Virulence Characteristic and MLST-\textit{agr} Genetic Background of High-Level Mupirocin-Resistant, MRSA Isolates from Shanghai and Wenzhou, China

Qingzhong Liu\textsuperscript{1}, Lizhong Han\textsuperscript{2}, Bin Li\textsuperscript{2}, Jingyong Sun\textsuperscript{2}, Yuxing Ni\textsuperscript{2*}

\textsuperscript{1}Department of Clinical Laboratory, Shanghai First People’s Hospital, School of Medicine, Shanghai Jiaotong University, Shanghai, China, \textsuperscript{2}Department of Clinical Microbiology, Ruijin Hospital, School of Medicine, Shanghai, China

Abstract

The emergence and prevalence of high-level mupirocin-resistant, methicillin-resistant \textit{Staphylococcus aureus} (MuH MRSA) is challenging the eradication of MRSA nasal carriage and the treatment of skin and soft tissue infections. To understand the potentially pathogenetic capacity and the genetic basis of MuH MRSA, it is important to have a detailed knowledge of the molecular traits of this organism. Fifty three MuH MRSA isolates were gathered from Shanghai (28 isolates) and Wenzhou (25 isolates) in China. These organisms, \textit{consisting of 27 different PFGE-SCCme\textsubscript{c}-spa patterns}, were examined by PCR for 35 virulence genes and further typed using \textit{agr} (accessory gene regulator) typing and MLST (multilocus sequence typing). All 53 strains were positive for the genes \textit{hlg} variant and \textit{icad}, and negative for \textit{seb}, \textit{sed}, \textit{seh}, \textit{eta}, \textit{eth}, \textit{hdl}, \textit{cap-5}, and \textit{ACME-arcA}. Compared with Wenzhou isolates, Shanghai isolates were more likely to carry \textit{seg} \textit{(P} = 0.002) and several other genes which were not found in Wenzhou strains such as \textit{sec}, \textit{sei}, \textit{tst} \textit{(P<0.001 each)}, and \textit{pvl} \textit{(P} = 0.012), and less likely to contain \textit{sea} \textit{(P}<0.001), \textit{cna} \textit{(P} = 0.031), and \textit{ebf} \textit{(P} = 0.045). MLST and \textit{agr} typing showed that ST239-\textit{agr1}, ST5-\textit{agr1}, and ST239-\textit{agr2} were the common lineages in MuH MRSA isolates from these two different regions. Our results indicated that MuH MRSA strains from two different geographic regions of China have differences in distribution of some virulence genes, while their major MLST-\textit{agr} genetic backgrounds were accordant.

Introduction

Methicillin-resistant \textit{Staphylococcus aureus} (MRSA) is still a leading pathogen of nosocomial infections in China. Because of its ability to produce virulence factors causing a variety of infections and its serious multidrug resistance, MRSA is one of the most frightening bacteria.

The pathogenesis of \textit{S. aureus} infections is involved in the expression of a wide range of virulence factors associated with attachment, persistence, evading/destroying host defenses, tissue invasion/penetration and toxin-mediated disease [1]. However, a majority of the serious \textit{S. aureus} infections are caused by the combined actions of several virulence factors. Otherwise, clinical isolates linked to \textit{S. aureus} infection may be naturally deficient in a scale of putative pathogenic determinants. Therefore, strains causing \textit{S. aureus} disease have variable combinations of virulence genes [2].

Mupirocin is a very effective topical antibiotic used to treat staphylococcal skin and soft tissue infections and eliminate MRSA from colonized nasal passages. However, the resistance to mupirocin has occurred, and its spreading is worrisome, with \textit{mupA}-mediated high-level resistance [3]. The \textit{mupA} gene is usually located on a large conjugative plasmid capable of mediating the co-transfer of other resistance genes. If high-level mupirocin resistance could not be controd, a highly effective means of decolonization of MRSA may be lost. Thus the high-level mupirocin resistance MRSA (MuH MRSA) may spread widely, and causes a range of infections. Therefore it is necessary to know the traits of virulence of clinical MuH MRSA strains more comprehensively.

The purpose of this study was to investigate the prevalence of putative virulence genes in a clinical population of MuH MRSA isolates with known types of PFGE-SCCme\textsubscript{c}-\textit{spa} from two different geographic areas in China, and explore the possible difference in virulence determinants combination between the strains from the both origins. Finally we further determined the genetic characteristics of these isolates by \textit{agr} (accessory gene regulator) typing and multilocus sequence typing (MLST).

Materials and Methods

MuH MRSA Isolates

Fifty three MuH MRSA isolates were selected from 5 university hospitals collection in Shanghai \textit{(n} = 28, 4 hospitals) and Wenzhou \textit{(n} = 25, 1 hospital), China. This collection comprised 803 MRSA, which were isolated from various clinical specimens of individual inpatients from August 2005 to May 2008. Most of the MuH isolates were gathered from respiratory samples (86.6%). Intensive
care units, surgical wards, burn wards and neurology wards were the major hospital units affected by MuH MRSA. All isolates had been previously described by PFGE, SCCmec and spa typing and these results are shown in Table 1 [4]. Because of being focused on bacteria, this study was exempted from review by the Ethics Committee of Shanghai First People’s Hospital.

Detection of Virulence Factor Genes and agr Alleles

Genomic DNA from MuH MRSA was extracted by the bacterial genomic DNA kit (Wuxi Institution of Clone and Genetic Technology, China) and then used for PCR amplification of 35 virulence genes involved in toxin mediated disease, attachment, evading/destroying host defenses, tissue invasion/penetration, persistence and others and agr alleles (allele 1 to 4) by the primers derived from the published sequences [2,5-14]. These genes were all listed in Table 2.

DNA Sequencing

One randomly chosen amplicon for the each gene tested was sequenced on an ABI 3730 sequencer (Applied Biosystems) by Shanghai Invitrogen Biotech to confirm that primers amplified the expected genes.

MLST

MLST was performed on 27 MuH MRSA isolates representative of each PFGE-SCCmec-spa type as described previously [15]. Sequence type (ST) of each strain was determined by sequencing internal fragments of 7 housekeeping genes (arcC, arcE, glpF, gmk, pta, yqiL and yqiM) according to the MLST database (http://www.mlst.net).

Statistical Analyses

Pearson’s chi-square test or Fisher’s exact test if necessary was used to compare distribution of the virulence determinants in clinical MuH MRSA strains investigated (SPSS version11.5). All statistical tests were two tailed, with P<0.05 considered statistically significant.

Results

Comparison of Virulence Genes in Shanghai and Wenzhou Isolates

Compared to Wenzhou isolates, Shanghai strains were significantly less likely to contain spa (32.1% versus 80.0%, P<0.001), cna (67.9% versus 92.0%, P = 0.031) and yqiB (64.3% versus 88.0%, P = 0.045), and more likely to host seg (57.1% versus 16.0%, P = 0.002). For Shanghai strains, 42.9%, 53.6%, 50.0%, 7.1% and 28.6% carried sec, sse, tst, seg and psv, respectively, whereas no isolate from Wenzhou possessed them, and those differences except that of seg were significant. No isolate from either origin was positive for seh, sed, sek, seh, eta, eib, hld, cap-5 and ACME-arcA (Table 3). The distribution of the remaining virulence genes in 53 MuH MRSA isolates were summarized in Table 3.

Virulence Genes Content and Combination in Shanghai and Wenzhou Isolates

Of 28 Shanghai strains, 13 (46.4%) possessed ≥5 virulence genes involved in toxin mediated disease (high toxin gene content), however, no Wenzhou strain carried ≥5 this type of genes. The most prevalent combination of this type of genes was hla+hlb+hlg/sbi+seg+agr (25.0% of strains, 7/28) in Shanghai strains. And the second frequent combination was sep/hir+hlb (17.9% of strains, 5/28), which was also the most main combination in Wenzhou strains (68.0% of strains, 17/25) (Table 4). As for the adhesion determinants, 17 (60.7%) Shanghai isolates and 20 (80.0%) Wenzhou isolates possessed ≥5 genes. The combinations of fnbA+clfA+clfB+ebpS+seg+spa and fnbA+clfA+clfB+ebpS+seg+spa were overrepresented in Shanghai (21.4%, 6/28) and Wenzhou strains (76.0%, 19/25), respectively (Table 4). As indicated in Table 4, there were 8 (28.6%, 8/28) Shanghai isolates harboring the pattern of hlg+shv+cap-8+map+pol, and 20 (80.0%, 20/25) Wenzhou isolates containing the combination of hlg/hlg+shv+cap-8+map. However, no Wenzhou strain hosted ≥5 this kind of determinants. The other combination of virulence genes were shown in Table 4.

Table 1. The PFGE-SCCmec-spa patterns of 53 MuH MRSA isolates [4].

| PFGE-SCCmec-spa type | No. of isolates (n) | Origination of isolates | PFGE-SCCmec-spa type | No. of isolates (n) | Origination of isolates |
|----------------------|---------------------|-------------------------|----------------------|---------------------|-------------------------|
| A1-IIIA-t030         | 12                  | Wenzhou                 | H-III-t037           | 3                   | Shanghai                |
| A2-IIIA-t030         | 4                   | Wenzhou                 | J-I189               | 1                   | Wenzhou                 |
| A3-IIIA-t030         | 1                   | Wenzhou                 | K-III-nontypeable     | 1                   | Shanghai                |
| A4-IIIA-t030         | 1                   | Wenzhou                 | L1-I-I002            | 1                   | Shanghai                |
| A5-IIIA-t037         | 2                   | Shanghai                | L2-III-t002          | 1                   | Shanghai                |
| B-IIIA-t030          | 4                   | Wenzhou                 | M1-IIIIA-t002        | 2                   | Shanghai                |
| C-IIIB-t037          | 1                   | Shanghai                | M2-IIIIA-t002        | 1                   | Shanghai                |
| D1-IIIA-t037         | 1                   | Shanghai                | N-I-I318             | 5                   | Shanghai                |
| D2-IIIA-t037         | 1                   | Shanghai                | N-III-A1318          | 1                   | Shanghai                |
| E-IIIA-1127          | 1                   | Shanghai                | O-III-1377           | 1                   | Shanghai                |
| F1-II-t002           | 1                   | Shanghai                | P1-III-t037          | 2                   | Shanghai                |
| F2-II-t002           | 1                   | Shanghai                | P2-III-t037          | 1                   | Shanghai                |
| G-IIIA-t030          | 1                   | Wenzhou                 | Q-III-A1985          | 1                   | Shanghai                |
| H-IIIA-12505         | 1                   | Wenzhou                 |                     |                     |                         |

doi:10.1371/journal.pone.0037005.t001
**Table 2.** The genes detected by PCR in this investigation.

| Virulence gene | Reference | Virulence gene | Reference |
|----------------|-----------|----------------|-----------|
| Involved in toxin mediated disease and/or sepsis | Capsular polysaccharide 5, 8 (cap5, cap8) | 9 |
| Staphylococcal enterotoxin A, B, C, D, E, G, H, I, J (sea, seb, see, sed, see, seg, seh, sei, sej) | 5,6,7 | Major-histocompatibility-complex class II-analogue protein (mmap) | 2 |
| Exfoliative toxin A, B (eta, etb) | 5 | IgG-binding protein SBI (sbi) | 10 |
| α, β, δ-hemolysins (Hla, hlb, hld) | 5 | Involved in tissue invasion/penetration |
| Toxic shock syndrome toxin-1 (tsf) | 6 | VII serine protease (isp) | 10 |
| Involved in attachment | Staphylokinase (sak) | 11 |
| Fibrinectin-binding protein A, B (fnbA, fnbB) | 2,8 | Involved in persistence |
| Clumping factor A,B (clfA, clfB) | 2 | Intercellular adhesion A, D (icaA, icaD) | 12 |
| Collagen adhesin (cna) | 2 | Others |
| Bone sialoprotein-binding protein (bbp) | 2 | Staphylococcal accessory regulator A (sarA) | 10 |
| Elastin-binding protein (ebpS) | 2 | Extracellular fibrinogen-binding protein (ebf) | 10 |
| Involved in evading/destroying host defenses | arca region of arginine catabolic mobile element (ACME-arcA) | 13 |
| Panton-Valentine leukocidin (pvl) | 5 | agr alleles |
| γ-hemolysin and variant (hlg, hlgv) | 5 | allele 1 to 4 | 14 |

**agr Allele Distribution**

Of the 53 isolates, 38 (16 from Shanghai and 22 from Wenzhou) were *agr-*1, 8 (5 Shanghai isolates and 3 Wenzhou isolates) were *agr-*2, and 7 (all from Shanghai) were *agr-*3. None was positive for *agr-*4 (Table 4).

**MLST**

A total of 27 isolates representative of each PFGE-SCCmec-spa profile were studied by MLST. And six ST types such as ST239, ST5, ST630, ST1, ST284 and ST188 were generated. ST239 was the most prevalent type (55.6%, 15/27), including A1 or A2 or A3 or A4 or B or G-IIIa-t030, A5 or D-I-IIIa-t037, C-IIIB-t037, A1-IIIA-t2505, H-IIIA-t030, I or P1 or P2-III-t037, K-IIII-nt and L2-III-t002. The association between the other 5 ST types and PFGE-SCCmec-spa types were displayed in Table 4.

**Discussion**

*S. aureus* produces numerous extracellular proteins which involve in the ability of this organism causing disease in the mammalian host. In this study, we detected six groups of pathogenic genes for 53 clinical MuH MRSA isolates from Shanghai and Wenzhou regions, China (Table 2). The first of these were the genes involved in toxin mediated disease. Previous study showed toxins encoded by *sec* and *sbi* tend to generate higher immune responses resulting in host tissue damage than do other enterotoxins [16]. However, we did not find the existence of *sec* in Wenzhou isolates. It is generally believed that the existence of the enterotoxin gene cluster (*egc*, containing *seg* and *sei*) is not related with severe infections, but probably contributes to the colonization potential of an *S. aureus* strain [16,17,18]. Because the toxins transcribed by the *egc* element appear to be generated in lower amounts compared to the other well-studied enterotoxins which may permit strains carrying the *egc* determinants to live together with healthy hosts [17]. However, Morgan’s report showed the products of *egc* may play a part in some cases, especially in immuno-compromised patients [19]. Due to the genes *seg* and *sei* being located on *egc* element [20], the combined occurrence of the toxin gene pair can generally be observed. Notably, 25.0% (5/20) *seg* positive isolates were not confirmed with the fixed *seg*-*sei* combination in our study. The *sed* and *seg* genes are encoded by a plasmid pIB483 [21]. However, the coexistence of the two determinants cannot also be certified with 2 *seg*-positive isolates (Table 4). The possible explanation for these opposite results is the existence of still-unknown variants of *sei* and *sed*.

The second group consisted of the determinants involved in attachment (*fnbA, fnbB, clfA, clfB, cna, cnA and ebpS* (Table 3), which may make them possess the ability to bind to fibronectin, fibrinogen and fibrin [22], collagen substrates and collagenous tissues [23], and soluble tropoelastin [24]. There is evidence that Bbp is a key factor in bone and joint infections produced by *S. aureus* [25,26]. However, the positive rate of Bbp implied most MuH MRSA strains of our collection might not have the ability to cause those diseases.

The third group included the genes *pvl, hlg, hlgv, cap-3, cap-8, map* and *sbi*. The toxins encoded by *pvl* and *hlg* are leukotoxic for neutrophils and macrophages [27]. Capsular polysaccharide (Cap) can protect the bacterium from phagocytic uptake and increases microbial virulence. Map may potentiate *S. aureus* survival by affecting protective cellular immunity [28,29]. Sbi has the ability to hinder phagocytosis and is implicated in blood coagulation [30]. In this study, we did not see any significant difference in the prevalence of this group of genes except *pvl* between the Shanghai and Wenzhou strains (Table 3).

Ssp can degrade host cell receptors and/or bacterial adhesins, and promote the spread and transmission of infection [31]. Sak may mediate bacterial invasion into the host tissues and enhance bacterial resistance to phagocytosis [32]. According to Table 3, most of our isolates may have the functions mentioned above.

The genes *icaA* and *icaD* belong to *ica* operon (*icaADB* and C), which is revealed to induce polysaccharide intercellular adhesin (PIA, associated with biofilm formation) synthesis in staphylococcus. However, expression of *icaA* alone leads only to low
Table 3. Distribution of 35 virulence genes among the isolates of MuH MRSA from Shanghai and Wenzhou, China.

| Gene           | No. of isolates positive for the gene [% of total (n = 53)] | No. of isolates positive for the gene in two regions (%) | $\chi^2$ (P value) | Shanghai (n = 28) | Wenzhou (n = 25) |
|----------------|------------------------------------------------------------|----------------------------------------------------------|-------------------|-------------------|-----------------|
|                |                                                             |                                                          |                   |                   |                 |
| Involved in toxin mediated disease and/or sepsis |                                                             |                                                          |                   |                   |                 |
| sea            | 29 (54.7)                                                   | 9 (32.1)                                                  | 20 (80.0)         | 12.208 (<0.001)  |                 |
| seb            | 0                                                          | 0                                                        | 0                 | NA                |                 |
| sec            | 12 (22.6)                                                   | 12 (42.9)                                                 | 0                 | 13.850 (<0.001)  |                 |
| sed            | 0                                                          | 0                                                        | 0                 | NA                |                 |
| see            | 0                                                          | 0                                                        | 0                 | NA                |                 |
| seg            | 20 (37.7)                                                   | 16 (57.1)                                                 | 4 (16.0)          | 9.515 (0.002)     |                 |
| seh            | 0                                                          | 0                                                        | 0                 | NA                |                 |
| sei            | 15 (28.3)                                                   | 15 (53.6)                                                 | 0                 | 18.680 (<0.001)  |                 |
| sej            | 2 (3.8)                                                     | 2 (7.1)                                                   | 0                 | 0.492*            |                 |
| tst            | 14 (26.4)                                                   | 14 (50.0)                                                 | 0                 | 16.987 (<0.001)  |                 |
| eta            | 0                                                          | 0                                                        | 0                 | NA                |                 |
| etb            | 0                                                          | 0                                                        | 0                 | NA                |                 |
| hla            | 52 (98.1)                                                   | 27 (96.4)                                                 | 25 (100)          | 1.000*            |                 |
| hib            | 43 (81.1)                                                   | 21 (75.0)                                                 | 22 (88.0)         | 0.733 (0.392)     |                 |
| hld            | 0                                                          | 0                                                        | 0                 | NA                |                 |
| Involved in attachment |                                                             |                                                          |                   |                   |                 |
| hbnA           | 48 (90.6)                                                   | 26 (92.9)                                                 | 22 (88.0)         | 0.018 (0.894)     |                 |
| hbnB           | 8 (15.1)                                                    | 7 (25.0)                                                   | 1 (4.0)           | 3.045 (0.081)     |                 |
| clfA           | 41 (77.4)                                                   | 21 (75.0)                                                 | 20 (80.0)         | 0.189 (0.664)     |                 |
| clfB           | 52 (98.1)                                                   | 27 (96.4)                                                 | 25 (100)          | 0.000 (1.000)     |                 |
| cna            | 42 (79.2)                                                   | 19 (67.9)                                                 | 23 (92.0)         | 4.681 (0.031)     |                 |
| btp            | 7 (13.2)                                                    | 6 (21.4)                                                   | 1 (4.0)           | 2.145 (0.143)     |                 |
| ebpS           | 50 (94.3)                                                   | 27 (96.4)                                                 | 23 (92.0)         | 0.010 (0.919)     |                 |
| Involved in evading/destroying host defenses |                                                             |                                                          |                   |                   |                 |
| pvi            | 8 (15.1)                                                    | 8 (28.6)                                                   | 0                 | 6.331 (0.012)     |                 |
| hlg            | 46 (86.8)                                                   | 22 (78.6)                                                 | 24 (96.0)         | 2.145 (0.143)     |                 |
| hlgv           | 7 (13.2)                                                    | 6 (21.4)                                                   | 1 (4.0)           | 2.145 (0.143)     |                 |
| capS           | 0                                                          | 0                                                        | 0                 | NA                |                 |
| capB           | 48 (90.6)                                                   | 23 (82.1)                                                 | 25 (100)          | 3.061 (0.080)     |                 |
| map            | 44 (83.0)                                                   | 23 (82.1)                                                 | 21 (84.0)         | 0.000 (1.000)     |                 |
| sbi            | 51 (96.2)                                                   | 28 (100)                                                  | 23 (92.0)         | 0.218*            |                 |
| Involved in tissue invasion/penetration |                                                             |                                                          |                   |                   |                 |
| esp            | 46 (86.8)                                                   | 23 (82.1)                                                 | 23 (92.0)         | 0.425 (0.515)     |                 |
| sak            | 45 (84.9)                                                   | 21 (75.0)                                                 | 24 (96.0)         | 3.054 (0.081)     |                 |
| Involved in persistence |                                                             |                                                          |                   |                   |                 |
| icaA           | 43 (81.1)                                                   | 21 (75.0)                                                 | 22 (88.0)         | 0.733 (0.392)     |                 |
| icaD           | 53 (100)                                                   | 28 (100)                                                  | 25 (100)          | NA                |                 |
| Others         |                                                             |                                                          |                   |                   |                 |
| sarA           | 46 (86.8)                                                   | 24 (85.7)                                                 | 22 (88.0)         | 0.000 (1.000)     |                 |
| efb            | 40 (75.5)                                                   | 18 (64.3)                                                 | 22 (88.0)         | 4.012 (0.045)     |                 |
| ACME-arcA      | 0                                                          | 0                                                        | 0                 | NA                |                 |

*P < 0.05 were considered statistically significant.  
NA, not available.  
*Fisher’s exact test.  
doi:10.1371/journal.pone.0037005.t003
| Strain | Virulence gene profile | MLST type* |
|--------|------------------------|------------|
| Shanghai strain | | |
| 1, 3, 4, 5, 6 | hla, hlb, seg, sei, tst, sec | N-I-t318 ST284 |
| 2 | hla, hlb, seg, sei, tst, sec | N-I-t318 ST284 |
| 7 | hla, hlb, sea | P2-II-1037 ST239 |
| 8 | hla, hlb, sea | P1-III-1037 ST239 |
| 10 | hla, hlb, sea | III-t377 ST630 |
| 12 | hla, hlb, sea | III-t377 ST630 |
| 13 | hla, hlb, sea | III-t377 ST630 |
| 14 | hla, hlb, sea | III-t377 ST630 |
| 16 | hla, hlb, sea | III-t377 ST630 |
| 17 | hla, hlb, sea | III-t377 ST630 |
| 18 | hla, hlb, sea | III-t377 ST630 |
| 19 | hla, hlb, sea | III-t377 ST630 |
| 20 | hla, hlb, sea | III-t377 ST630 |
| 21 | hla, hlb, sea | III-t377 ST630 |
| 22 | hla, hlb, sea | III-t377 ST630 |
| 23 | hla, hlb, sea | III-t377 ST630 |
| 24 | hla, hlb, sea | III-t377 ST630 |
| 25 | hla, hlb, sea | III-t377 ST630 |
| 26 | hla, hlb, sea | III-t377 ST630 |
| 27 | hla, hlb, sea | III-t377 ST630 |
| 28 | hla, hlb, sea | III-t377 ST630 |
| 29 | hla, hlb, sea | III-t377 ST630 |
| 30 | hla, hlb, sea | III-t377 ST630 |
| 31, 32, 33 | hla, hlb, sea | III-t377 ST630 |
| 35, 40, 42, 43 | hla, hlb, sea | III-t377 ST630 |
| 50, 51, 53 | hla, hlb, sea | III-t377 ST630 |

Wenzhou strain | | |
| 29 | hla, hlb, sea | J1-t189 ST188 |
| 30 | hla, hlb, sea | A1-IIA-1030 ST239 |
| 31, 32, 33 | hla, hlb, sea | A1-IIA-1030 ST239 |
| 35, 40, 42, 43 | hla, hlb, sea | A1-IIA-1030 ST239 |
| 50, 51, 53 | hla, hlb, sea | A1-IIA-1030 ST239 |
Table 4. Cont.

| Strain     | Virulence gene profile | Involved in tissue invasion/penetration | Involved in adherence/defence | Involved in toxin mediated disease and/or sepsis | Involved in persistence | Involved in evading/persistence | Involved in destroying host defenses |
|------------|------------------------|----------------------------------------|-------------------------------|-----------------------------------------------|------------------------|-------------------------------------|-------------------------------------|
|            |                        | PFGE-SCC mec-spa agr                      |                               |                                               |                        |                                     |                                     |
| 32         |                        | A1-IIIA-t030 ST239                        | hla, clfB, ebpS, cna, hlg, cap-8, sak, icaD, sarA | 52                                             |                        |                                     |                                     |
| 34, 37, 38, 39 |                        | A1-IIIA-t030 ST239                        | hla, clfB, ebpS, cna, hlg, cap-8, sak, icaD, sarA | 2                                               |                        |                                     |                                     |
| 41, 44, 46 |                        | A1-IIIA-t030 ST239                        | hla, clfB, ebpS, cna, hlg, cap-8, sak, icaD, sarA | 34                                              |                        |                                     |                                     |
| 45         |                        | A1-IIIA-t030 ST239                        | hla, clfB, ebpS, cna, hlg, cap-8, sak, icaD, sarA | 45                                             |                        |                                     |                                     |
| 47         |                        | A1-IIIA-t030 ST239                        | hla, clfB, ebpS, cna, hlg, cap-8, sak, icaD, sarA | 47                                             |                        |                                     |                                     |
| 48         |                        | A1-IIIA-t030 ST239                        | hla, clfB, ebpS, cna, hlg, cap-8, sak, icaD, sarA | 48                                             |                        |                                     |                                     |
| 49         |                        | A1-IIIA-t030 ST239                        | hla, clfB, ebpS, cna, hlg, cap-8, sak, icaD, sarA | 49                                             |                        |                                     |                                     |

MLST (multilocus sequence typing) was performed on representative isolates for each PFGE-SCC mec-spa-agr type.

acr: accessory gene regulator; PFGE: pulsed-field gel electrophoresis; SCC mec: staphylococcal chromosomal cassette mec.

Production of PIA. It was demonstrated that coexpression of icaA with icaD will promote the biosynthesis of capsular polysaccharide [33]. In this study, a small part of our strains were negative for icaD (Table 3). This phenomenon may be the deficiency of icaA gene or the existence of point mutations in the primer binding sites causing a negative PCR reaction.

It has been hypothesized that Efb might benefit the bacterium by interacting with fibrinogen and preventing the clotting process, thereby delaying the healing process [34]. sar (containing 3 transcripts designated sarA, sarB and sarC) is a global regulatory locus, and controls the production of many virulence factors in S. aureus. Among which the sarC encodes the major effector molecule [35]. ACME can encode an arginine deaminase pathway and an oligopeptide permease system that could enhance the ability of S. aureus, especially for USA300 clone, to grow and survive within the host [36]. Table 3 showed the genes efb and sarC were prevalent in most our strains; however, no isolate studied possessed the fomulation of ACME gene.

agr is another important global regulatory locus controlling the production of most staphylococcal exoproteins. In S. aureus, four different agr alleles have been described and agr is regarded as a slowly evolving genetic marker to investigate hospital-acquired MRSA [37]. A report by Van Leeuwen et al. [38] suggested that the agr-1 is the most prevalent agr group in MRSA isolates. We found similar in our isolates (71.7%, 38/53) (Table 4) and by Liu et al. [39] (96.4%, 134/139) in Beijing MRSA strains. Besides agr-1, 28.3% (15/53) of our strains belonged to agr-2 or agr-3. Previous investigation has demonstrated the association between agr-3 and TSST-1 [40], whereas the findings of this study revealed that only 6 of 14 tsst-positive strains were agr-3, and the remainder belonged to agr-1 (7 isolates) and agr-2 (1 isolate) (Table 4). Likewise, the data reported by Ben Nejma et al. [41] could also not reveal the relationship between agr-3 and this toxin.

Researchers based on MLST exhibited that the predominant MRSA clone was ST239-MRSA in Asian countries besides Japan and South Korea (ST5). Yu et al. [42] and Yao et al. [43] reported that ST239-MRSA was the most commonly detected clone in MRSA obtained from Wenzhou, and Liu et al. [44] also discovered the prevalence of this clone in fourteen cities of mainland China, including Shanghai. Our study displayed the same results among the 27 MuH MRSA isolates representative (55.6%, 15/27). However, the ST5 isolates also accounted for 25.9% (7/27) of the representative strains (Table 4). Table 4 showed that the ST239-agr1 (44.4%, 12/27), ST5-agr1 (22.2%, 6/27) and ST239-agr2 (18.5%, 5/27) were the common lineages in the two regions’ isolates.

For the determinants involved in toxin mediated disease, all the isolates from the two regions contained at least one gene of this group. However, there were 6 Shanghai strains and 4 Wenzhou isolates that did not carry any staphylococcal enterotoxin gene (Table 4). Table 4 shows there were 3 Shanghai isolates lacking genes involved in tissue invasion/penetration and the ‘others’ group genes (sarC and ebp). In addition, 1 Shanghai isolate and 1 Wenzhou isolate were only absent from the genes divided into the ‘other’ group. In Shanghai and Wenzhou strains hosting ≥2 genes involved in toxin mediated disease, most of them also carried fewer other virulence genes (Table 4). In addition, Some MuH isolates yielded different virulence genes patterns even though they were of the same PFGE-SCC mec-spa-agr-ST type, and the same virulence determinants combination can belong to different genotype patterns (Table 4). In respect to these results, the possible explanation may be that some virulence determinants are located on mobile genetic elements and can be horizontally transmitted among bacteria.
In summary, our study showed there were some differences in virulence profiles between MuH MRSA isolates from Shanghai and Wenzhou, and the differences mainly existed in the genes sec, sig, seq, siaB, ecp and felt. The results of MLST and agr typing displayed that the two regions’ isolates were genetically less diverse.

Acknowledgments

We are grateful to Tieli Zhou (First Affiliated Hospital, Wenzhou Medical College, Wenzhou, Zhejiang, China), Yanqun Jiang (Shanghai Sixth People’s Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China) and Hong Zhang (Shanghai Children’s Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China) for obtaining some clinical strains.

Author Contributions

Conceived and designed the experiments: QL YN. Performed the experiments: QL LH BL. Analyzed the data: QL JS. Contributed reagents/materials/analysis tools: LH JS. Wrote the paper: QL.

References

1. Gordon RJ, Lowy FD (2008) Pathogenesis of methicillin-resistant Staphylococcus aureus infection. Clin Infect Dis 46 (suppl): pp 350–359.
2. Pe´rez-Roth E, Lo´pez-Aguilar C, Alcoba-Florez J, Me´ndez-Alvarez S (2006) High-level mupirocin resistance within methicillin-resistant Staphylococcus aureus pandemic lineages. Antimicrob Agents Chemother 50: 3207–3211.
3. Pérez-Redi E, Lopez-Aguilar C, Alonso-Herrera J, Ménendez-Alvarez S (2006) High-level mupirocin resistance within methicillin-resistant Staphylococcus aureus strains. Antimicrob Agents Chemother 50: 3207–3211.
4. Moore PC, Lindsay JA (2001) Genetic variation among hospital isolates of Staphylococcus aureus. J Med Microbiol 50: 171–177.
5. Jarraud S, Mougel C, Thioulouse J, Lina G, Meugnier H, et al. (2002) Comparative analysis of clinical and hospital isolates of Staphylococcus aureus in France. J Clin Microbiol 40: 2235–2241.
6. Monday SR, Bohach GA (1999) Use of multiplex PCR to detect classical and nery syngeneic pyrogenic toxin genes in staphylococcal isolates. J Clin Microbiol 37: 3411–3419.
7. Holtfreter S, Grumann D, Schmudde M, Nguyen HT, Eichler P, et al. (2007) Clonal distribution of superantigen genes in clinical Staphylococcus aureus isolates. J Clin Microbiol 45: 2699–2700.
8. Salasta SI, Kinnunen Z, Lammel UR, Czeckh M (2004) Comparative studies on phage and genetic typing properties of Staphylococcus aureus isolates from bovine subclinical mastitis in central Java in Indonesia and Hesse in Germany. J Vet Med C 5: 103–109.
9. Verderi J, Durand G, Bes M, Taylor KL, Lina G, et al. (2007) Identification of the capsular polysaccharides in Staphylococcus aureus clinical isolates by PCR and strainification tests. J Clin Microbiol 45: 725–729.
10. Moore PC, Lindsay JA (2001) Genetic variation among hospital isolates of methicillin-susceptible Staphylococcus aureus: evidence for horizontal transfer of virulence genes. J Clin Microbiol 39: 2760–2767.
11. Gorsek C, Kolzer J, Wolz C (2006) CfDo-oxacillin and trimethoprim cause phage induction and virulence modulation in Staphylococcus aureus. Antimicrob Agents Chemother 50: 3301–3306.
12. Cramton SE, Gerke C, Schmoll NF, Nichols WW, Gotz F (1999) The intercellular adhesion (ica) locus is present in Staphylococcus aureus and is required for biofilm formation. Infect Immun 67: 5427–5435.
13. Murray CK, Holmes RL, Ellis MW, Mende K, Wolf SE, et al. (2009) Twenty-five year epidemiology of invasive methicillin-resistant Staphylococcus aureus (MRSA) isolates retrieved at a burn center. Burns 35: 1112–1117.
14. Cattoir V, Bertuccio T, Tantagati M, Demodei V, Spina D, et al. (2007) Aggregation and immunomodulatory function of Staphylococcus aureus (MRSA) isolates from hospitalised patients in China. Clin Microbiol Infect 14: 381–384.
15. Eslaki MC, Day NP, Davies CE, Peacock SJ, Spratt BG (2000) Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of Staphylococcus aureus. J Clin Microbiol 38: 1008–1015.
16. Ferry T, Thomas D, Genestier A, Bes M, Lina G, et al. (2005) Comparative prevalence of superantigen genes in Staphylococcus aureus isolates causing sepsis with and without severe shock. Clin Infect Dis 41: 771–777.
17. Hoffertser S, Bauer K, Thomas D, Feig C, Lorenz V, et al. (2004) Genetic variation and transcriptional analysis of biofilm-producing Staphylococcus aureus. FEMS Immunol Med Microbiol 35: 220–227.
18. Eunghi MC, Day NP, Davies CE, Peacock SJ, Spratt BG (2000) Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of Staphylococcus aureus. J Clin Microbiol 38: 1008–1015.
19. Ferry T, Thomas D, Genestier A, Bes M, Lina G, et al. (2005) Comparative prevalence of superantigen genes in Staphylococcus aureus isolates causing sepsis with and without severe shock. Clin Infect Dis 41: 771–777.
20. Jarraud S, Peyrat MA, Lim A, Tristan A, Bes M, et al. (2001) qgy, a highly prevalent operon of enterotoxin gene, forms a putative nursey of superantigens in Staphylococcus aureus. J Clin Microbiol 39: 1008–1012.
21. Jarraud S, Iandolo JJ, Stewart GC (1998) The enterotoxin D plasmid of Staphylococcus aureus encodes a second enterotoxin determinant (spt). FEMS Microbiol Lett 168: 227–233.
43. Yao D, Yu FY, Qin ZQ, Chen C, He SS, et al. (2010) Molecular characterization of *Staphylococcus aureus* isolates causing skin and soft tissue infections (SSTIs). BMC Infect Dis 10: 133.

44. Liu Y, Wang H, Du N, Shen E, Chen H, et al. (2009) Molecular evidence for spread of two major methicillin-resistant *Staphylococcus aureus* clones with a unique geographic distribution in Chinese hospitals. Antimicrob Agents Chemother 53: 512–518.