Research Article

iMedPub Journals
http://www.imedpub.com/

Archives of Clinical Microbiology
ISSN 1989-8436

2019
Vol.10 No.4:94

Penicillin Binding Proteins3 and 4 Relations between Resistance Phenotypes and *mecA*, *TEM* Genes Expression in *Staphylococcal aureus*

Yae Sung Mun[1], Yuan Li[1], Yiel-Hea Seo[2] and You Jin Hwang[1,3*]

[1]Department of Life Science, College of Bio-nano Technology, Gachon University, 191, Hambangmoe-ro, Yeonsu-gu, Incheon, 21936, Republic of Korea

[2]Department of Biomedical Engineering, College of Health Science, Gachon University, 191, Hambangmoe-ro, Yeonsu-gu, Incheon, 21936, Republic of Korea

[3]Department of Laboratory Medicine, Gil Hospital, Gachon University College of Medicine, Incheon, Republic of Korea

[*]**Corresponding author**: You Jin Hwang, Department of Life Science, College of Bio-nano Technology, Gachon University, 191, Hambangmoe-ro, Yeonsu-gu, Incheon, 21936, Republic of Korea, Tel: +82328204545; E-mail: gene@gachon.ac.kr

Received date: July 17, 2019; **Accepted date:** August 24, 2019; **Published date**: July 30, 2019

Citation: Mun YS, Li Y, Seo YH, Hwang YJ (2019) Penicillin binding proteins3 and 4 relations between resistance phenotypes and *mecA*, *TEM* genes expression in *Staphylococcal aureus*. Arch Clin Microbiol Vol. 10 No. 4:94

Abstract

This study was to determine the relationship between *pbp3* and *pbp4 gene* compared with *mec*A and TEM resistance genes expression patterns. Total 134 clinical *S. aureus* strains were subjected to 19 antimicrobial susceptibility tests. We detected resistance to methicillin (*mec*A), penicillin (*blaTEM*) and expression of *pbp* (Penicillin-binding proteins) genes. We were compared *blaTEM*, extended spectrum, carbapenem related genes and types of SCC*mec* identified. Total of 134 clinical *S. aureus* strains, 79 (58.96%) in methicillin resistance, and 77 strains carried *mec*A. Prevalence rates of *blaTEM* and *pbp genes* were 107/134 (79.85%) and 128/134 (95.52%). Multiplex PCR results revealed that the predominant SCC*mec* type among 77 *mec*A-positive MRSA strains were similer too SCC*mec* type II 41.56% (32/77) and type IVA 40.26% (31/77). Prevalence rates of type IVb, IVd and non-typable were 18.18% (14/77), respectively. From a total of 77/134 (57.46%) MRSA isolate strains, 35/77 (45.46%) were positive for extended spectrum, 40/77 (51.95%) for cephalosporins, and 35/77 (45.46%) for carbapenems. The predominant SCC*mec* type II had more carbapenem resistances than IVA, IVb and IVd. TEM and *mec*A gene expression were not correlated with *pbp gene*, and the properties of drug resistance were appeared not associated with *pbp3, 4 genes*.

Keywords: MRSA; blaTEM; CCmec type II; type IVA; *pbp* (Penicillin-binding proteins) gene

Introduction

Antimicrobial resistance (AMR) is a major public health concern globally and methicillin resistant *Staphylococcus aureus* (MRSA) is one of the most important pathogens worldwide [1,2]. MRSA a prominent pathogen that causes severe infections from healthcare settings to various community settings over recent decades has raised considerable concern [1]. The resistance of *S. aureus* to methicillin is mainly mediated by the gene *mecA*, which is located on *Staphylococcus* cassette chromosome *mec* (SCC*mec*), a mobile genetic element that encloses a modified penicillin-binding protein with reduced affinity to β-lactam antibiotics, which contributes to inactivating antibiotics [3].

MRSA in hospital settings is more prevalent in Asian countries such as South Korea, China, and Japan, with reported rates of 70-80% and Europe (25.1%) [4,5]. In one recent study, the proportion of MRSA in Health care-associated (HA) isolates was very high, 73.3% [6]. Although rates of Community-associated (CA) MRSA infections are still very low in South Korea, recent rates of MRSA isolates have been unclear [7,8].

Resistance to antimicrobial agents has become one of the most serious problems worldwide, especially resistance to nosocomial pathogens.

Excessive therapeutic usage of antimicrobial agents in both humans and animals has contributed to the development of widespread antibiotic resistance in bacteria [9], and multidrug-resistant *S. aureus* is causing public health problems that should arouse societies attention [10].

MRSA can lead to difficult-to-treat infections because they are resistant to many groups of antibiotics such as β-lactams, tetracyclines, aminoglycosides, and macrolides. The principal mechanism of aminoglycoside resistance in *S. aureus* is drug inactivation mediated by aminoglycoside-modifying enzymes (AMEs) encoded by various genes such as *aac(6')-aph(2")* and *ant(4')-Ia* [11]. The most prevalent AME in *S. aureus* is bifunctional enzyme *AAC(6')-APH(2")*, which is encoded by *aac(6')-aph(2")* [12]. In addition, *ANT(4')-I* encoded by ant*(4')-Ia, erm(A), erm(C)* and tetM has been found in *S. aureus* [13-15].

MRSA is resistant to all penicillins including semisynthetic penicillinase-resistant congeners, carbapenems, cephalosporins, and penems [16]. The principal mechanism of penicillin

resistance in MRSA is mediated by *mecA*, which encodes a modified penicillin-binding protein with reduced affinity to β-lactam antibiotics [17,18]. Another mechanism of penicillin resistance is the expression of penicillinase, which hydrolyzes the β-lactam ring, which in turn inactivates penicillin [18]. The resistance of *S. aureus* to methicillin is caused by the presence of the *mecA* gene, which encodes the 78-kDa penicillin-binding protein (*pbp*) *2a* (or *pbp2a*). Than b-lactam antibiotics cannot bind to *pbp2a*, synthesis of the peptidoglycan layer and cell wall synthesis are able to continue [19,20].

S. aureus can acquire antibiotic resistance genes through horizontal gene transfer using mobile genetic elements include SCC*mec*, plasmid, transposon, insertion sequence, and bacteriophage [21]. SCC*mec* elements are important for MRSA because they usually serve as determinants of antibiotic resistance patterns. Health care-associated MRSA strains usually harbor type I-III SCC*mec* elements that confer Multidrug Resistance (MDR) [22].

However, community-associated strains are generally non-MDR strains that carry small SCC*mec* elements; most of these elements are types IV and V [23,24]. There have, however, been recent reports from clinical trials of the efficacy of beta-lactams and carbapenems in *S. aureus* [25-27].

Our objectives with this study were to compare the relationship between phenotypic antimicrobial susceptibility patterns and *pbp* genes were present in bacteria isolated strains. Also to compare the prevalence of genes with SCC*mec* resistance with *blaTEM* and *pbp* genes among clinical *S. aureus* isolate strains.

Materials and Methods

Bacterial isolates

A total of 134 *S. aureus* strains were obtained from clinical patients at Gachon University Gil Medical Center in South Korea between April 2016 and June 2018. The research was approved by the ethics committee of Gil Hospital, Gachon University of Medicine. *S. aureus* strains identification and antimicrobial susceptibility testing of *S. aureus* isolated from blood culture were performed using MicroScan Pos Breakpoint Combo panel type 28 (PBC28; Beckman Coulter, West Sacramento, CA, USA).

Sample strains were streaked onto sheep blood agar (Sinyang Diagnostics, Seoul, Korea) and transported to our laboratory after culture. One colony was picked from each blood agar plate and incubated in lysogeny broth with shaking (80 rpm) at 37°C overnight. Isolates were preserved in 20% glycerol (vol/vol) and stored at -80°C freezer until further use.

Antimicrobial susceptibility testing

We tested for antimicrobial susceptibility using the Kirby-Bauer disc diffusion method described by Clinical and Laboratory Standard Institute (CLSI) guidelines; 2015 [28]. Each bacterial suspension was adjusted to McFarland 0.5 turbidity, swabbed onto lysogeny broth agar, and incubated in the presence of antibiotic discs at 37°C for 18 hours. We tested the following 19 antibiotic discs (Liofilchem, Roseto degli Aburzzi, Italy): penicillin G (10 IU), methicillin (5 μg), kanamycin (30 μg), gentamicin (10 μg), streptomycin (10 μg), tetracycline (30 μg), erythromycin (15 μg), vancomycin (30 μg), chloramphenicol (30 μg), amoxicillin (25 μg), ticarcillin (75 μg), piperacillin (100 μg), cefepime (30 μg), cefotaxime (30 μg), ceftazidime (30 μg), imipenem (10 μg), ertapenem (10 μg) and meropenem (10 μg).

We measured the diameters of inhibition zones ≤ 10-13 mm and determined each isolate as resistant or susceptible to antimicrobial agents based on CLSI 2015 and Liofilchem (Liofilchem, Roseto degli Aburzzi, Italy) guidelines. We obtained *S. aureus* control strain *Staphylococcus aureus* ATCC 29213 (Korean Culture Center of Microorganisms, Seodaemun-gu, Seoul, Korea).

Genomic DNA isolation

Genomic DNA was isolated after alkaline cell lysis, phenol-chloroform DNA extraction, and ethanol DNA precipitation. A single colony was picked from each blood agar plate and then incubated in lysogeny broth at 37°C overnight. Then 1.5 ml of the bacterial suspension was harvested by centrifugation at 14,000 rpm for 30 s.

The harvested bacterial pellet was proceeded protocol alkaline phenol chloroform method. We were used fresh tube and phenol-chloroform (1:1) solution (Bioneer, Daejeon, Korea). DNA pellet was then dissolved in 30 μl autoclaved tri-distilled water. DNA concentrations were determined using a NanoDrop™ spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA).

Identifying *mecA*, *blaTEM* and SCC*mec* typing by multiplex real time-PCR

We have used to detect *mecA* and *blaTEM* gene list in **Table 1** [12,15,29,30]. The following reaction mixture was added to each sample: 10 pmol of each primer, 2 μl DNA (100 ng), and 10 μl iQ™ SYBR® Green supermix (2×reaction buffer with dNTPs, iTaq DNA polymerase, SYBR® Green I, fluorescein, and stabilizers, Bio-Rad, Hercules, CA, USA). The volume was adjusted to 20 μl by adding autoclaved triple-distilled water. PCR cycling conditions on a thermal cycler (iQ5, Bio-Rad and TC-512, TECHNE, Cambridge, UK) were as follows: 94°C for 3 min followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 56°C for 30 s, and extension at 72°C for 45 s.

The reaction was ended with a final extension step at 72°C for 10 min. Multiplex PCR was carried out for SCC*mec* typing using nine pairs of primers specific for SCC*mec* types I, II, III, IVa, IVA, IVb, IVc, IVd, and V primer sets by Zhang et al. [30]. PCR products were subjected to electrophoresis using 2% agarose gel in 1×TBE buffer at 100 V for 25 min. The 100 bp DNA ladder (Bioneer, Daejeon, Korea) was used as a molecular size maker. PCR

This article is available from: 10.4172/1989-8436.100094

products in gels were then visualized with Safe Green loading dye (Applied Biological Materials Inc, Vancouver, Canada).

Table 1. Primers used for detecting antibiotic resistance determinants in *S. aureus* isolates

Antibiotic	Primer	Oligonucleotide sequence (5'→3')	Amplicon size (bp)	specific gene	Reference	GenBank
β-lactams	TEM-F	GCA CGA GTG GGT TAC ATC GA	311	*blaTEM*	This study	NG_050162.1
	TEM-R	GGT CCT CCG ATC GTT GTC AG				
Tetracyclines	tet(M)-F	GGT TGG AAT GTG ACG GAC TG	200	*tetM*	This study	LS483319.1
	tet(M)-R	ATC GTT GTA TGC TCG TGA AAG A				
Aminoglycosides	kan-F	GAA GCA GAG TTC AGC CAT GA	390	*ant(4')-Ia*	This study	CP019563.1
	kan-R	CGA AGC GCT CGT CGT ATA AC				
	AAC(6')-APH(2")-F	CCA AGA GCA ATA AGG GCA TA	222	*aac(6')-aph(2")*	[12]	
	AAC(6')-APH(2")-R	CAC TAT CAT AAC CAC TAC CG				
Macrolides	erm(A)-F	AAG CGG TAA ACC CCT CTG A	199	*ermA*	[15]	
	erm(A)-R	ACAATGATGGACAATGACTGTGA				
	erm(C)-F	AAT CGT CAA TTC CTG CAT GT	299	*ermC*	[15]	
	erm(C)-R	TAA TCG TGG AAT ACG GGT TTG				
SCC*mec*	TypeIVA-F	TTACCACGCTTGTTGATGGTA	1752	*SCCmec IVA*	This study	EU437549.2
	TypeIVA-R	ACAATGATGGACAATGACTGTGA				

Detecting genes associated with carbapenem related genes and *pbp genes*

We performed PCR to detect genes associated with antimicrobial resistance; oligonucleotide primer sequences and specific genes are listed in **Table 2.** These products were determined the existence of carbapenem related genes and *pbp* genes PCR result and DNA sequencing.

Table 2. Primers used for detecting *pbp* (penicillin binding proteins) genes determinants in *S. aureus* isolates

Primers name	Oligonucleotide sequence (5'→3')	Amplicon size (bp)	Specificity	Reference/ GenBank
pbp1-F	AGCAACAACCACAAACTAAGC	2690	This study	CP034441
pbp1-R	CCTCGTCTACCTTAAAATTCTC			
pbp2-F	TGCATATCAACAAAAAGGTATTG	2567	This study	CP039759
pbp2-R	CTATTTAGATGTTTCAAAATGTATG			
pbp3-F	GTTTGTTTTCACGTGAACAGAA	2489	This study	CP039848
pbp3-R	ATTTTGGAATGTAGTTAACTGGG			
pbp4-F	GACATGACTGGGAAGGTGAATT	1711	This study	CP039156
bp4-R	TAACACCTTTAGCTACACACGT			
pbp1s-F	AGGTAGCGGTTTTGTGTCC	169	This study	AY920399
pbp1s-R	TATCCTTGTCAGTTTTACTGTC			
pbp2s-F	TATTTAGCCGGTTTACCTCA	193	This study	AY920400
pbp2s-R	TTTTGACGTTCTTCAGCAGT			
pbp3s-F	GTGGACCAACCTCATCTTTA	317	This study	AY920401
pbp3s-R	CGGGAGACCCTTATTATTCT			

pbp4s-F	TGGTGCTAACTGCTTTGTAA			
pbp4s-R	GCTAAAGCTATCGGAATGAA	199	This study	AY920402

Results

We tested for antimicrobial susceptibility using Kirby-Bauer disc diffusion and determined the isolates as resistant or susceptible to antimicrobial agents based on the diameters of the inhibition zones ≤ 10-13 mm. Our susceptibility testing showed that 58.96% (79/134) of *S. aureus* strains were resistant to methicillin; our results showed high rates of susceptibility to chloramphenicol 132/134 (98.51%) and vancomycin 132/134 (98.51%), but *S. aureus* strains showed resistance against streptomycin 128/134 (95.52%) and penicillin 111/134 (82.84%). The overall rates of resistance to kanamycin, gentamicin, erythromycin, and tetracycline were 55.97%, 45.52%, 34.34%, and 24.63% (**Table 3**).

Our susceptibility testing also showed that 81/134 (60.45%) of *S. aureus* strains were susceptible to amoxicillin (AML), and we found resistance against piperacillin 42/134 (31.34%) and cefotamxime 27/134 (20.15%) as well. **Table 3** displays the results for correlations between methicillin resistance and the presence of *mecA* gene. A total of 79 MRSA strains resistant to methicillin, 77 strains were *mecA* positive and 2 strains were *mecA* negative (**Table 3, Figure 1a**). Fifty-seven (42.54%) strains of *S. aureus* were susceptible to methicillin. The relationship between penicillin resistance and the presence of *blaTEM* is also summarized in **Table 3**. One hundred-eleven (82.84%) *S. aureus* strains were resistant to penicillin based on disk diffusion, and 107 of them were positive for *blaTEM* (**Table 3, Figure 1a**).

Tables 3 shows the correlations between kanamycin resistance and the presence of *ant(4')-Ia* and *aac(6')-aph(2")* in *S. aureus*; a total of 68/134 (50.75%) strains carried at least one of the genes. Seventy-five *S. aureus* strains were resistant to kanamycin, including 48 that carried resistance genes, and 16 strains were positive for *ant(4')-Ia* and *aac(6')-aph(2")* by PCR. Sixty-one (45.52%) *S. aureus* strains were resistant to gentamycin as determined by disk diffusion, and 36 of these were positive for *aac(6')-aph(2")* (**Table 3, Figures 1b-1d**).

The correlations between erythromycin resistance and the presence of ermA and ermC are summarized in **Table 3**. A total of 46 (34.34%) *S. aureus* were resistant to erythromycin determined by disc diffusion, including 38 that were positive for ermA and two that had carried ermC (**Table 3**); however, 88/134 (65.67%) susceptible strains did not harbor either of these two genes associated with erythromycin resistance based on multiplex PCR. There were correlations between tetracycline resistance and the presence of tetM (**Table 3**): Thirty-three (24.63%) *S. aureus* strains were resistant to tetracycline on the susceptibility test, but 45 were positive for tetM by PCR (**Table 3**).

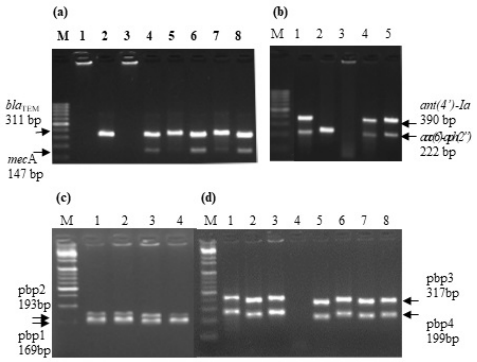

Figure 1. Detecting *mecA*, *blaTEM, ant(4')-Ia* and *aac(6')-aph(2")* by Polymerase Chain Reaction (PCR). The PCR results were visualized by 2% agarose gel and stained with Safe Green loading dye-Lane M, 100 bp DNA ladder, (a) Multiplex PCR for detecting line no 1-8 *mecA* (147 bp) and *blaTEM* (311 bp), (b) Multiplex PCR for detecting line no 1-*5, ant(4')-Ia (390 bp)* and *aac(6')-aph(2") (222 bp)* genes in *S. aureus* strains, (c) Multiplex PCR for *pbp1* and *2* typing, Lane M: 100 bp DNA ladder; Lane 1-4, *pbp* type I (169 bp), *pbp* type 2 (193 bp, (d) Detection of *pbp3* (317bp) and *pbp4* (199bp) line 1-8, line 4 was not detected *pbp3, 4* gene.

Table 3. Phenotypic antibiotic resistance patterns and rates of antibiotic resistance genes and *pbp* genes in *S. aureus.*

Antibiotic	Resistant strains No=134 (%)	PCR positive strains No=134 (%)
Methicillin	79 (58.96%)	*mecA* 77 (57.46%)
Penicillin G	111 (82.84%)	*blaTEM* 107 (79.58%)
		ant(4')-Ia 32 (23.88%)
Kanamycin	75 (55.97%)	aac(6')-aph(2") 32 (23.88%)
		total 52 (38.81%)
		ermA 36 (26.87%)

This article is available from: 10.4172/1989-8436.100094

Erythromycin	46 (34.34%)	ermC 2 (1.49%)
		total 38 (28.36%)
Gentamicin	61 (45.52%)	aac(6')-aph(2") 32 (23.88%)
Tetracycline	33 (24.63%)	tetM 45 (33.58%)
Streptomycin	128 (95.52%)	
Vancomycin	2 (1.49%)	vanA, vanB (not detected)
chloramphenicol	2 (1.49%)	
pbp genes		128/134 (95.52%)

We used multiplex PCR to determine SCC*mec* types in 77 *mecA*-positive strains (**Figure 1a**). The prevalence of different SCC*mec* types in *mecA*-positive MRSA strains is summarized; the predominant type was SCC*mec* type II 32/77 (41.56%). The prevalence rates of type IVA and non-typable were 40.26% (31/77) and 18.18% (14/77) by multiplex PCR.

The correlations between carbapenem resistances and the presence of SCC*mec* types are shown in **Table 4**. A total of 32/77 (41.56%) SCC*mec* type II strains were resistant to piperacillin 21/32, cefotaxime 22/32, and imipenem 22/32, and 31/77 (40.26%) SCC*mec* type IVA strains were resistant to piperacillin 11/31, cefotaxime 9/31, and imipenem 5/31. Fourteen 14/77 (18.18%) non-typable strains were resistant to ticarcillin 5/14, cefepime 5/14, and meropenem 3/14; SCC*mec* type II had higher carbapenem resistance than did type IVA and non-tapable strains (**Table 4**). We have analysed relationship between carbapenems related resistance phenotypes and *pbp1,2,3,4* genes expression in total 134 *S. aureus* (**Table 5**).

Table 4. Antimicrobial resistance patterns of *S. aureus* isolates, Extended-spectrum, carbapenem and *mecA*-positive patterns of MRSA strains

Antibiotics	**Antimicrobial resistance (n=134)**		***blaTEM* gene positive (n=111)**		***mecA* gene positive (n=77)**	
	Resistance		**Resistance**		**Resistance (n=60)**	
	No	%	No	%	No	%
Amoxicillin	16	11.94%	15	11.19%	16	11.94%
Ticarcillin	28	20.89%	26	19.40%	28	20.89%
Piperacillin	42	31.34%	38	28.36%	31	50.00%
Cefepime	36	26.87%	33	24.53%	36	26.87%
Cefotaxime	27	20.15%	26	19.40%	27	20.15%
Ceftazidime	32	23.88%	31	23.13%	32	23.88%
Imipenem	30	22.39%	27	20.15%	30	22.39%
Ertapenem	31	23.13%	28	20.89%	31	23.13%
Meropenem	29	21.64%	27	20.15%	29	21.64%
Aztreonam	127	94.78%	82	61.19%	58	43.28%

Table 5. Relationship between resistance phenotypes and gene expression

Group	**TEM**	**Penicillin**	*mecA*	**methicillin**	**carbapenems**	**penicillins**	**cephalosporins**	*pbp*(-)	**No**
TMall	+	+	+	+	+	+	+		35
TM4	+	+	+	+	-	-	-		16
TP	+	+	-	-	-	-	-	2	20
AM	-	+	+	+	-	-	-	1	9
TEM	+	-	-	-	-	-	-		7

Penic	-	+	-	-	-	-	-	1	6
ETC	+/-	+/-	+/-	+/-	-	-	-	1	16

*Abbreviation: TMall is all positive; TM4 is TEM; penicillin; mecA and methicillin positive; TP is TEM and penicillin positive; AM is penicillin; mecA and methicillin positive; TEM is only positive; Penic is Penicillin positive; ETC is rest strains

Discussion and Conclusion

In the present study, we compared the results of antimicrobial susceptibility determined by disc diffusion with PCR analysis results for *S. aureus* strains (**Table 3).** Although results of the present study showed almost perfect correlation between phenotypic methicillin susceptibility and *mecA*, two strains presented discrepancies between genotype and phenotype, as did two methicillin-resistant *mecA*-negative strains. Previous researchers have reported that *S. aureus* isolates that carry *mecA* are sensitive to oxacillin, and thus, *mecA* might be heterogeneously expressed; therefore, some *S. aureus* strains that carry *mecA* might not be detectable with phenotypical methods [12,31]. The possibility of selecting resistant cells from originally susceptible strains has been demonstrated; some strains do not express their *mecA* unless they are provided with selective pressure *via* increasing gradients of the antibiotic agent. The second case of discrepancy occurred in two *mecA*-negative *S. aureus* strains that were phenotypically resistant to methicillin and *mecA* gene was not detected in these isolates. We will proceed investigation with further study in these two isolates (continue to study, approximate type mecC). Researchers have reported that penicillin resistance in *S. aureus* is commonly mediated by the expression of penicillinase encoded by blaZ and hydrolyze the β-lactam ring and contribute to the inactivation of penicillin [9,16,32,33].

However, others have investigated the presence of *blaTEM* were unclear. It is known that *blaTEM* encodes a series of class A plasmid-mediated enzymes belonging to extended-spectrum β-lactamases that are associated with penicillin resistance and are frequently present in *Klebsiella pneumoniae* and *Escherichia coli* [34,35]. In addition, three strains that showed penicillin resistance were *blaTEM*-negative and *pbp3* gene negative; thus, penicillin resistance in these strains might not be associated with *mecA* but other resistance genes. We result of *pbp3* and *pbp4* have been considered not so important for *mecA* and *TEM* resistance in *S. aureus* sample strains.

In harbored *ant(4')-Ia* were resistant to kanamycin, and all strains that carried *aac(6')-aph(2")* were clearly resistant to gentamicin and kanamycin in susceptibility testing [11]. Our results were phenotypically resistant to kanamycin, including three that showed kanamycin resistance in susceptibility testing but did not carry *ant(4')-Ia* or *aac(6')-aph(2")*. The prevalence of phenotypic tetracycline resistance and carried tetM were discrepances. These discrepancies also suggested that some strains might harbor tetracycline resistance genes and variable measured the diameters of inhibition zones ≤ 13 mm.

We evaluated the prevalence of different types of *SCCmec* by multiplex PCR. Commonly, HA-MRSA strains carry SCC*mec* types I-III with multidrug resistance while CA-MRSA strains harbor types IV and V. Previous researchers in South Korea have indicated that SCC*mec* type II is the most prevalent among HA-MRSA strains while SCC*mec* type IVA is predominant in CA-MRSA strains, but other researcher were different higher prevalence types IV [8,36].

Multiplex PCR results revealed that the predominant SCC*mec* type among 77 *mecA*-positive MRSA strains were similer too SCC*mec* type II (32/77) and type IVA (31/77). The predominant SCC*mec* type II had more carbapenem resistances than IVA, IVb and IVd. *TEM* and *mecA* gene expression were not correlated with *pbp* gene, and the properties of drug resistance were appeared not associated with *pbp3, 4* genes.

The strains of SCC*mec* type II had higher carbapenem resistance than did type IVA (**Table 4**). Excessive therapeutic usage of antimicrobial agents in hospital environments might have contributed to the development of resistance and the widespread distribution of SCC*mec* type II MRSA strains. Recent clinical trials ongoing demonstrate the efficacy of beta-lactams and carbapenems in *S. aureus* [25-27]. However, this efficacy remains to be tested in future studies using phenotype–genotype pairs for the diagnostic microbiology and monitor resistance trends in infection control.

Conflict of Interest

The authors declare that they have no conflicts of interest.

Financial Support Statement

This study was partially supported by a National Research Foundation grant funded by the Korean government (MSIT, No. 103120) and a Regional Innovation System grant (No. R003942) funded by the Ministry of Trade, Industry and Energy, Republic of Korea.

References

1. World Health Organisation (2014) Global report on surveillance. Geneva: World Health Organisation 109-116.
2. Jean SS, Hsueh PR (2011) High burden of antimicrobial resistance in Asia. Int J Antimicrob Agents. 37: 291-295.
3. Matthews P, Tomasz A (1994) Molecular aspects of methicillin resistance in Staphylococcus aureus. J Antimicrob Chemother 33: 7-24.
4. Reinert RR, Low DE, Rossi F, Zhang X (2007) Antimicrobial susceptibility among organisms from the Asia/Pacific Rim, Europe and Latin and North America collected as part of TEST and the in vitro activity of tigecycline. J Antimicrob Chemother 60: 1018-1029.
5. Inomata S, Yano H, Tokuda K, Kanamori H, Endo S, et al. (2015) Microbiological and molecular epidemiological analyses of community-associated methicillin-resistant Staphylococcus aureus

This article is available from: 10.4172/1989-8436.100094

at a tertiary care hospital in Japan. J Infect Chemother 21: 729-736.

6. Song JH, Hsueh PR, Chung DR, Ko KS, Kang CI, et al. (2011) Spread of methicillin-resistant Staphylococcus aureus between the community and the hospitals in Asian countries: an ANSORP study. J Antimicrob Chemother 66: 1061-1069.

7. Moon HW, Kim HJ, Hur M, Yun YM (2014) Antimicrobial susceptibility profiles of Staphylococcus aureus isolates classified according to their origin in a tertiary hospital in Korea. Am J Infect Control 42: 1340-1342.

8. Kim ES, Song JS, Lee HJ, Choe PG, Park KH, et al. (2007) A survey of community-associated methicillin-resistant Staphylococcus aureus in Korea. J Antimicrob Chemother 60: 1108-1114.

9. Xu J, Shi C, Song M, Xu X, Yang P, et al. (2014) Phenotypic and genotypic antimicrobial resistance traits of foodborne Staphylococcus aureus isolates from Shanghai. J Food Sci 79: M635-M642.

10. Coast J, Smith RD (2003) Solving the problem of antimicrobial resistance: is a global approach necessary? Drug Discov Today 8: 1-2.

11. Ramirez MS, Tolmasky ME (2010) Aminoglycoside modifying enzymes. Drug Resist Update 13: 151-171.

12. Martineau F, Picard FJ, Lansac N, Ménard C, Roy PH, et al. (2000) Correlation between the Resistance Genotype Determined by Multiplex PCR Assays and the Antibiotic Susceptibility Patterns of Staphylococcus aureus and Staphylococcus epidermidis. Antimicrob Agents Chemother 44: 231-238.

13. Schmitz FJ, Fluit AC, Gondolf M, Beyrau R, Lindenlauf E, et al. (1999) The prevalence of aminoglycoside resistance and corresponding resistance genes in clinical isolates of staphylococci from 19 European hospitals. J Antimicrob Chemother 43: 253-259.

14. Trzcinski K, Cooper BS, Hryniewicz W, Dowson CG (2000) Expression of resistance to tetracyclines in strains of methicillin-resistant Staphylococcus aureus. J Antimicrob Chemother 45: 763-770.

15. Varaldo PE, Montanari MP, Giovanetti E (2009) Genetic elements responsible for erythromycin resistance in Streptococci. Antimicrob Agents Chemother 53: 343-353.

16. Olsen JE, Christensen H, Aarestrup FM (2006) Diversity and evolution of blaZ from Staphylococcus aureus and coagulase-negative staphylococci. J Antimicrob Chemother 57: 450-460.

17. Page MG (2006) Anti-MRSA beta-lactams in development. Curr Opin Pharmacol 6: 480-485.

18. Stapleton PD, Taylor P (2002) Methicillin resistance in Staphylococcus aureus: mechanisms and modulation. Sci Prog 85: 57-72.

19. Fishovitz, J, Hermoso JA, Chang M, Mobashery S (2014) Penicillin-binding protein 2a of methicillin-resistant Staphylococcus aureus. IUBMB Life 66: 572-577.

20. Lowy FD (2003) Antimicrobial resistance: the example of Staphylococcus aureus. J Clin Invest 111: 1265-1273.

21. McCarthy AJ, Loeffler A, Witney AA, Gould KA, Lloyd DH, et al. (2014) Extensive horizontal gene transfer during Staphylococcus aureus co-colonization in vivo. Genome Biol Evol 6: 2697-2708.

22. Uhlemann AC, Otto M, Lowy FD, DeLeo FR (2014) Evolution of community-and healthcare-associated methicillin-resistant Staphylococcus aureus. Infect Genet Evol 21: 563-574.

23. Kondo Y, Ito T, Ma XX, Watanabe S, Kreiswirth BN, et al. (2007) Combination of multiplex PCRs for staphylococcal cassette chromosome mec type assignment: rapid identification system for mec, ccr, and major differences in junkyard regions. Antimicrob Agents Chemother 51: 264-274.

24. Orlin I, Rokney A, Onn A, Glikman D, Peretz A (2017) Hospital clones of methicillin-resistant Staphylococcus aureus are carried by medical students even before healthcare exposure. Antimicrob Resist Infect Control 6: 15.

25. Lee H, Yoon EJ, Kim D, Jeong SH, Won EJ, et al. (2018) Antimicrobial resistance of major clinical pathogens in South Korea, May 2016 to April 2017: first one-year report from Kor-Glass. Euro Surveill 23: 1800047.

26. Saeki M, Shinagawa M, Yakuwa Y, Nirasawa S, Sato Y (2018) Inoculum effect of high concentrations of methicillin-susceptible Staphylococcus aureus on the efficacy of cefazolin and other beta-lactams. J Infect Chemother 24: 212-215.

27. Wootton M, MacGowan AP, Howe RA (2017) Towards better antimicrobial susceptibility testing: impact of the Journal of Antimicrobial Chemotherapy. J Antimicrob Chemother 72: 323-329.

28. Wayne PA, (2015) Clinical and laboratory standards institute. Performance standards for antimicrobial susceptibility testing. Twenty-five Inform Suppl M100-S25.

29. Strommenger B, Kettlitz C, Werner G, Witte W (2003) Multiplex PCR assay for simultaneous detection of nine clinically relevant antibiotic resistance genes in Staphylococcus aureus. J Clin Microbiol 41: 4089-4094.

30. Zhang K, McClure JA, Elsayed S, Louie T, Conly JM (2005) Novel multiplex PCR assay for characterization and concomitant subtyping of staphylococcal cassette chromosome mec types I to V in methicillin-resistant Staphylococcus aureus. J Cinic Microbiol 43: 5026-5033.

31. Kolbert C, Arruda J, Varga-Delmore P, Zheng X, Lewis M, et al. (1998) Branched-DNA Assay for Detection of the mecA Gene in Oxacillin-Resistant and Oxacillin-Sensitive Staphylococci. J Clin Microbiol 36: 2640-2644.

32. Bradford PA (2001) Extended-spectrum β-lactamases in the 21st century: characterization, epidemiology, and detection of this important resistance threat. Clin Microbiol Rev 14: 933-951.

33. Ferreira AM, Martins KB, Silva VR, Mondelli AL, Cunha ML (2017) Correlation of phenotypic tests with the presence of the blaZ gene for detection of beta-lactamase. Braz J Microbiol 48: 159-166.

34. Chong Y, Ito Y, Kamimura T (2011) Genetic evolution and clinical impact in extended-spectrum β-lactamase-producing Escherichia coli and Klebsiella pneumoniae. Infect Genet Evol 11: 1499-1504.

35. Dahms C, Hübner NO, Kossow A, Mellmann A, Dittmann K, et al. (2015) Occurrence of ESBL-producing Escherichia coli in livestock and farm workers in Mecklenburg-Western Pomerania, Germany. PLoS One 10: e0143326.

36. Park C, Lee DG, Kim SW, Choi SM, Park SH, et al. (2007) Predominance of community-associated methicillin-resistant Staphylococcus aureus strains carrying staphylococcal chromosome cassette mec type IVA in South Korea. J Clin Microbiol 45: 4021-4026.