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RESEARCH ARTICLE

Genotypes, Virulence Factors and Antimicrobial Resistance Genes of *Staphylococcus aureus* Isolated in Bovine Subclinical Mastitis from Eastern China

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ABSTRACT

This study was carried out to determine the genotypes, virulence factors and antimicrobial resistance traits of 34 *Staphylococcus aureus* isolated from subclinical mastitis in Eastern China. Minimal inhibitory concentration (MIC) results showed resistance to erythromycin in all isolates. A high frequency of Methicillin resistant *S. aureus* (MRSA; 29%) was observed and these isolates were also highly resistant to penicillin, oxacillin, oxytetracycline and chloramphenicol than methicillin sensitive *S. aureus* (MSSA) isolates. Thirteen pathogenic factors and seven resistance genes including *mecA* and *blaZ* gene were checked through PCR. The *spaX* gene was found in all isolates, whereas *cna*, *spaIg*, *nuc*, *clfA*, *fnbpB*, *hlA*, *hlB* and *seA* were present in 35, 79, 85, 59, 35, 85, 71 and 38% isolates, respectively. Nine isolates carried a group of 8 different virulence genes. Moreover, macrolide resistance genes *ermB* and *ermC* were present in all isolates. High resistance rate against methicillin was found but no isolate was positive for *mecA* gene, whereas *blaZ* and *tetK* were detected in 82 and 56% isolates, respectively. Genes; *fnbpA*, *seB*, *seC, seD, dfrK* and *tetM* were not found in any isolate. The statistical association between phenotypic resistance and virulence genes showed, *clfA*, *fnbpB, hlB* and *seA,* were potentially associated with penicillin G, ciprofloxacin, methicillin, chloramphenicol, trimethoprim and oxytetracycline resistance ($P \leq 0.05$). REP-PCR based genotyping showed seven distinct genotypes (A-G) prevalent in this region. This study reports the presence of multidrug resistant *S. aureus* in sub-clinical mastitis which were also highly virulent that could be a major obstacle in the treatment of mastitis in this region of China.

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INTRODUCTION

Staphylococcal mastitis is a major concern in dairy farming and critical source of subclinical and clinical intra-mammary infections in dairy cows leading to severe economic losses to the dairy industry, worldwide (Momtaz *et al*., 2010; Atasever, 2012; Hussain *et al*., 2012a). Naturally, *Staphylococcus aureus* isolates are inhabitants of mucous epithelia and skin of human, dairy cattle and other mammalians (Chu *et al*., 2012), and spread by virtue of milker's hand/milking machines (Seki *et al*., 1998). β-lactams antibiotics are frequently used for treatment of *S. aureus* mastitis as well as intra-mammary infusion for preventive measures in dry cows. Improper use of antimicrobials has resulted in augmenting the bacterial resistance mechanism including the β-lactamase production and low-affinity penicillin binding protein 2a (PBP2a). The *S. aureus* exhibited resistance to methicillin was first reported in 1960, by the time MRSA gradually developed multiple resistances and became a source of causing serious nosocomial infections, worldwide (David and Daum, 2010). The pathogenic potential of *S. aureus* depends on numerous cell surface virulence factors and it has capability of producing a variety of exotoxins and cell surface-associated proteins that enhances the cellular attachment, organism invasion to host immune system and

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stimulation of toxic tissue reactions (Kalorey *et al*., 2007; Hussain *et al*., 2012b). It has been reported that in divergent geographical areas a limited diversity of *S. aureus* strains is involved in mastitis infection (Moon *et al*., 2007). Therefore, genotyping of isolates is necessary to identify the genetic relatedness of strains and their source of spread; and one of the reliable and broad genotyping methodologies is repetitive element sequence-based PCR or REP-PCR (Del Vecchio *et al*., 1995). *S. aureus* strains are capable of mutation, clonal evolution and horizontal gene transfer that boost up the virulence and drug resistance (Brody *et al*., 2008). Hence, identification of pathogenic and resistant *S. aureus* from intra mammary infection at herd level is of vital importance for successful treatment. A little information was available on diversity of bovine mastitis *S. aureus* isolates in China, particularly in eastern region. This study reports the genotypic distribution, virulence and resistance patterns of *S. aureus* strains isolated from mastitic cattle in east China.

MATERIALS AND METHODS

Identification of *S. aureus* isolates: All the subclinical mastitis derived 34 *S. aureus* isolates identified by a chain of laboratory techniques and their confirmation through PCR is reported in our earlier study (Memon *et al*., 2013). *S. aureus* isolates were retrieved from microorganism storage system and cultured overnight in TSB broth at 37°C for further experimental use. All the isolates were tested for their Coagulase reaction according to the standard procedure. Briefly, *S. aureus* isolates along with ATCC 29213 (positive control) were cultured in TSB medium overnight. All the 36 tubes were filled with 0.5 ml of 1 in 10 diluted rabbit plasma and labeled. To the tubes labeled as test; 0.1ml of fresh culture of test bacteria were added, to the tubes labeled as positive control; 0.1ml of ATCC strain culture was added and to the tubes labeled as negative control; 0.1ml of sterile broth was added and incubated at 37°C for four hours. After four hours, all the tubes were examined for formation of gel by inverting them.

Antimicrobial susceptibility test: Antimicrobial susceptibility test of *S. aureus* isolates was determined by standard broth dilution method on Muller–Hinton (MH) medium (Oxide, UK). A concentration of 1280µg/ml for erythromycin (Sigma, USA) was used to screen the drug sensitivity of isolates. MIC results were interpreted in accordance with Clinical Laboratory Standards Institute standards (Anonymous, 2010) and ATCC 29213 was used as quality control strain for MIC interpretation of isolates.

Virulence genes (VGs) and Antimicrobial resistance genes (ARGs): Using conventional PCR, all the *S. aureus* isolates were assessed for the presence of putative pathogenic factors including surface protein in the X-region of protein A (*spa*), immunoglobulin-binding region (*Ig*), adhesions including clumping factor A (*clfA*), fibronectin-binding proteins A and B (*fnbpA* and *fnbpB*), hemolysins (*hlA* and *hlB*) and enterotoxins (*seA, seB, seC and seD*), *nuc* gene encodes the thermostable nuclease and *cna* encodes for collagen-binding protein. Antimicrobial resistance genes including *mecA* encoding for methicillin resistance, *blaZ* for β-lactams, *ermB* and *ermC* for erythromycin, *tetK* and *tetM* for tetracycline, *dfrK* encoding for trimethopirm resistance were screened. Oligonucleotide sequences of VGs and ARGs, their amplicon sizes and annealing temperatures are enlisted in Table 1.

Genotyping: Genomic DNA was extracted by using DNA purification kit (Geneaid Biotech, Taiwan). The REP-PCR used for amplifying RW3A primers in 50µl reaction was mixture containing 5µl DNA template. The amplification conditions were: initial denaturing step of 5 min at 94°C, following 35 cycles; each consists of 1.30 min at 94°C, annealing of 1min at 54°C and extension at 72°C for 2.30 min and final extension at 72°C for 20 min. On accomplishment of PCR, the product was electrophorased in 2% agarose gel stained with gold view at 60 V for approximately 4 hrs. The DNA fingerprints were visualized using UV light trans-illuminator (BIO-RAD, USA) and photographed for further analysis. Quantity-1 software used to analyze the banding patterns on gel and SPSS data editor was used for statistical analysis and plotting a dendrogram using Hierarchical Cluster Analysis method (average linkage between groups).

Statistical association between phenotypic resistance and Virulence genes: To examine the significance of the association (contingency) between two kinds of classification (phenotypic resistance and VGs), the Fisher Exact Tests online model was used (Danielsoper.com, Statistics calculator for 2×2 contingency table, Beta 3 version). The association was considered as significant when $P \leq 0.05$.

RESULTS

All the *S. aureus* isolates were coagulase positive and were resistant to erythromycin. The recorded resistance to enrofloxacin was (3%), penicillin G (47%), ampicillin (91%), oxacillin (9%), ciprofloxacin (26%), trimethoprim (56%), methicillin (29%), chloramphenicol (32%) and (59%) against oxytetracycline (Memon *et al*., 2013). Furthermore, MIC results of MRSA and MSSA isolates were separated; MRSA isolates were highly resistant as compare to MSSA (Fig. 1). Of thirteen virulence genes investigated, only nine were found. All the *S. aureus* were positive for the *spaX* gene but all the MRSA were negative for *mecA* gene. While 9 virulence and 4 resistance genes were present in the isolates (Fig. 2 & 3). None of virulent genes (VG) found alone in any strain, majority of *S. aureus* isolates conserved at least two and maximum 8 VGs in a group, whereas most of isolates harbored 2-4 resistance genes in a group. Nine isolates carried 8 virulence genes and 6 virulence genes were harbored by nine isolates (Fig. 4). The statistical analysis of association between virulence genes and recorded phenotypic resistance showed that *clfA* was potentially associated with penicillin G and oxytetracyline, *fnbpB* was significantly associated with penicillin G, ciprofloxacin, methicillin and chloramphenicol, *hlB* was potentially associated with trimethoprim and oxytetracycline and *seA* showed strong association with penicillin G and methicillin, in all cases ($P \leq 0.05$). Whereas, *seA* showed possible association with

Table 1: Oligonucleotide sequences, size of fragment, annealing temperature and references for VGs and ARGs in *S. aureus* isolates

Gene	Oligonucleotide sequences	Size in bp	Annealing	Reference
SpaX	CAA GCA CCA AAA GAG GAA CAC CAG GTT TAA CGA CAT	150-315	60	(Fre´nay *et al*., 1996)
spaIg	CAC CTG CTG CAA ATG CTG CG GGC TTG TTG TTG TCT TCC TC	900-1000	58	(Seki *et al*., 1998)
clfA	GGC TTC AGT GCT TGT AGG TTT TCA GGG TCA ATA TAA GC	900-1000	57	(Stephan *et al*., 2001)
blaZ	AAG AGA TTT GCC TAT GCT TC GCT TGA CCA CTT TTA TCA GC	517	55	(Vesterholm-Nielsen *et al*., 1999)
mecA	GTG AAG ATA TAC CAA GTG ATT ATG CGC TAT AGA TTG AAA GGA T	147	55	(Zhang *et al*., 2005)
Nuc	GCGATTGATGGTGATACGGTT ACGCAAGCCTTGACGAACTAAAGC	280	55	(Brakstad *et al*., 1992)
FnbpB	GGAGAAGGAATTAAGGCG GCCGTCGCCTTGAGCGT	820-1000	50	(Booth *et al*., 2001)
hlA	GGTTTAGCCTGGCCTTC CATCACGAACTCGTTCG	550	53	(Booth *et al*., 2001)
hlB	GCCAAAGCCGAATCTAAG GCGATATACATCCCATGG C	840	62	(Booth *et al*., 2001)
seA	GCAGGGAACAGCTTTAGGC GTTCTGTAGAAGTATGAAACACG	521	68	(Monday and Bohach, 1999)
seB	ACATGTAATTTTGATATTCGCACTG TGCAGGCATCATGTCATACCA	667	68	(Monday and Bohach, 1999)
seC	CTT GTA TGT ATG GAG GAA TAA CAA TGC AGG CAT CAT ATC ATA CCA	284	66	(Monday and Bohach, 1999)
seD	GTG GTG AAA TAG ATA GGA CTG C ATA TGA AGG TGC TCT GTG G	385	66	(Monday and Bohach, 1999)
FnbpA	CCGGAGAGGAGACTTCACAGA TCCACGATTTCCCAGAGAAC	1214	62	(Palma *et al*., 2001)
*erm*B	ACGACGAAACTGGCTAA TGGTATGGCGGGTAA	409	53	(Gao *et al*., 2011)
*erm*C	CTTGTTGATCACGATAATTTCC ATCTTTTAGCAAACCCGTATTC	190	55	(Gao *et al*., 2011)
SpaX	CAA GCA CCA AAA GAG GAA CAC CAG GTT TAA CGA CAT	150-315	60	(Fre´nay *et al*., 1996)
spaIg	CAC CTG CTG CAA ATG CTG CG GGC TTG TTG TTG TCT TCC TC	900-1000	58	(Seki *et al*., 1998)
clfA	GGC TTC AGT GCT TGT AGG TTT TCA GGG TCA ATA TAA GC	900-1000	57	(Stephan *et al*., 2001)
blaZ	AAG AGA TTT GCC TAT GCT TC GCT TGA CCA CTT TTA TCA GC	517	55	(Vesterholm-Nielsen *et al*., 1999)
mecA	GTG AAG ATA TAC CAA GTG ATT ATG CGC TAT AGA TTG AAA GGA T	147	55	(Zhang *et al*., 2005)

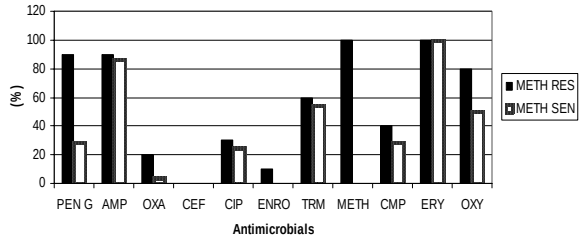


Fig. 1: MIC interpretation of Methicillin resistance (10) and sensitive (24) isolates

trimethoprim and ciprofloxacin, as P values were 0.053 and 0.059, respectively (Table 2). REP-PCR generated banding patterns of *S. aureus* isolates ranges from 150bp to 1800bp in size. The DNA polymorphism based genotype analysis suggested the existence of 34 REP-profiles, which were arranged by dendrogram analysis in seven distinct genotypes (A, B, C, D, E, F and G) (Fig. 4).

DISCUSSION

S. aureus is a major causative organism of mastitis, its emergence as multi drug resistant has become a deep concern for dairy industry worldwide. Considering the MRSA and its zoonotic importance, we probed *S. aureus* for presence of MRSA. This study reports 100% isolates were multi-drug resistant that is alarming, while comparatively a less percentage 52% of isolates were also reported as multi drug resistant in Ethiopia (Sori *et al*., 2011). Methicillin resistance was found in high percentage of *S. aureus* isolates (29%) which is greater than reported in Korea and India (Moon *et al*., 2007; Kumar *et al*., 2010), but there was no *mecA* gene found in any isolate. It is well reported that emergence of drug resistance is the consequence of the improper use of antimicrobials (Kumar *et al*., 2010; Kenar *et al*., 2012). Resistance against beta-lactams and presence of *blaZ* gene in our isolates is in agreement with the Green and Bradley (2004), who reported that *S. aureus* resistance to beta-lactams is due to production of beta-lactamase. The *blaZ* gene detected in majority of isolates and *mecA* was not found in any isolates, these findings are consistent with previous report (Haveri *et al*., 2007). MIC results revealed that phenotypically methicillin resistant isolates were more resistant to the other tested antimicrobials if compared to the methicillin susceptible (MSSA) isolates; these results support the finding of Moon *et al*. (2007). Moreover, all the isolates were resistant to erythromycin and were positive for *ermB* and *ermC* genes which were also found in high frequency among mastitis *S. aureus* isolated in northern China (Gao *et al*., 2011). Tetracycline resistance encoding gene *tetK* was present in 56% of isolates which is lower than previously detected in 96%

Table 2: Statistical association of virulence genes and phenotypic resistance in mastitis *S. aureus* isolates

Antibiotic	Criteria	Virulence gene (Positive isolates)						
	(Positive isolates)	*nuc* (29)	*spa-IG* (27)	*clfA* (20)	*fnbpB* (12)	*hlA* (29)	*hlB* (24)	*seA* (13)
Penicillin G	Res (16)	16 (100)	13 (81)	4 (25)	2 (12)	15 (94)	14 (87)	10 (62)
	Sus (18)	13 (72)	14 (78)	16 (89)	10 (55)	14 (78)	10 (55)	3 (17)
	P value	0.163	0.202	0.043*	0.051*	0.186	0.148	0.050*
Ampicillin	Res (31)	28 (90)	27 (100)	18 (58)	12 (39)	29 (100)	21 (68)	13 (42)
	Sus (3)	1 (33)	0 (0)	2 (67)	0 (0)	0(0)	3 (100)	0 (0)
	P value	0.291	0.166	0.359	0.394	0.151	0.299	0.369
Oxacillin	Res (3)	3 (100)	3 (100)	1 (33)	3 (100)	3 (100)	1 (33)	1 (33)
	Sus (31)	26 (84)	24 (77)	19 (61)	9 (29)	26 (84)	23 (74)	12 (39)
	P value	0.321	0.315	0.378	0.140	0.321	0.338	0.436
Ciprofloxacin	Res (9)	7 (78)	5 (56)	9 (100)	7 (78)	9 (100)	7 (78)	7 (78)
	Sus (25)	22 (88)	22 (88)	11 (44)	5 (20)	20 (80)	17 (68)	6 (24)
	P value	0.223	0.188	0.090	0.034*	0.203	0.227	0.059
Trimethoprim	Res (19)	14 (74)	16 (84)	12 (63)	10 (53)	18 (95)	19 (100)	11 (58)
	Sus (15)	15 (100)	11 (73)	8 (53)	2 (13)	11 (73)	5 (33)	2 (13)
	P value	0.167	0.199	0.215	0.070	0.179	0.043*	0.053
Methicillin	Res (10)	10 (100)	6 (60)	6 (60)	8 (70)	10 (100)	8 (80)	8 (80)
	Sus (24)	19 (79)	21 (87)	14 (58)	4 (21)	19 (79)	16 (67)	5 (21)
	P value	0.194	0.191	0.240	0.023	0.194	0.214	0.036*
Chloramphenicol	Res (11)	8 (73)	5 (45)	7 (64)	8 (73)	10 (91)	6 (36)	6 (54)
	Sus (23)	21 (91)	22 (96)	13 (56)	4 (17)	19 (83)	18 (87)	7 (30)
	P value	0.200	0.114	0.228	0.034*	0.207	0.195	0.179
Oxytetracycline	Res (20)	19 (95)	16 (80)	17 (85)	10 (50)	20 (100)	20 (100)	8 (40)
	Sus (14)	10 (71)	11 (79)	3 (21)	2 (14)	9 (64)	4 (28)	5 (36)
	P value	0.177	0.206	0.034*	0.093	0.148	0.033*	0.256

Values in parenthesis indicate percentage. Res = resistant isolates (No. of positive isolates); Sus = susceptible isolates (No. of positive isolates). The association deemed as statistical significant when *P≤0.05

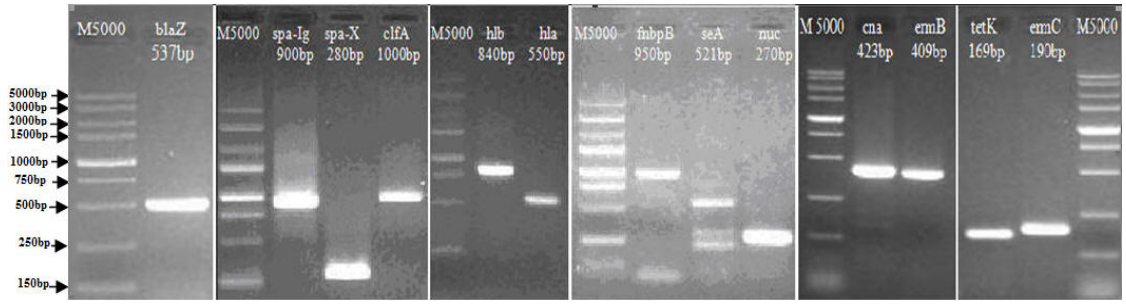


Fig. 2: All detected virulence and antimicrobial genes (M is DNA ladder 5000)

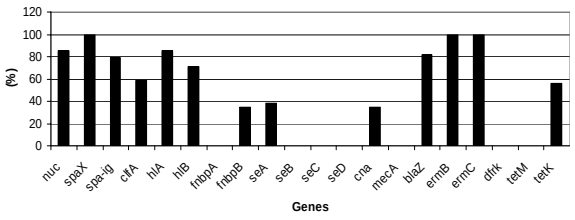
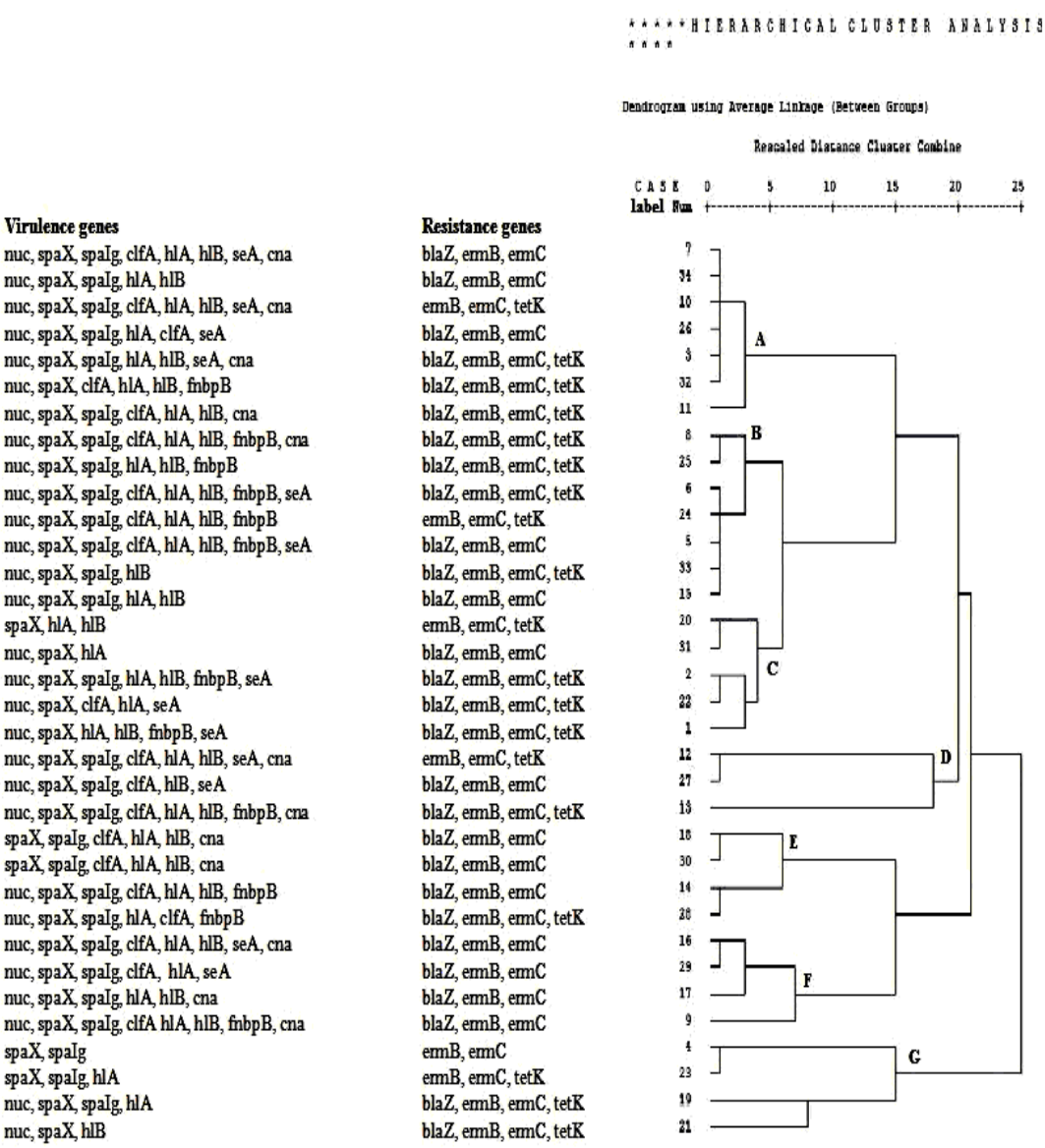


Fig. 3: Percentage of detected virulence and resistance genes in mastitis *S. aureus* isolates

isolates (Gao *et al*., 2011). Li *et al*. (2009) reported that there is a common use of penicillin, tetracycline and erythromycin for the treatment of mastitis in China. Acquisition of resistance in *S. aureus* isolates attributed to mutation in gene or due to exchange of genetic material between organisms, since resistance genes carrying mobile genetic elements of *S. aureus* have exceedingly been explored (Teruyo *et al*., 2003).

In this study, most of the isolates harbored at least two and maximum eight virulence factor genes, suggesting that mastitis *S. aureus* isolates in this region were highly pathogenic. Almost all isolates were positive for *spaX* and *spaIg*, similarly high prevalence of these spa proteins observed in a Turkish study (Karahan *et al*., 2011). Normally, the *spaIg* amplicon size ranges from 900-1000bp; though in two isolates we found a smaller size of about 700bp, which is only reported in human origin *S. aureus* strains (Reinosoa *et al*., 2008), this result indicates that there may be a cross contamination between human and bovine strains and these strains may be the source of spreading infection in human through milk or milk products. Majority of studied isolates were positive for *nuc*, *hlA* and *hlB* gene, their significant pathogenic role in mastitis infection has been documented (Kumar *et al*., 2011). The clumping factor encoding *clfA* gene detected in 59% of isolates, which is less than 91% reported earlier by Karahan *et al*. (2011) and 73% reported in Iran (Momtaz *et al*., 2010). *FnbpB* and *cna* present in 35% of isolates, although their role in pathogenesis of mastitis infection is not well established (Salasia *et al*., 2004). All the isolates were tested for enterotoxin genes and *seA* was frequently encountered in this investigation. Our isolates did not carry *fnbpA*, *seB*, *seC* and *seD* genes, while their prevalence in mastitis *S. aureus* isolates has been reported (Kumar *et al*., 2011; Salasia *et al*., 2004).

Some genes including *clfA, fnbpB, hlB* and enterotoxin (*seA*) were present in both antimicrobial resistant and susceptible isolates, statistical analysis showed their strong relationship of co-presence with resistance patterns. Although, the pathogenic traits and genetic determinants of antimicrobials of mastitis *S.*



Virulence genes	Resistance genes
nuc, spaX, spaIg, clfA, hlA, hlB, seA, cna	blaZ, ermB, ermC
nuc, spaX, spaIg, hlA, hlB	blaZ, ermB, ermC
nuc, spaX, spaIg, clfA, hlA, hlB, seA, cna	ermB, ermC, tetK
nuc, spaX, spaIg, hlA, clfA, seA	blaZ, ermB, ermC
nuc, spaX, spaIg, hlA, hlB, seA, cna	blaZ, ermB, ermC, tetK
nuc, spaX, clfA, hlA, hlB, fnbpB	blaZ, ermB, ermC, tetK
nuc, spaX, spaIg, clfA, hlA, hlB, cna	blaZ, ermB, ermC, tetK
nuc, spaX, spaIg, clfA, hlA, hlB, fnbpB, cna	blaZ, ermB, ermC, tetK
nuc, spaX, spaIg, hlA, hlB, fnbpB	blaZ, ermB, ermC, tetK
nuc, spaX, spaIg, clfA, hlA, hlB, fnbpB, seA	blaZ, ermB, ermC, tetK
nuc, spaX, spaIg, clfA, hlA, hlB, fnbpB	ermB, ermC, tetK
nuc, spaX, spaIg, clfA, hlA, hlB, fnbpB, seA	blaZ, ermB, ermC
nuc, spaX, spaIg, hlB	blaZ, ermB, ermC, tetK
nuc, spaX, spaIg, hlA, hlB	blaZ, ermB, ermC
spaX, hlA, hlB	ermB, ermC, tetK
nuc, spaX, hlA	blaZ, ermB, ermC
nuc, spaX, spaIg, hlA, hlB, fnbpB, seA	blaZ, ermB, ermC, tetK
nuc, spaX, clfA, hlA, seA	blaZ, ermB, ermC, tetK
nuc, spaX, hlA, hlB, fnbpB, seA	blaZ, ermB, ermC, tetK
nuc, spaX, spaIg, clfA, hlA, hlB, seA, cna	ermB, ermC, tetK
nuc, spaX, spaIg, clfA, hlB, seA	blaZ, ermB, ermC
nuc, spaX, spaIg, clfA, hlA, hlB, fnbpB, cna	blaZ, ermB, ermC, tetK
spaX, spaIg, clfA, hlA, hlB, cna	blaZ, ermB, ermC
spaX, spaIg, clfA, hlA, hlB, cna	blaZ, ermB, ermC
nuc, spaX, spaIg, clfA, hlA, hlB, fnbpB	blaZ, ermB, ermC
nuc, spaX, spaIg, hlA, clfA, fnbpB	blaZ, ermB, ermC, tetK
nuc, spaX, spaIg, clfA, hlA, hlB, seA, cna	blaZ, ermB, ermC
nuc, spaX, spaIg, clfA, hlA, seA	blaZ, ermB, ermC
nuc, spaX, spaIg, hlA, hlB, cna	blaZ, ermB, ermC
nuc, spaX, spaIg, clfA hlA, hlB, fnbpB, cna	blaZ, ermB, ermC
spaX, spaIg	ermB, ermC
spaX, spaIg, hlA	ermB, ermC, tetK
nuc, spaX, spaIg, hlA	blaZ, ermB, ermC, tetK
nuc, spaX, hlB	blaZ, ermB, ermC, tetK

Fig. 4: REP-PCR base dendrogram, VSs and ARGs are placed in front of isolate number for understanding the genetic similarity of isolates.

aureus have not been reported to reside on same loci (Brody *et al*., 2008), but the majority of resistance genes and virulence factors have reportedly reside on mobile genetic elements especially on plasmid and they can be linked (Brody *et al*., 2008; Kumar *et al*., 2010). Interestingly, the high prevalence of pathogenic factors like *clfA*, *fnbpB* and *seA* in MRSA isolates showed significant correlation with resistance patterns.

The REP-PCR generated phylogenetic tree typed all isolates into seven distinct genotypes (A-G) and all the genotypes can be differentiated by the presence or absence of virulence genes. In general, there was a clear difference in virulence genes combinations of genotypes except genotype-A and B isolates, which remained in separate genotype despite of relatively same virulence patterns; there may be some other undetected genes role in differentiating these genotypes. While, isolates clustered in genotype-G were less virulent as compared to other genotypes, having only few VGs. MRSA isolates conserved highly virulent profile and found related with each other and grouped in genotype (A, B and F) regardless a little variation in virulence properties.

Conclusion: This study reports increasing prevalence of MRSA isolates without having *mecA* gene. High frequency of virulence genes and genetic resistance in the isolates is a main reason for treatment failure and possibly leads to spread of resistance. Presence of anti-phagocytosis activity bearing polymorphic spa proteins and comparatively low frequency of adhesins, binding proteins and enterotoxin showed typical characteristics of *S. aureus* isolates in this region of China. Phylogeny grouped the *S. aureus* isolates having similar genetic profiles and should be consider as essential tool for epidemiological studies. These findings can be considered in designing strategic plans for treatment, prevention and control of *S. aureus* mastitis in this region of China.

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