nj	AMP P	6	0	0	0	6	6	0
				Sz	AMP AZM CIP CN DA P S TET	4	0	0	0	4	4	0
				Sz	AMP AZM CIP CN DA P S	1	0	0	0	1	1	0
				Nj	AMP AZM CIP DA P TET	1	0	0	0	1	1	0
				Nj	AMP KAN P S	1	0	0	0	1	1	0
				Nj	AMP KAN P	1	0	0	0	1	1	0
				Nj	KAN	1	0	0	0	1	1	0
				Nj		2	0	0	0	2	2	0
				Nj		1	0	1	0	0	0	1
ST630 (n=24)	(3,1,1,4,4,3)			Yz	AMP AZM CIP KAN P S	1	0	0	0	1	1	0
		+	+	Yz	AMP AZM CIP KAN P S	1	0	0	0	1	1	0
				Yz	AMP CIP CTX KAN P	1	0	0	1	0	1	0
		+	+	Yz	AMP CIP CTX KAN P	1	0	0	0	1	1	0
				Yz	AMP CIP KAN P	5	0	0	0	5	5	0
				Yz	AMP CIP KAN P	1	0	0	1	0	1	0
		+	+	Yz	AMP CIP KAN P	9	0	0	0	9	9	0
			+	Yz	AMP CIP OFX P	4	0	0	0	4	4	0
			+	Yz	AMP CIP OFX P	1	0	1	0	0	0	1

[a]Antimicrobial resistance: P, penicillin; AMP, ampicillin; AZM, azithromycin; KF, cephalothin; CTX, cefotaxime; CIP, ciprofloxacin; DA, clindamycin; CN, gentamicin; KAN, kanamycin; OFX, ofloxacin; STR, streptomycin; TET, tetracycline; SXT, sulfamethoxazole; VA, vancomycin.

Table 2. MLST, MRSA, agr group, biofilm formation and intracellular survival frequency distribution

				Biofilm formation				Intracellular survival[a]	
MLST		**MRSA**	**agr I**	**No**	**Weak**	**Moderate**	**Strong**	**<1%**	**>1%**
ST1	1	0	1	0	0	0	1	1	0
ST5	1	0	1	0	0	0	1	1	0
ST6	4	0	4	0	0	1	3	4	0
ST71	3	0	3	0	0	2	1	3	0
ST97	7	0	7	3	0	1	3	5	2
ST188	18	0	18	0	0	1	17	17	1
ST630	24	11	24	0	1	2	21	23	1
Total	58	11	58	3	1	7	47	54	4
	Intracellular survival		**<1%**	4	1	9	40		
			>1%	2	1	1	0		

[a]**:** Intracellular survival is presented as the % of the initial inoculum.

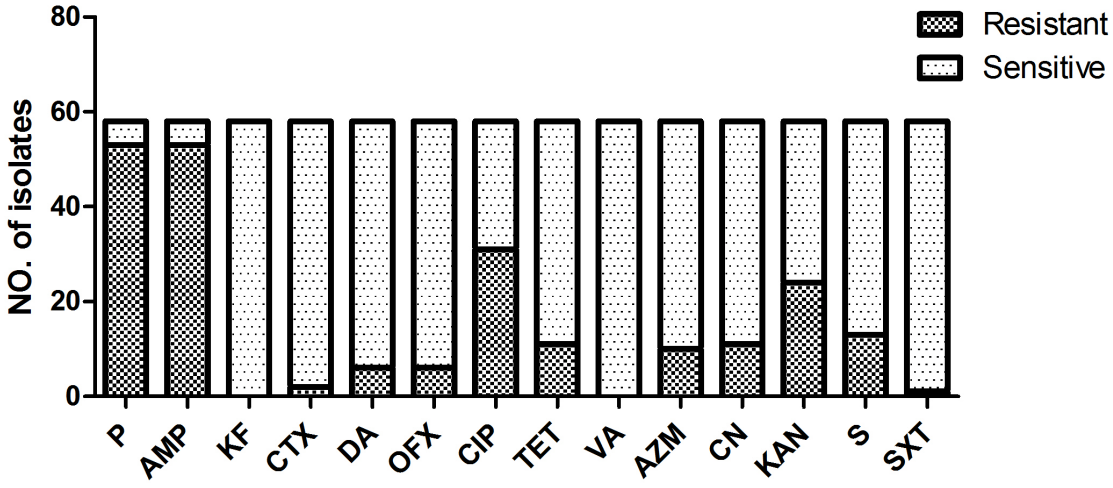

Fig.1

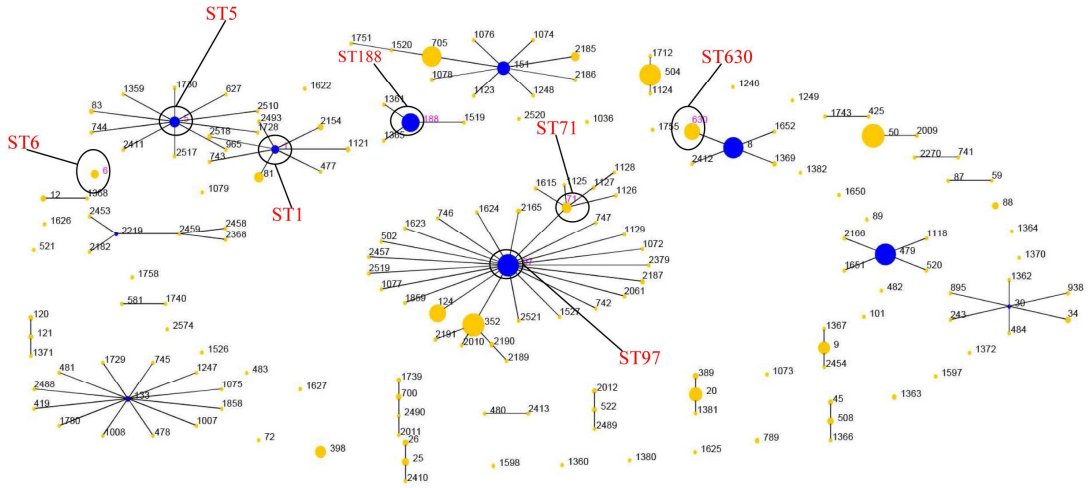

Fig. 2

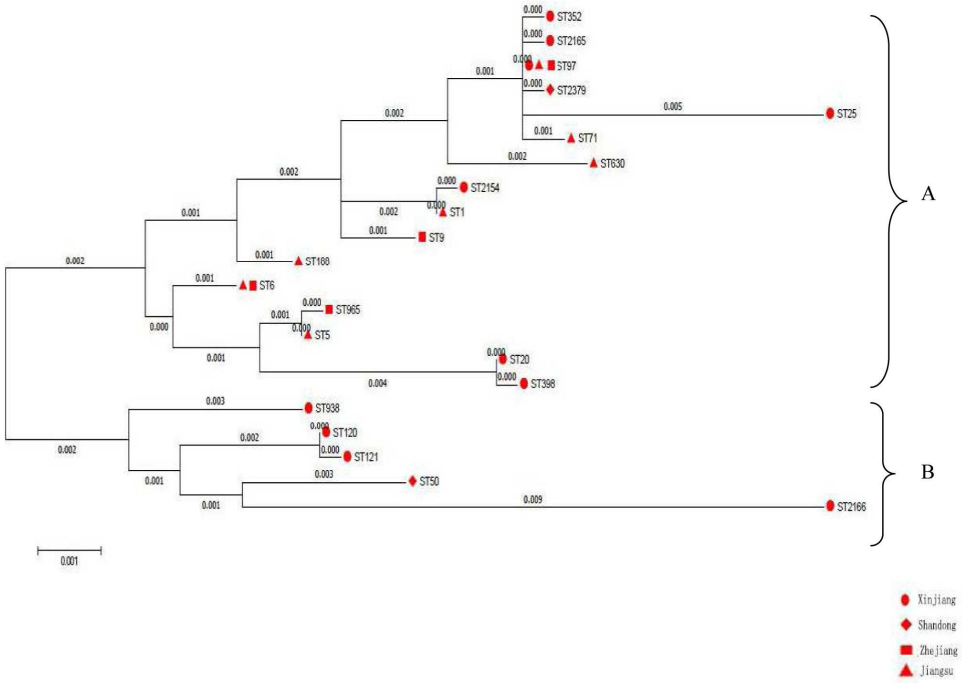

Fig. 3

Highlights

The 58 (29.0%) *S. aureus* strains causing bovine mastitis were isolated from Jiangsu of China, and 19.0% was MRSA.

All of the 58 *S. aureus* isolates were classified in *agr* group I.

ST630 and ST188 were the prominent STs in this study.

Approximately 22.4% of isolates was resistant to ≥ 5 antimicrobials.

Most strains showed a low invasion rate in MAC-T cells and formed a strong biofilm in TSB_{glc}.