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First Report on Multidrug-Resistant Methicillin-Resistant *Staphylococcus aureus* Isolates in Children Admitted to Tertiary Hospitals in Vietnam[S]

Nguyen Thai Son[1], Vu Thi Thu Huong[2], Vu Thi Kim Lien[2], Do Thi Quynh Nga[2], Tran Thi Hai Au[2], Tang Thi Nga[2], Le Nguyen Minh Hoa[3], and Tran Quang Binh[2,4*]

[1]*Vietnam Military Medical University, Hanoi 151000, Vietnam*

[2]*National Institute of Hygiene and Epidemiology, Hanoi 100000, Vietnam*

[3]*National Hospital for Tropical Diseases, Hanoi 100000, Vietnam*

[4]*Dinh Tien Hoang Institute of Medicine, Hanoi 155300, Vietnam*

The extensive distribution of multidrug-resistant (MDR) methicillin-resistant *Staphylococcus aureus* (MRSA) poses a threat to healthcare worldwide. This study aimed to investigate the MDR and molecular patterns of MRSA isolates in children admitted to the two biggest tertiary care pediatric hospitals in northern and southern Vietnam. A total of 168 MRSA strains were collected to determine antibiotic susceptibility by minimum inhibitory concentration tests. Antibiotic-resistant genes, pulsed-field gel electrophoresis, staphylococcal cassette chromosome *mec* (SCC*mec*) typing, and multilocus sequence typing were used for the molecular characterization of MRSA. Among the total strains, the MDR rate (51.8%) was significantly higher in the northern hospital than in the southern hospital (73% *vs.* 39%, $p <$ 0.0001). The MDR-MRSA with the highest rates were "ciprofloxacin-erythromycin-gentamicin-tetracyclines" (35.6%), followed by "erythromycin-tetracycline-chloramphenicol" (24.1%), and "ciprofloxacin-erythromycin-gentamicin" (19.5%), showing an accumulative total of 79.3%. The most susceptible antibiotics were rifampicin (100%) and vancomycin (100%), followed by doxycycline (94.0%), meropenem (78.0%), and cefotaxime (75.0%). The SCC*mec*II strains showed greater resistance to gentamicin, ciprofloxacin, tetracycline, meropenem and cephalosporins compared with the other strains. The SCC*mec*II strains exhibited the highest rate in the tested genes (*aac*A/*aph*D: 55.2%, *erm*A/B/C: 89.7%, and *tet*K/M: 82.8%). ST5-SCC*mec*II was the predominant clone in the northern hospital, whereas SCC*mec*IVa was more pronounced in the southern hospital. In conclusion, our results raised concerns about the predominant MDR-MRSA strains in the pediatric hospitals in Vietnam. The north–south difference in the antibiotic resistance patterns and genetic structure of MRSA suggests different MRSA origins and various uses of antimicrobial agents between the two regions.

Keywords: Methicillin-resistant, multidrug-resistant, *Staphylococcus aureus*, molecular pattern, children, Vietnam

Introduction

Staphylococcus aureus is a common bacterium that can cause a variety of diseases from localized to systemic infections [1]. Methicillin-resistant *S. aureus* (MRSA) is a major cause of nosocomial infections worldwide and is becoming increasingly prevalent in community settings [2,

3]. The emergence and spread of multidrug-resistant (MDR) MRSA pose a serious problem in the treatment and control of staphylococcal infections, thereby threatening global human health [4, 5]. In northern Vietnam, the nasopharyngeal carriage of *S. aureus* is observed in one-third of the Vietnamese population [6], and the population structure of MRSA differs from that in other Asian

countries [7, 8]. In southern Vietnam, the MRSA rates were 74.1% in hospitals and 30.1% in communities in 2004–2006 [9]. Most of the abovementioned studies were conducted on adult samples. An outbreak of severe community-acquired MRSA infections was reported in nine children in Ho Chi Minh City [10]. However, information about MDR and the molecular epidemiology of MRSA infections in Vietnamese children remains scarce.

The antimicrobial susceptibility patterns of *S. aureus* were identified using minimum inhibitory concentrations (MICs), and the presence of genes conferring resistance to *S. aureus* was detected by multiplex PCR. The different types of staphylococcal cassette chromosome *mec* (SCC*mec*) were identified to check for the distribution of SCC*mec* types among MRSA isolates. Multilocus sequence typing (MLST) was used to determine the sequence type (ST) to understand the geographical spread of MRSA isolates. Pulsed-field gel electrophoresis (PFGE) was used to assess the genetic similarity among MRSA isolates [11, 12]. In this study, we applied the above methods to investigate MDR and characterize MRSA isolates in children admitted to the biggest tertiary care pediatric hospitals in northern and southern Vietnam.

Materials and Methods

Ethics Statement

Isolates were obtained from patients with verbal consent from their parents as part of the standard care for patients in pediatric hospitals. Isolates were coded to protect the patients' identities and stored for treatment and study purposes. This study protocol was reviewed and approved by the Ethical Review Board (IRB No. 18IRB) of the National Institute of Hygiene and Epidemiology, Hanoi, Vietnam.

Bacterial Isolates and Clinical Information

A total of 168 non-duplicate clinical strains of MRSA were collected retrospectively between December 2011 and May 2014 in the two biggest pediatric hospitals located in two distinct geographic regions in Vietnam: 63 strains were obtained from the National Hospital of Pediatrics in Hanoi in northern Vietnam, and 105 strains were obtained from the Children's Hospital No. 2 in Ho Chi Minh City in southern Vietnam (Table S1). The two hospitals were tertiary care general hospitals with 1,400–2,000 beds. The strains were previously isolated from blood samples (N=77), other body fluids (N=9), respiratory samples (N=25), and skin lesions (N=57). The median age (interquartile range) of children was 9.6 (1.3–26.8) months. Isolates were identified as *S. aureus* using Gram staining, positive catalase test, coagulase test, and a commercial latex agglutination kit (Pastorex Staph Plus, Bio-Rad, France). *S. aureus* isolates were further confirmed

by ID-GP Card in VITEK 2 Compact (BioMérieux) in the two hospitals. MRSA isolates were identified using the MIC breakpoint of cefoxitin in accordance with the criteria of Clinical and Laboratory Standards Institute [13] and confirmed by the presence of the *mecA533* or *mecA310* genes using multiplex PCR [14, 15]. Clinical data, including general demographic information, laboratory results, and treatment, were collected from medical records. MRSA infections that occurred either more than 48 h after hospital admission or with healthcare risk factors were classified as healthcare-associated (HA) MRSA infection as reported previously, and those that occurred in inpatients within 48 h of admission without healthcare risk factors were classified as community-associated (CA) MRSA infections [16]. If an *S. aureus* infection did not meet these criteria, then it was considered a CA infection.

Antimicrobial Susceptibility Tests

Antibiotic susceptibility testing of all the bacterial strains was performed using microdilution and agar dilution methods in accordance with the M100-S26 Guidelines of the Clinical and Laboratory Standards Institute [13]. The 12 tested antibiotics were cefuroxime (CFR), cefotaxime (CFT), cefepime (CFP), meropenem (MER), gentamicin (GEN), ciprofloxacin (CIP), erythromycin (ERY), rifampicin (RIF), tetracycline (TET), doxycycline (DOX), chloramphenicol (CHL), and vancomycin (VAN). The MICs of the tested antimicrobials were measured by agar dilution. MIC90 and MIC50 were defined as the lowest concentrations of the antibiotic at which 90% and 50% of the isolates were inhibited, respectively.

On the basis of the standardized international terminology to describe acquired resistance profiles in 2012 [17], all the MRSA isolates in the present study were classified as MDR. No isolate was considered extensively drug-resistant. By contrast, many previous studies used the MDR definition for MRSA (MDR-MRSA) such that MRSA isolates were classified as MDR if they were non-susceptible to three or more different classes without beta-lactams [4, 9, 18, 19]. To compare our findings with those of previous studies, we used the same previous MDR-MRSA definition with non-beta-lactam antimicrobial types, including fluoroquinolones (CIP), macrolides (ERY), aminoglycosides (GEN), TETs (TET and/or DOX), phenicols (CHL), ansamycins (RIF), and glycopeptides (VAN) [18]. *S. aureus* ATCC 29213 was used as a quality control strain in the MIC experiments.

Analysis of Antibiotic-Resistant Genes

All the *S. aureus* strains were screened for the presence of the *mec*A gene with 533 bp PCR product (*mecA533*) as previously described [14]. Thirty-four *mecA533*-negative isolates were screened for the presence of the *mecA310* gene using another PCR method [15]. Genes conferring resistance to other antibiotics of TET (*tet*K and *tet*M), aminoglycosides (*aac*A/*aph*D), and macrolides and lincosamide (*erm*A, *erm*B, and *erm*C) were detected by multiplex PCR assays as previously reported [20].

Molecular Characterization of the MRSA Strains

All the MRSA isolates from the two hospitals were selected for SCCmec typing. SCCmec types were determined by multiplex PCR as previously reported [12]. SCCmec type IV was further tested by the subtyping method [21]. SCCmec types that could not be assigned to any known type by the above methods were classified as non-typeable.

PFGE was performed using 30 units of SmaI enzyme per sample [22] for selected MRSA strains that were classified either as MDR or identified as causes of sepsis, shock, or death. The PFGE patterns of the MRSA isolates were determined by cluster analysis using BioNumerics software version 6.6 (Applied-Maths, Sint-Martens-Latem, Belgium). Pairwise similarities were calculated using the Dice coefficient (optimization, 1%; band tolerance, 1.5%). The unweighted-pair group method using average algorithm was used to generate a dendrogram. Isolates with ≥ 80% similarity were assigned in a PFGE cluster. Clusters that included ≥ 10 isolates were considered major lineages. Sixteen strains were randomly selected in all clusters for MLST as described elsewhere. [11]. The allelic profiles and STs were assigned by the MLST website (http://saureus.mlst.net).

Statistical Analysis

Categorical variables were expressed in numbers and proportions (%). The chi-square test or Fisher's exact test was used to compare the distribution of the categorical variables. All statistical procedures were performed using Stata software version 11.0. A p value less than 0.05 was considered statistically significant.

Results

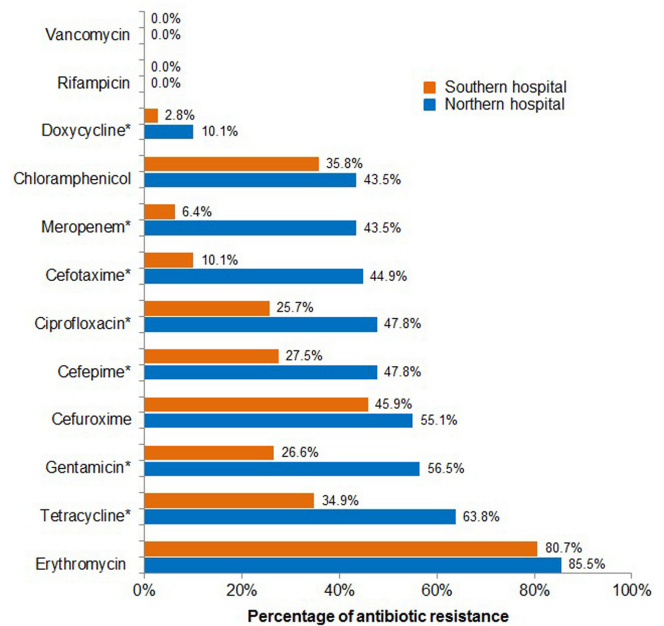In the total sample count, the highest antibiotic resistance

Fig. 1. Comparison of antibiotic resistance methicillin-resistant *Staphylococcus aureus* isolates between the two hospitals.
*Significant difference between northern and southern hospitals at $p < 0.05$.

frequency was found in ERY (81.5%). Antibiotic resistance rates of one-third to half of the tested isolates were observed in CFR (52.4%), TET (45.2%), GEN (38.1%), CFP (37.5%), CHL (36.3%), and CIP (35.7). The most susceptible antibiotics were RIF (100%) and VAN (100%), followed by

Table 1. Antimicrobial resistance profiles by HA-MRSA and CA-MRSA in two pediatric hospitals, Vietnam.

Antimicrobial category	Antimicrobial agent	HA-MRSA		p-value	CA-MRSA		p-value
		Northern hospital (N=48)	Southern hospital (N=82)		Northern hospital (N=15)	Southern hospital (N=23)	
Aminoglycosides	Gentamicin	30 (62.5)	24 (29.3)	<0.0001	5 (33.3)	5 (21.7)	0.473*
Ansamycins	Rifampicin	0 (0)	0 (0)	-	0 (0)	0 (0)	-
Fluoroquinolones	Ciprofloxacin	28 (58.3)	24 (29.3)	0.001	4 (26.7)	4 (17.4)	0.687*
Glycopeptides	Vancomycin	0 (0)	0 (0)	-	0 (0)	0 (0)	-
Macrolides	Erythromycin	40 (83.3)	67 (81.7)	0.815	13 (86.7)	17 (73.9)	0.440*
Phenicols	Chloramphenicol	14 (29.2)	29 (35.4)	0.468	10 (66.7)	8 (34.8)	0.054
Tetracyclines	Tetracycline	30 (62.5)	26 (31.7)	0.001	10 (66.7)	10 (43.5)	0.162
	Doxycycline	5 (10.4)	2 (2.4)	0.100*	2 (13.3)	1 (4.3)	0.550*
Carbapenems	Meropenem	26 (54.2)	5 (6.1)	<0.0001	4 (26.7)	2 (8.7)	0.188*
Cephalosporins	Cefuroxime	31 (64.6)	42 (51.2)	0.138	7 (46.7)	8 (34.8)	0.464
	Cefotaxime	27 (56.2)	9 (11.0)	<0.0001	4 (26.7)	2 (8.7)	0.188*
	Cefepime	29 (60.4)	25 (30.5)	0.001	4 (26.7)	5 (21.7)	1.000*

*Fisher's exact test. CA, community-associated; HA, hospital-associated; MRSA, methicillin-resistant *S. aureus*.

Table 2. Antimicrobial resistance profiles by *SCCmec* types of methicillin-resistant *Staphylococcus aureus* isolates in two pediatric hospitals, Vietnam.

Antimicrobial category	Antimicrobial agent	SCC*mec*II (N=29)	SCC*mec*III (N=64)	SCC*mec*IV (N=66*)	Others (N=9)	*p*-value
Aminoglycosides	Gentamicin	28 (96.6)	9 (14.1)	23 (34.8)	4 (44.4)	<0.0001
Ansamycins	Rifampicin	0 (0)	0 (0)	0 (0)	0 (0)	
Fluoroquinolones	Ciprofloxacin	25 (82.6)	8 (12.5)	23 (34.8)	4 (44.4)	<0.0001
Glycopeptides	Vancomycin	0 (0)	0 (0)	0 (0)	0 (0)	
Macrolides	Erythromycin	28 (96.6)	61 (95.3)	39 (59.1)	9 (100)	<0.0001
Phenicols	Chloramphenicol	5 (17.2)	39 (60.9)	12 (18.2)	5 (55.6)	<0.0001
Tetracyclines	Tetracycline	23 (79.3)	41 (64.1)	11 (16.7)	1 (11.1)	<0.0001
	Doxycycline	5 (17.2)	3 (4.7)	0 (0)	2 (22.2)	0.001
Carbapenems	Meropenem	25 (86.2)	8 (12.5)	1 (1.5)	3 (33.3)	<0.0001
Cephalosporins	Cefuroxime	27 (93.1)	16 (25.0)	40 (60.6)	5 (55.6)	<0.0001
	Cefotaxime	25 (86.2)	10 (15.6)	6 (9.1)	1 (11.1)	<0.0001
	Cefepime	26 (89.7)	13 (20.3)	21 (31.8)	3 (33.3)	<0.0001

*66 SCC*mec*IV: 59 SCC*mec*IVa, 6 SCC*mec*IVc/IVe, 1 SCC*mec*IVg. Others: Non-typeable SCC*mec* and SCC*mec*I.

DOX (94%), MER (78.0%), and CFT (75.0%). A comparison of the antibiotic resistance of MRSA isolates between the two hospitals is presented in Fig. 1. The MRSA isolates exhibited significantly higher antibiotic resistance in the northern hospital than in the southern hospital to DOX, MER, CFT, CIP, CFP, GEN, and TET ($p < 0.05$).

Table 1 shows the antimicrobial resistance profiles of HA-MRSA and CA-MRSA in the two pediatric hospitals. With regard to HA-MRSA, a significant north–south difference of antimicrobial agents in the two regions was found in GEN (62.5% *vs.* 29.3%), CIP (58.3% *vs.* 29.3%), TET (62.5% *vs.* 31.7%), MER (54.2% *vs.* 6.1%), CFT (56.2% *vs.* 11%), and CFP (60.4% *vs.* 30.5%). The antimicrobial resistance profiles of CA-MRSA isolates in the two pediatric hospitals in all antibiotics tested were not significantly different.

The distribution of SCC*mec* types of MRSA isolates in the northern and southern hospitals is shown in Table S2. The majority of the MRSA isolates belonged to SCC*mec*IV (39.3%), followed by SCC*mec*III (38.1%) and SCC*mec*II (17.3%) in the total samples. The north–south difference in the SCC*mec* types was observed in the present study: SCC*mec*II (41.3%), SCC*mec*III (33.3%), and SCC*mec*IV (22.2%) were found in northern Vietnam, whereas SCC*mec*II (2.9%), SCC*mec*III (41.0%), and SCC*mec*IV (49.5%) were observed in southern Vietnam ($p < 0.0001$).

Table 2 shows the antimicrobial resistance profiles by SCC*mec* types of MRSA isolates in the two pediatric hospitals. Overall, the SCC*mec*II strains showed greater resistance to GEN, CIP, TET, MER, and cephalosporins

Table 3. Distribution of antibiotic-resistant genes by SCC*mec* types of methicillin-resistant *Staphylococcus aureus* strains.

Gene	SCC*mec*II	SCC*mec*III	SCC*mec*IV	Others	Total	*p*-value
*mec*A	29 (100)	64 (100)	66 (100)	9 (100)	168 (100)	1.00
*aac*A/*aph*D	16 (55.2)	6 (9.4)	24 (36.4)	3 (36.4)	49 (29.2)	< 0.0001
*erm*A	24 (82.8)	4 (6.2)	0 (0)	1 (11.1)	29 (17.3)	< 0.0001
*erm*B	4 (13.8)	45 (70.3)	29 (43.9)	6 (56.7)	84 (50.0)	< 0.0001
*erm*C	0 (0)	0 (0)	12 (18.2)	2 (22.2)	14 (8.3)	< 0.0001
*erm*A/B/C	26 (89.7)	48 (75.0)	40 (60.6)	8 (88.9)	122 (72.6)	0.015
*tet*K	1 (3.4)	43 (67.2)	10 (15.2)	2 (22.2)	56 (33.3)	<0.0001
*tet*M	24 (82.8)	3 (4.7)	1 (1.5)	0 (0)	28 (16.7)	< 0.0001
*tet*K/M	24 (82.8)	44 (68.8)	11 (16.7)	2 (22.2)	81 (42.8)	<0.0001

Data are number (%). Others: Non-typeable SCC*mec* and SCC*mec*I.

(CFR, CFT, and CFP) than the other strains. The SCC*mec*II strains also had the highest rate in the tested genes (*aac*A/*aph*D: 55.2%, *erm*A/B/C: 89.7%, *tet*K/M: 82.8%; Table 3). A statistically significant difference was observed in the distribution of the tested genes (except for *mec*A) among the SCC*mec* types ($p < 0.0001$).

The MIC values by the *SCCmec* types of MRSA isolates from the children in the two pediatric hospitals are shown in Table S3. The SCC*mec*II isolates showed the highest MIC50 and MIC90 for almost all antibiotics tested among the SCC*mec* types. Increased MIC50 levels were observed in ERY (> 128 µg/ml), CHL (8 µg/ml), CFR (32 µg/ml), CFT (8 µg/ml), and CFP (16 µg/ml). MIC90 ≥ 128 µg/ml was noted in GEN, ERY, CHL, CFR, CFT, and CFP.

The MDR-MRSA rates significantly decreased with age: 77.1% in neonates, 54.7% in infants, and 36.2% in pediatrics ($p < 0.0001$). Table 4 shows the MDR patterns by the SCC*mec* types of the MDR-MRSA isolates in the northern and southern hospitals in Vietnam. The rate of the MDR-MRSA isolates was 51.8% (87/168). The MDR-MRSA isolates had the highest co-resistance rate to four antibiotic classes "CIP-ERY-GEN-TETs" (31/87, 35.6%), followed by "ERY-TET-CHL" (21/87, 24.1%) and "CIP-ERY-GEN" (17/87, 19.5%), with an accumulative total of 79.3%. Among 87 MDR-MRSA strains, the frequencies of the isolates that showed co-resistance to five, four, and three classes of antibiotics without beta-lactams were 1 (1.1%), 40 (46%), and 46 (52.9%), respectively. In addition, significantly higher MDR-MRSA isolates were found in the northern hospital than in the southern hospital (73 *vs.* 39%, $p <$
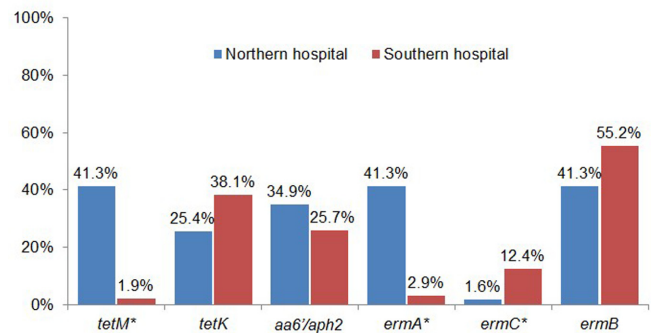

Fig. 2. Distribution of genes conferring antibiotic resistance among methicillin-resistant *Staphylococcus aureus* isolates in the northern and southern hospitals, Vietnam.
*Significant difference between northern and southern hospitals at $p < 0.05$.

0.0001). The northern hospital exhibited a higher rate of co-resistance to four antibiotics "CIP-ERY-GEN-TETs" (SCC*mec*II: 43.5% in northern hospital *vs.* 4.9% in southern hospital) and a lower rate of three co-resistance agents "CIP-ERY-GEN" (SCC*mec*IVa: 2.2% in northern hospital *vs.* 26.8% in southern hospital) in comparison with the southern hospital.

Fig. 2 shows the distribution of genes conferring antibiotic resistance among the MRSA isolates in the northern and southern hospitals in Vietnam. A significant difference was found between the two regions in the distribution of *tet*M, *erm*A, and *erm*C ($p < 0.01$). Among 168 MRSA isolates, the most predominant genes conferring

Table 4. Multidrug-resistant patterns by SCC*mec* types of methicillin-resistant *Staphylococcus aureus* isolates in two pediatric hospitals, Vietnam.

No. of co-resistance agent	Antimicrobial resistance phenotype	Total (N=87)	Northern hospital (N=46)	Southern hospital (N=41)
5	CIP-ERY-GEN-TET-CHL	II(1)	II(1)	0
4	CIP-ERY-GEN-TETs	II(22), III(3), IVa(5), NA(1)	II(20)*, III(3)	II(2), IVa(5), NA(1)
	CIP-ERY-GEN-CHL	II(1), IVa(2)	II(1)	IVa(2)
	ERY-GEN-TET-CHL	I(1), III(3), IVa(1), IVg(1)	I(1), III(1), IVa(1)	III(2), IVg(1)
3	ERY-TET-CHL	III(20), IVc/VIe(1)	III(10); IVc/VIe(1)	III(10)
	CIP-ERY-GEN	II(1), III(2), IVa(12), NA(2)	II(1), III(1), IVa(1), NA(1)	III(1), IVa(11)*, NA(1)
	CIP-ERY-TET	III(1), IVa(1)	III(1)	IVa(1)
	CIP-ERY-CHL	IVa(1), NA(1)	0	IVa(1), NA(1)
	GEN-CHL-DOX	II(1)	II(1)	0
	ERY-GEN-CHL	II(1), III(1)	0	II(1), III(1)
	ERY-GEN-TET	IVa (1)	IVa (1)	0

CHL: Chloramphenicol; CIP: ciprofloxacin; ERY: erythromycin; GEN: gentamicin; TETs (TET: tetracycline and/or DOX: doxycycline). Roman numerals indicate SCC*mec* types. Data in parentheses are number of isolates. *Statistically significant difference between Northern and Southern hospitals at $p < 0.01$.
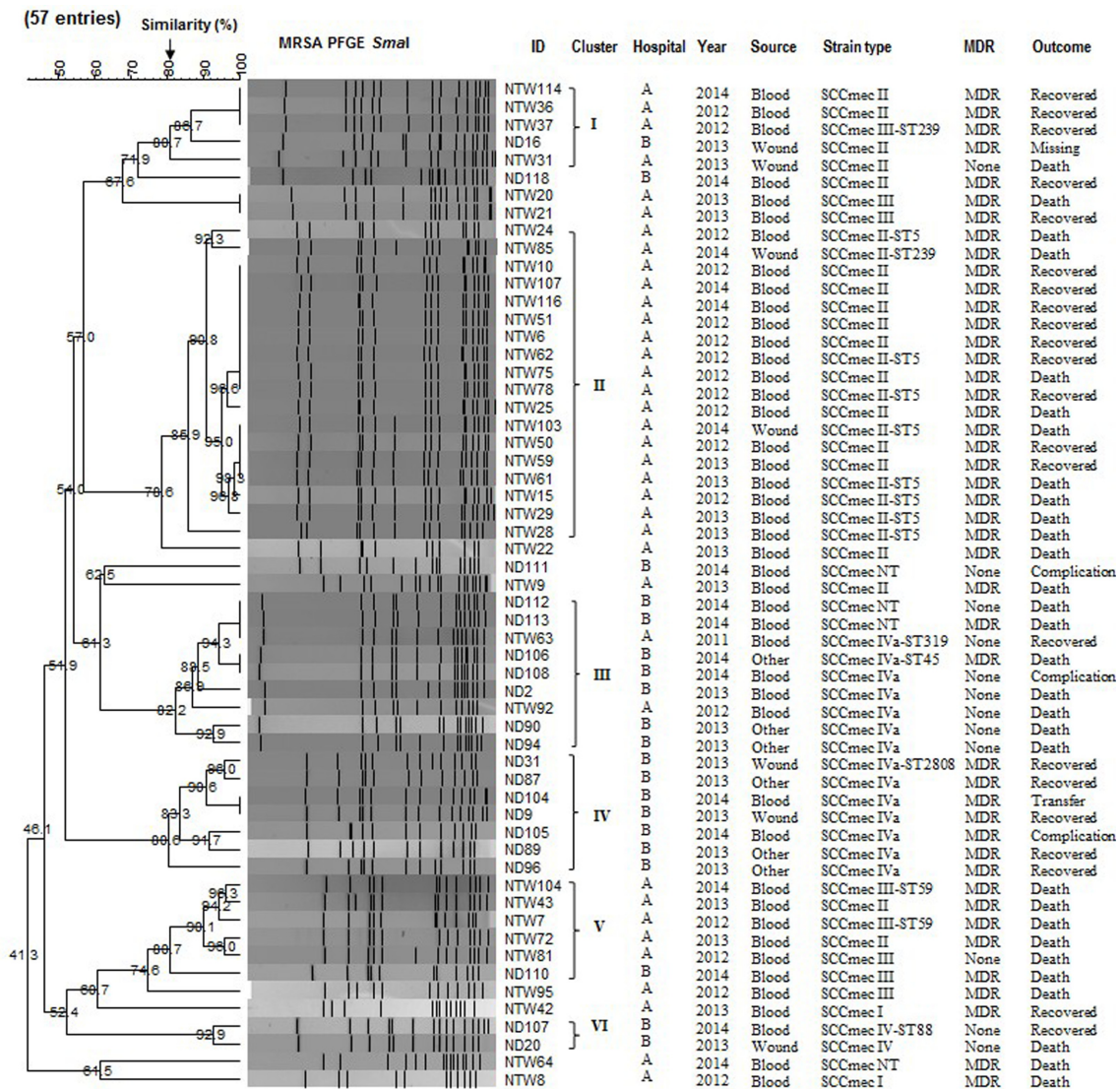
Fig. 3. Dendrogram based on PFGE patterns of 57 multidrug-resistant and/or death-associated MRSA isolates in the pediatric hospitals, Vietnam.

ID	Cluster	Hospital	Year	Source	Strain type	MDR	Outcome
NTW114	I	A	2014	Blood	SCCmec II	MDR	Recovered
NTW36	I	A	2012	Blood	SCCmec II	MDR	Recovered
NTW37	I	A	2012	Blood	SCCmec III-ST239	MDR	Recovered
ND16	I	B	2013	Wound	SCCmec II	MDR	Missing
NTW31	I	A	2013	Wound	SCCmec II	None	Death
ND118		B	2014	Blood	SCCmec II	MDR	Recovered
NTW20		A	2013	Blood	SCCmec III	MDR	Death
NTW21		A	2013	Blood	SCCmec III	MDR	Recovered
NTW24		A	2012	Blood	SCCmec II-ST5	MDR	Death
NTW85		A	2014	Wound	SCCmec II-ST239	MDR	Death
NTW10		A	2012	Blood	SCCmec II	MDR	Recovered
NTW107		A	2014	Blood	SCCmec II	MDR	Recovered
NTW116		A	2014	Blood	SCCmec II	MDR	Recovered
NTW51		A	2012	Blood	SCCmec II	MDR	Recovered
NTW6		A	2012	Blood	SCCmec II	MDR	Recovered
NTW62		A	2012	Blood	SCCmec II-ST5	MDR	Recovered
NTW75	II	A	2012	Blood	SCCmec II	MDR	Death
NTW78		A	2012	Blood	SCCmec II-ST5	MDR	Recovered
NTW25		A	2012	Blood	SCCmec II	MDR	Death
NTW103		A	2014	Wound	SCCmec II-ST5	MDR	Death
NTW50		A	2012	Blood	SCCmec II	MDR	Recovered
NTW59		A	2013	Blood	SCCmec II	MDR	Recovered
NTW61		A	2013	Blood	SCCmec II-ST5	MDR	Death
NTW15		A	2012	Blood	SCCmec II-ST5	MDR	Death
NTW29		A	2013	Blood	SCCmec II-ST5	MDR	Death
NTW28		A	2013	Blood	SCCmec II-ST5	MDR	Death
NTW22		A	2013	Blood	SCCmec II	MDR	Death
ND111		B	2014	Blood	SCCmec NT	None	Complication
NTW9		A	2013	Blood	SCCmec II	MDR	Death
ND112		B	2014	Blood	SCCmec NT	None	Death
ND113		B	2014	Blood	SCCmec NT	MDR	Death
NTW63		A	2011	Blood	SCCmec IVa-ST319	None	Recovered
ND106	III	B	2014	Other	SCCmec IVa-ST45	MDR	Death
ND108		B	2014	Blood	SCCmec IVa	None	Complication
ND2		B	2013	Blood	SCCmec IVa	None	Death
NTW92		A	2012	Blood	SCCmec IVa	None	Death
ND90		B	2013	Other	SCCmec IVa	None	Death
ND94		B	2013	Other	SCCmec IVa	None	Death
ND31		B	2013	Wound	SCCmec IVa-ST2808	MDR	Recovered
ND87		B	2013	Other	SCCmec IVa	MDR	Recovered
ND104		B	2014	Blood	SCCmec IVa	MDR	Transfer
ND9	IV	B	2013	Wound	SCCmec IVa	MDR	Recovered
ND105		B	2014	Blood	SCCmec IVa	MDR	Complication
ND89		B	2013	Other	SCCmec IVa	MDR	Recovered
ND96		B	2013	Other	SCCmec IVa	MDR	Recovered
NTW104		A	2014	Blood	SCCmec III-ST59	MDR	Death
NTW43		A	2013	Blood	SCCmec II	MDR	Death
NTW7		A	2012	Blood	SCCmec III-ST59	MDR	Death
NTW72	V	A	2013	Blood	SCCmec II	MDR	Death
NTW81		A	2012	Blood	SCCmec III	None	Death
ND110		B	2014	Blood	SCCmec III	MDR	Death
NTW95		A	2012	Blood	SCCmec III	MDR	Death
NTW42		A	2013	Blood	SCCmec I	MDR	Recovered
ND107		B	2014	Blood	SCCmec IV-ST88	None	Recovered
ND20	VI	B	2013	Wound	SCCmec IV	None	Death
NTW64		A	2014	Blood	SCCmec NT	MDR	Death
NTW8		A	2012	Blood	SCCmec I	MDR	Death

Numbers along the branches indicate percentage of similarity among isolates. The isolates with the similarity over 80% were clustered from I to VI. MRSA: methicillin-resistant *Staphylococcus aureus*; PFGE: pulsed-field gel electrophoresis; A: National Hospital of Pediatrics; B: Children's Hospital No. 2; MDR: multidrug-resistant.

antibiotic resistance were as follows: *erm*A/B/C (72.6%), *tet*K/M (42.8%), and *aac*A/*aph*D (29.2%), which corresponded to the antibiotic resistance phenotypes ERY (81.5%), TET (46.4%), and GEN (38.1%), respectively.

PFGE analysis of the MDR and/or death-associated isolates recovered in this study revealed six PFGE clusters (Fig. 3). Cluster II was the largest and the only major lineage that contained 18 MDR-MRSA isolates from the National Institute of Pediatrics in northern Vietnam. All of the 18 isolates in cluster II were SCC*mec*II. Two STs (ST5 and ST239) were identified by MLST among 9 out of 18 isolates in the major cluster II, in which ST5-SCC*mec*II was the predominant profile (8 isolates). Sporadic STs were observed in minor clusters: ST239-SCC*mec*III in cluster I, ST59-SCC*mec*III in cluster V, ST88-SCC*mec*IV in cluster VI, SCC*mec*IVa with ST45 or ST319 in cluster III, and ST2808-SCC*mec*IVa in cluster IV. Clusters III and IV contained 14 (out of 16) MDR-MRSA isolates from Children's Hospital No. 2 in southern Vietnam between 2012 and 2014. SCC*mec*IVa was predominant in clusters III and IV (14/16).

Discussion

S. aureus silently exists as a commensal bacterium that colonizes human mucosal surfaces, but it can also threaten our life as an opportunistic, conditionally pathogenic microorganism. MRSA is responsible for several infections that are difficult to treat in humans. The first MRSA was found among *S. aureus* clinical isolates in 1961 [23], less than 1 year after the introduction of methicillin into clinical practice; however, MRSA was likely acquired in the mid-1940s [24]. In the Vietnamese population, the first MRSA report in 2004–2006 [9] showed that MRSA rates were 30.1% in communities and 74.1% in hospitals in southern Vietnam. These rates were higher than those in general Asians: MRSA accounted for 25.5% of CA *S. aureus* infections and 67.4% of HA infections. In Asian populations, the MDR rates were 73.1% and 83.7% in CA-MRSA and HA-MRSA isolates, respectively ($p = 0.001$), but no such data were reported in Vietnam [9]. Several subsequent studies did not report MDR-MRSA isolates [6–8, 10, 25]. The present study is the first report of MDR-MRSA isolates in Vietnamese children. Our results raise concerns about the predominant MDR-MRSA isolates in pediatric hospitals in Vietnam. The MDR rate was 51.8% in MRSA isolates; it was significantly higher in the northern hospital compared with that in the southern hospital (73 *vs.* 39%, $p < 0.0001$), possibly reflecting differences in the use of antimicrobial agents between the two regions. The MDR rate in MRSA isolates in this study was lower than that in other countries. It varied in nosocomial children worldwide: 100% in China [19], versus 66.7% in Chicago, USA [18]. The HA-MRSA isolates from children and adults exhibited similar non-β-lactam antimicrobial drug resistance rates in Chicago, USA [18].

One of the major findings of the present study is the detailed MIC profile by the SCC*mec* types of MRSA isolates in 12 commonly used antibiotics in Vietnamese pediatric hospitals. Among the SCC*mec* types, SCC*mec*II showed the highest MIC50 and MIC90 for almost all antibiotics tested. Increased MIC50 and MIC90 levels were observed in ERY (MIC50>128 μg/ml), CHL, and cephalosporins (CFR, CFT, and CFP). Fortunately, RIF (100% sensitive, MIC range: 0.06–2 μg/ml) and VAN (100% sensitive, MIC range: 0.5–2 μg/ml) were fully susceptible antibiotics to MRSA isolates, and DOX was susceptible in most cases (94%) in the Vietnamese pediatric hospitals. VAN has been considered the standard therapy for serious MRSA infections for a long time. The decreased susceptibility of *S. aureus* to VAN has been reported since 1997 [26]. Our study showed that VAN was fully sensitive to MRSA, which was consistent with previous reports from many Asian countries (Vietnam, Hong Kong, Philippines, Sri Lanka, Korea, China, Thailand, Nepal, Taiwan, and India) [8, 9, 22, 25, 27, 28], whereas its high doses have been recommended to maintain efficacy in other countries [29]. VAN-sensitive MRSA was reported in eight pediatric hospitals in mainland China [19], whereas reduced VAN susceptibility was found in MRSA and methicillin-sensitive *S. aureus* clinical strains in two adult hospitals in northeast China [30]. A national antimicrobial resistance monitoring system for *S. aureus* must be maintained.

In terms of the SCC*mec* types in MRSA isolates, a previous study on adult subjects in southern Vietnam reported [9] that the most frequent SCC*mec* type is SCC*mec*III (CA: 51.6%, HA: 47.6%), followed by SCC*mec*II (CA: 15.6%, HA: 33.3%), SCC*mec*I (CA: 12.9%, HA: 4.8%), and SCC*mec*IV (CA: 3.2%, HA: 0%). The present study on pediatric subjects in southern Vietnam reported a different trend: SCC*mec*IV (CA: 34.8%, HA: 53.7%), SCC*mec*III (CA: 47.8%, HA: 39%), and SCC*mec*II (CA: 13%, HA: 0%). Only one study analyzed the SCC*mec* types in children: an outbreak of CA-MRSA [10] reported ST59-SCC*mec*V (N=5), ST59-SCC*mec*IVa (N=1), and one allele variant of ST45 (N=1) in nine children. Notably, the north–south difference of the SCC*mec* types was observed in the present study: SCC*mec*II (40.6%) was predominant in northern Vietnam, whereas SCC*mec*IV (47.3%) was more pronounced in southern Vietnam ($p < 0.0001$), showing the different genetic structures of MRSA between the two regions. Indeed, a significant difference was observed between the two regions in the distribution of genes (*tet*M, *erm*A, and *erm*C) conferring antibiotic resistance ($p < 0.01$).

The observed differences in the antimicrobial susceptibilities and genotypes between northern and southern MRSA isolates may be explained by the following factors. (i) Farmers from the north and south have different knowledge and practices on antibiotic use (many antibiotics used to treat human diseases are used in agriculture) [31]. (ii) Antibiotic prescription in private clinics is not based on antibiotic susceptibility tests, and private clinics are abundant in the Red River Delta (58%) compared with that the Mekong River Delta (27%) [32]. (iii) The prevalence of inappropriate indications for antibiotic prescriptions is high in hospitals in Vietnam [33], and most antibiotics are sold without a prescription in pharmacies: 88% and 91% in urban and rural areas, respectively [34].

Among 16 MDR-MRSA isolates from the southern pediatric hospital in Ho Chi Minh City, SCC*mec*IVa was

predominant (14/16) in clusters III and IV, indicating that SCC*mec*IVa could be expanded clonally in the hospital and may result in intra-hospital patient-to-patient spread. SCC*mec* type IVa isolates are known to be more prevalent in South Korea than in other Asian countries [35]. The strain NN47 was isolated in 2008 from a Japanese girl and described as the first USA300 transmitted among people in Japan [36]. The USA300 clone, that is, ST8-MRSA-IVa, has become the prevalent CA-MRSA strain in the United States since it was first reported in 2000 as a cause of skin and soft tissue infections. It is also recognized as an emerging nosocomial MRSA [37]. SCC*mec*IVa was reported to be predominant in a major hospital in Lebanon [38] and in a community environment in Australia [39]. Given that the USA300 clone is highly transmissible and virulent, surveillance in Vietnam is of utmost importance.

MLST STs, namely, CA-MRSA-ST59, CA-MRSA-ST239, and HA-MRSA-ST239, were previously reported in southern Vietnam [9,40]. In northern Vietnam, HA-MRSA: ST59 (15/25), ST45 (5/25), ST239 (2/25), ST188 (2/25), and ST834 (1/25); and CA-MRSA: ST45 (11/28), ST59 (8/28), ST188 (1/28), ST25 (1/28), ST88 (1/28), ST121 (2/28), ST1232 (1/28), ST15 (1/28), ST406 (1/28), and ST942 (1/28) were described [7]. Previous studies reported the predominant STs of ST59 and ST45 in the north and ST59 and ST239 in the south for both HA-MRSA and CA-MRSA isolates from adults. In 2006, an outbreak of severe CA-MRSA pediatric infections of ST59 occurred in southern Vietnam following routine vaccination injection [10]. ST45 and ST239 were also found in this study. The present study indicated the predominant clone of ST5-SCC*mec*II. It initially recognized ST239-SCC*mec*II and ST319-SCC*mec*VIa in the northern hospital and ST2808-SCC*mec*IVa in the southern hospital.

In Asian countries, ST239-SCC*mec*III and ST5-SCC*mec*II have been reported as the two major endemic MRSA clones prevalent in hospitals with a unique geographic distribution and evolutionary MRSA clone patterns. Almost all MRSA strains from Korea and Japan belong to ST5-SCC*mec*II (named as the New York/Japan MRSA clone), whereas most MRSA isolates from other Asian countries belong to ST239-SCC*mec*III [40]. After 15 years, these HA-MRSA clones possibly spread to communities in countries including Korea, Taiwan, Thailand, Vietnam, and Sri Lanka [9]. The major clonal lineages of HA-MRSA isolates were ST239-MRSA-SCC*mec*III in Thailand, Korea, Vietnam, Taiwan, and India and ST5-MRSA-SCC*mec* type II in Taiwan, Philippines, Hong Kong, Sri Lanka, and Korea. ST5-SCC*mec*II (USA100) has not been previously reported

in Vietnam. In the present study, ST5-SCC*mec*II was initially recognized as the predominant clone in the northern hospital, suggesting the intra-hospital transmission of this clone.

The ST239-MRSA clone was first discovered in Brazil [41], and it has since become widely spread in various hospitals. ST239-SCC*mec*III is commonly found in HA-MRSA in Asian populations [9,40,42]. Interestingly, ST239-SCC*mec*II was first reported in this study, in line with a report in a Malaysian hospital [43]. This ST239-SCC*mec*II clone is more frequent (8.1%, 16/198) in HA-MRSA isolates in Chinese hospitals than in other Asian countries [44]. Thus, evaluations of the geographic distribution and evolutionary pattern of this MRSA clone are crucial.

The present study has some limitations. First, as a retrospective study, the database collected from medical records was subject to missing data, and the incidence rates of *S. aureus* infection outbreak and non-MDR-MRSA infections could not be identified. Second, MLST and *spa* and *arg* typing methods could not be performed in all samples although the ST types were identified in all clusters. Third, the sample size of CA-MRSA was relatively small, so significant differences in antibiotic resistance of CA-MRSA between the two regions could not be detected. Finally, our work included only some genes conferring antibiotic resistance. A comprehensive image of the genetic patterns of MRSA isolates in Vietnam may be necessary in the future.

In conclusion, the present study highlighted concerns about the predominant MDR-MRSA isolates from pediatric patients in Vietnam. The most effective antibiotics to treat *S. aureus* infections in children were found to be VAN and RIF. These findings support a different characteristic of the molecular structure and antibiotic resistance of MRSA isolates from pediatric hospitals between northern and southern Vietnam.

Acknowledgments

design, data collection and analysis, decision to publish, or preparation of the manuscript.

Conflict of Interest

The authors have no financial conflicts of interest to declare.

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