EPIDEMIOLOGICAL ANALYSIS OF METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* IN THAILAND

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Abstract. The geographical distribution of 65 clinical isolates of methicillin resistant *Staphylococcus aureus* (MRSA) recovered from 7 hospitals in Thailand was investigated. The presence of *mec*A gene in MRSA was determined by specific PCR with the use of primers 5´-GTAGTTGTCGGGTTTGGT-3´ and 5´-GGTATCATCTTGTACCCA-3´. Chromosomal DNA restriction analysis with *Sma*I was resolved by pulsed-field gel electrophoresis (PFGE) compared with antibiotype analysis and phage type analysis. All 65 strains carried *mec*A gene. They all were resistant to penicillin, tetracycline, erythromycin, amoxicillin/clavulanic acid and variably resistant to gentamicin, ofloxacin, trimethoprim-sulfamethoxazole, chloramphenicol, fosfomycin and clindamycin; and all isolates were susceptible to vancomycin. A total of 19 PFGE patterns designated as type A, A1, A2, A3, A4, B, B1, C, D, E, E1, E2, F, F1, F2, G, H, I and J was identified. Type A4 and E were commonly found in every studied areas. Phage typing showed even greater variability that 52 (80%) isolates belonged to 25 different phage types; 13 (20%) isolates were non-typable. The clarity and polymorphism of the PFGE patterns enable us to discriminate between isolates which could not be differentiated by antibiogram or phage type analysis. The findings demonstrate the existence of a common epidemic MRSA clone in Thailand.

INTRODUCTION

Methicillin resistant *Staphylococcus aureus* (MRSA) is recognized as a common cause of both hospital- and community-acquired infections worldwide. A total of 1,783 (25%) isolations of MRSA were reported from 23 hospitals to the National Institute of Health, Thailand in 1998. The increased proportion was much more evenly distributed around the country than before. Between 1988 and 1998 the MRSA proportion rose from 11% to 23.4% in the northeast region, from 16% to 30.5% in the central region, and from 21% to 30.3% in the southern region (The Committee on Using Computer in Clinical Microbiology, 1991; (National Antimicrobial Resistance Surveillance Center, 1999). Multiple techniques have been used to identify distinct strains of MRSA and to resolve epidemiologically related cases (Wei and Grubb, 1992; Jordensen *et al*, 1996). Comparison of the patterns of PFGE of DNA fragments from MRSA with the various phage type and resistance levels can be used to elucidate the epidemiology of MRSA strains.

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The aim of the present study was to investigate the geographical distribution of the MRSA clinical isolates recovered from different hospitals located in four regions in Thailand.

MATERIALS AND METHODS

Bacterial strains

From January 1996 to December 1998, 65 MRSA isolates (isolate no.W-1 to W-65) from sporadic infections and from outbreaks in hospitals from four regions (north, northeast, central and south) of Thailand were sent to a national reference center for staphylococci (Fig 1). All isolates were confirmed as *S. aureus* based on coagulase production using rabbit plasma, DNase production and standard biochemical methodologies (Kloss and Bannerman, 1995). Strains were defined as MRSA by disk diffusion with inhibition zone diameter ≤10 mm for oxacillin (1 μg). Of these isolates, 65 single-patient isolates of MRSA (isolate no. W-1 to W-65) from 7 hospitals (hospital 1-7) located in four regions in Thailand were subjected to molecular typing.

Antibiograms

All isolates were tested by the Kirby-Bauer disk diffusion according to the National Committee for Clinical Laboratory Standards (NCCLS, 1997) recommendations to determine the susceptibility

pattern against a panel of 11 antibiotics. The antibiotics were penicillin, amoxicillin/clavulanic acid, gentamicin, tetracycline, ofloxacin, trimethoprim-sulfamethoxazole, erythromycin, chloramphenicol, vancomycin, fosfomycin and clindamycin. The MICs of vancomycin against 65 MRSA isolates (isolate no. W-1 to W-65) were determined by using Etest.

Methicillin resistance of 65 isolates (isolate no. W-1 to W-65) was confirmed by using Etest with MIC ≥ 4 mg/l for oxacillin.

Detection of the *mec*A gene

The presence of *mec*A gene in 65 isolates of MRSA was determined by specific PCR with the use of primers: 5´-GTAGTTGTCGGGTTT-GGT-3´ and 5´-GGTATCATCTTGTACCCA-3´. The samples were amplified for 25 cycles, with each cycle consisting of 30 seconds at 94ºC, 30 seconds at 55ºC, and 30 seconds at 72ºC. The *mec*A specific PCR product was 589-bp long.

Bacteriophage typing

Bacteriophage typing was performed by using the international bacteriophage typing set issued by the International Center, Colindale, UK. All phage were used at 100x routine test dilution (RTD). Phage types that differed by the presence or absence of one phage were considered related. Differences by the presence or absence of two or more phages were considered to be unrelated strains.

PFGE analysis

DNA extraction and PFGE were performed by a modification of the method described by Smith *et al* (1988). Briefly, MRSA cells grown overnight in 10 ml of brain heart infusion broth were resuspend in TES buffer to achieve a 3 McFarland turbidity and mixed with an equal volume of 1.6 % low melting agarose (Bio-Rad Laboratories, Richmond, CA, USA.) to make plugs for PFGE. The cells in the plugs were treated with lysis solution and then with protease. The DNA was digested overnight with *Sma*I (New England Biolabs Inc, Beverly, MA, USA). For PFGE, a CHEF DR II system (Bio-Rad Laboratories, Richmond, CA, USA) was used with switch time 10 and 20 seconds for 22 hours at 6 V/cm. The gels were stained with ehidium bromide and photographed under UV light. Chromosomal DNA of *Saccharomyces cerevisiae* (Bio-Rad Laboratories) was used for molecular standard markers. The interpretation of the banding patterns was done visually according to the guidelines of Bannerman *et al*. (1995).

RESULTS

Detection of the *mec*A gene

All the 65 studied isolates carried *mec*A gene. An example of the positive and negative results is shown in Fig 2.

Antibiograms

Sixty-five MRSA isolates obtained during 1997-1998 were analyzed to detect the patterns of resistance against a panel of 11 antimicrobial agents. A large diversity of antibiograms was found, thirteen patterns were identified and all isolates were resistant to penicillin, tetracycline, erythromycin, amoxicillin/clavulanic acid and variably resistant to gentamicin (95.4%), ofloxacin (93.8%), trimethoprim-sulfamethoxazole (81.5%), chloramphenicol (84.6%), fosfomycin (3.1%) and clindamycin (56.9%); and all isolates were susceptible to vancomycin (Table 1). The MICs for 65 MRSA isolates (isolate no. W-1 to W-65) ranges from 0.75-2.00 μg/ml.

Bacteriophage typing

Among the 65 MRSA isolates studied, phage typing showed a great variability that 22 (33.8%) isolates belonged to 8 different single phage types; 30 (46.2%) belonged to 17 different mixed phage types; and 13 (20%) isolates were non-typable (Table 1). The majority of the MRSA isolates were restricted to specific areas of Thailand. For example, MRSA with phage types 85, 77, 6/54 and 55/6/75/85 were restricted to the northern province (Chiang Mai), the northeasthern province (Khon Kaen), the southern province (Songkhla) and the central province (Bangkok), respectively. Only a few MRSA isolates were distributed throughout a number of regions.

PFGE analysis

Sixty-five MRSA isolates were tested for PFGE patterns of *Sma*I-digested genomic DNA. Ten major PFGE patterns designated as type A, B, C, D, E, F, G, H, I and J; 9 subtypes A1, A2, A3, A4, B1, E1, E2, F1, F2 were identified (Table 1 and Fig 3). The PFGE pattern A and related subtypes were represented by 36.9% of the isolates (24 of 65). The most frequent among these subtypes was A4,

Table 1
PFGE, phage type, antibiogram and source of 65 MRSA isolates studied.

Isolate	PFGE	Phage type	Resistant to a panel of 11 antibiotics*expept for:	Antibiogram	Origin Province(hospital no.)
W-48	A	55/6/75	SXT C V FOS DA	12	Bangkok(HP5)
W-49	A	55/6/75/85	SXT C V FOS DA	12	Bangkok(HP5)
W-50	A	55/6/75/85	SXT V FOS	3	Bangkok(HP5)
W-l	A	54/85	V FOS	1	Udon Thani(HP3)
W-9	Al	47	V FOS	1	Chaing Mai(HP1)
W-24	A2	NT	V FOS	1	Nan(HP2)
W-62	A2	54	C V FOS DA	11	Songkhla(HP7)
W-63	A2	6/54	C V FOS DA	11	Songkhla(HP7)
W-64	A2	6/54	OFX V DA	13	Songkhla(HP7)
W-65	A2	6/54	OFX V DA	13	Songkhla(HP7)
W-51	A3	79/55/6/54/75/83A/84/85	V FOS DA	5	Bangkok(HP5)
W-10	A4	85	V FOS	1	Chaing Mai(HP1)
W-11	A4	NT	CN OFX V FOS	7	Chaing Mai(HP1)
W-12	A4	6/54/85	V FOS	1	Chaing Mai(HP1)
W-13	A4	6/47/53/54/75/84/85	SXT V FOS DA	10	Chaing Mai(HP1)
W-2	A4	54/85	SXT V FOS	3	Udon Thani(HP3)
W-29	A4	77	V FOS	1	Khon Kaen(HP4)
W-30	A4	77	V FOS	1	Khon Kaen(HP4)
W-31	A4	84	V FOS DA	5	Khon Kaen(HP4)
W-52	A4	55/6/75/85	V FOS DA	5	Bangkok(HP5)
W-53	A4	55/6/75/85	SXT C V FOS DA	12	Bangkok(HP5)
W-54	A4	55/6/75/85	SXT V FOS	3	Bangkok(HP5)
W-55	A4	55/6/75/85	SXT C V FOS	9	Bangkok(HP5)
W-56	A4	55/6/75	SXT V FOS DA	10	Bangkok(HP5)
W-25	B	NT	V FOS DA	5	Nan(HP2)
W-26	B	NT	V FOS DA	5	Nan(HP2)
W-27	B	47/75	V FOS DA	5	Nan(HP2)
W-3	B	NT	OFX V FOS	6	Udon Thani(HP3)
W-4	B	NT	V FOS	1	Udon Thani(HP3)
W-32	B	NT	V FOS	1	Khon Kaen(HP4)
W-33	B	77	V FOS DA	5	Khon Kaen(HP4)
W-34	B	77	V FOS	1	Khon Kaen(HP4)
W-35	B	85	V FOS DA	5	Khon Kaen(HP4)
W-36	B1	77/85	V FOS DA	5	Khon Kaen(HP4)
W-14	C	85	C V FOS DA	11	Chaing Mai(HP1)
W-37	D	85	V FOS DA	5	Khon Kaen(HP4)
W-15	E	85	V FOS DA	5	Chaing Mai(HP1)
W-16	E	47	V FOS	1	Chaing Mai(HP1)
W-28	E	NT	V FOS	1	Nan(HP2)
W-5	E	54/85	V FOS	1	Udon Thani(HP3)
W-38	E	6	V FOS	1	Khon Kaen(HP4)
W-39	E	NT	V FOS	1	Khon Kaen(HP4)
W-40	E	85/96	V FOS DA	5	Khon Kaen(HP4)
W-60	E	55/6/47/75/85/96	V FOS	1	Nonthaburi(HP6)
W-61	E	55/6/47/75/85/96	V FOS DA	5	Nonthaburi(HP6)
W-57	E	55/6	C V FOS	4	Bangkok(HP5)
W-58	El	55	V FOS	1	Bangkok(HP5)
W-59	E2	NT	CN V FOS DA	8	Bangkok(HP5)
W-6	F	54/85	V FOS	1	Udon Thani(HP3)
W-41	F	NT	V FOS	1	Khon Kaen(HP4)
W-42	F	77	V FOS DA	5	Khon Kaen(HP4)
W-43	F	77/84/85	V FOS DA	5	Khon Kaen(HP4)
W-44	F	84/85	V FOS DA	5	Khon Kaen(HP4)
W-45	F	96	V FOS DA	5	Khon Kaen(HP4)
W-46	Fl	53/77	V FOS	1	Khon Kaen(HP4)
W-7	F2	54/85	V FOS	1	Udon Thani(HP3)
W-17	G	85	SXT V FOS DA	10	Chaing Mai(HP1)
W-18	H	85	V FOS	1	Chaing Mai(HP1)
W-l9	H	85	SXT C V FOS	9	Chaing Mai(HP1)
W-20	H	47/84/85	V FOS	1	Chaing Mai(HP1)
W-21	H	NT	SXT V FOS	3	Chaing Mai(HP1)
W-47	H	NT	V FOS	1	Khon Kaen(HP4)
W-8	I	54/83A/84/85	V FOS	1	Udon Thani(HP3)
W-22	I	85	CN V FOS	2	Chaing Mai(HP1)
W-23	J	85	C V FOS DA	11	Chaing Mai(HP1)

*penicillin, amoxicillin/clavulanic acid, gentamicin(CN), tetracycline, ofloxacin(OFX), trimethoprim-sulfamethoxazole(SXT), erythromycin, chloramphenicol(C), vancomycin(V), fosfomycin(FOS) and clindamycin(DA).

identified in 13 (20%) of the 65 MRSA isolates. Type E and related subtypes were identified in 18.5 % of the isolates (12 of 56). Both types and related subtypes were common PFGE patterns found in 7 different provinces studied that are hundreds of kilometers apart (Table 1, Fig 1).

DISCUSSION

In 1996, Hiramatsu and colleagues reported the first clinical isolate of MRSA with reduced susceptibility to vancomycin (MIC=8 μg/ml). Subsequently, similar organisms with reduced susceptibility to glycopeptides were reported from Michigan and New Jersey (CDC, 1997). No such organisms have been observed in our study (MICs of vancomycin 0.75-2 μg/ml). Resistance to fosfomycin were observed in 2 (3.1%) MRSA isolates.

Recently, pulsed-field gel electrophoretic (PFGE) analysis of *Sma*I-restricted genomic DNA has proven to be an adequate means for the detection and typing of a multitute of microorganisms (Wei and Grubb, 1992; Jordensen *et al*, 1996). In this study we analyzed a number of isolates from seven hospitals in four different regions (north, northeast, central and south) of Thailand, using antibiograms, phage typing and PFGE in an attempt to identify related isolates of MRSA which may be epidemic. We showed, like others that the PFGE analysis using *Sma*I is very effective typing method. PFGE requires preparation of DNA in agarose blocks and the use of specialized electrophoretic equipment. However, time and labor are required to maintain phage stocks and propagating strains. Interpretation of phage typing results was difficult when multiple, related patterns were ob-

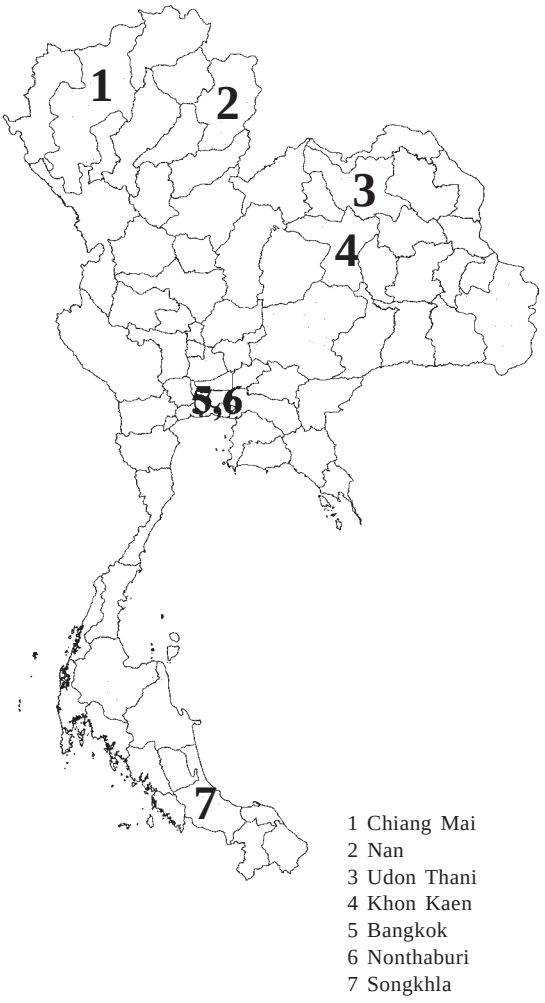

Fig 1–The Map shows the locations and names of the 7 provinces in Thailand included in study.

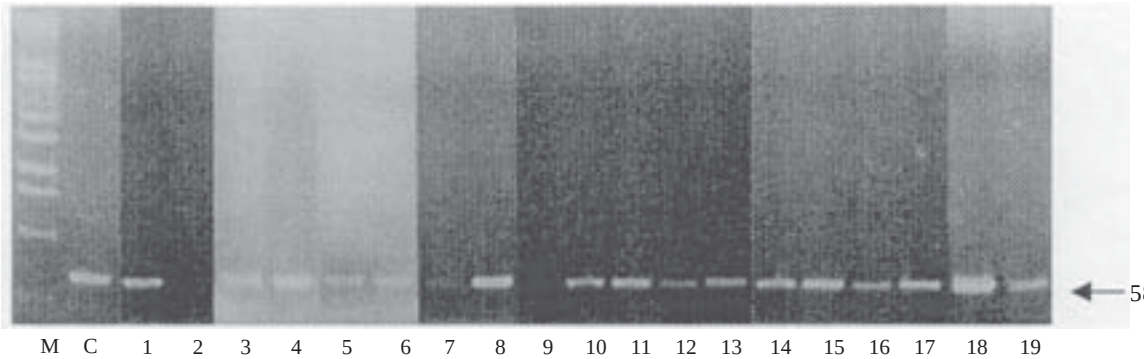

Fig 2–Amplified *mec*A of clinically isolated *Staphylococcus aureus* by PCR. Lane M, DNA marker ladder. Lane C, positive control. Lane 1, 3-8, 10-19, positive isolates. Lane 2 and 9, negative isolates.

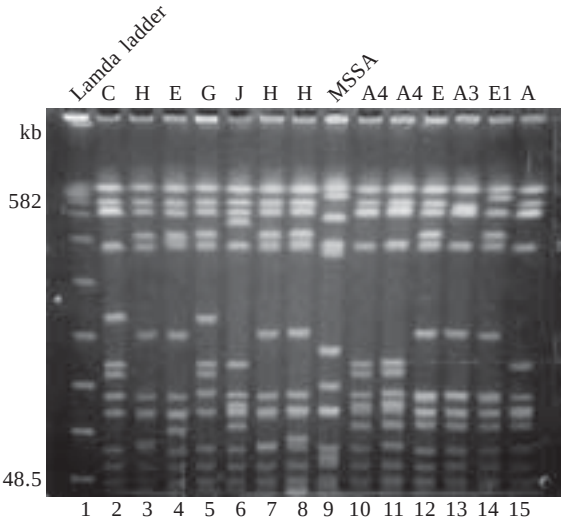

Fig 3–PFGE major patterns and subtypes of *Sma*I digests. The PFGE types are shown above the lanes.

served. Antibiograms are the simplest method to perform and the results were the simplest to interpret; no equipment was required. But they do not provide a high degree of specificity. All of the 65 MRSA isolates obtained during 1997-98 were multiresistant (resistance to six to nine antibiotics). The antibiogram pattern showed 13 different types. In the present study, 20% of the MRSA isolates were non-typable with the international panel of phages. Ten MRSA isolates studied (W-10, W-35, W-14, W-37, W-15,W-17, W-18, W-19, W-22 and W-23) had the same single phage type (type 85) but were divided by PFGE into 9 PFGE patterns (A4, B, C, D, E, G, H, I and J). On the other hand, 4 MRSA isolates (W-10, W-29, W-30 and W-31) had the single PFGE pattern (A4) but were divided by bacteriophage into 3 single-phage types (type 85, 77 and 84). Five non-typable isolates (W-25, W-26, W-3,W-4, and W-32) and three different phage types (W-27, W-33 or W-34 and W-35) could be classified into the major PFGE pattern B. Therefore, a combination of both PFGE and phage typing methods may be most efficacious for discriminating between MRSA isolates.

The clarity and polymorphism of the PFGE patterns enable us to discriminate between isolates which could not be differentiated by antibiogram or phage type analysis. The molecular findings demonstrate the existence of a common epidemic MRSA clone, which is widely dispersed in provincial hospitals hundreds of kilometers apart.

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