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A major methicillin-resistant *Staphylococcus aureus* clone predominates in Malaysian hospitals

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SUMMARY

This study was conducted to determine the molecular epidemiology of methicillin-resistant *Staphylococcus aureus* (MRSA) in Malaysian hospitals. A total of 264 MRSA isolates from eight hospitals were subjected to typing by pulsed-field gel electrophoresis (PFGE) of *Sma*I restricted DNA. Antibiotic disk susceptibility testing was also carried out to determine their resistance patterns. Thirty-one PFGE pattern types were identified. Three major pattern types A, ZC and K were found with type A the predominant profile in *c.* 80% of strains and present in all hospitals. Unlike type A, other DNA pattern types were unique to the hospitals in which they were isolated. PFGE type A also consisted of strains that were multiply antibiotic resistant. The presence of a single predominant PFGE type in Malaysian hospitals is an important finding which suggests that inter-hospital spread of MRSA had occurred frequently and regularly.

INTRODUCTION

Molecular typing techniques such as chromosomal DNA typing and PCR-based typing methods have provided insights to the molecular epidemiology of methicillin-resistant *Staphylococcus aureus* (MRSA). The technique of pulsed-field gel electrophoresis (PFGE) of *Sma*I restricted DNA has been widely used to demonstrate the existence of common epidemic MRSA clones in a geographical area [1, 2]. The spread of some MRSA epidemic clones such as the 'Iberian MRSA' in Portuguese and Spanish hospitals [3] and the Brazilian epidemic clone in Argentinian hospitals [4] has also been well documented at the molecular level. These and other studies have confirmed that certain MRSA clones are widely distributed internationally, while others are confined to a particular geographical area.

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MRSA is a major nosocomial pathogen in Malaysian hospitals where a gradual increase has been observed in its frequency in eight major hospitals rising from 20% in 1990 to 32% in 1998 (National Surveillance of Antibiotic Resistance (NSAR) System, Ministry of Health, Malaysia). No data are available on the clonality of our local MRSA strains and so this study was conducted to provide information on the prevalence of MRSA clones currently in Malaysian hospitals and to determine their distribution country wide.

MATERIALS AND METHODS

Hospitals

Eight major hospitals (700–900 beds) were included in this study. Six of these hospitals are situated in different states in Malaysia, namely Alor Star (HAS) in Kedah; Seremban (HSE) in Negeri Sembilan;

Sultanah Aminah (HSA) in Johore; Kota Bharu (HKB) in Kelantan; Sarawak (HUS) in Sarawak and Queen Elizabeth (HQE) in Sabah. Two teaching hospitals, Universiti Kebangsaan Malaysia (HUKM) and Kuala Lumpur (HKL) are situated in Kuala Lumpur. All the states are located in Peninsular Malaysia except for Sarawak and Sabah, which are in East Malaysia and separated from Peninsular Malaysia by the South China Sea.

Bacterial strains

MRSA isolates were recovered from clinical samples and identified in the microbiology laboratory of the respective hospitals. The isolates were collected from 1997 until 1999 and a random selection from single patients from each hospital was examined (total 264), namely 28 isolates from HAS, 26 from HSE, 23 from HAS, 22 from HKB, 47 from HUKM, 38 from HKL, 40 from HUS and 40 from HQE. Of the 264 isolates, 92 (34·3%) were from skin and wound swabs, 69 (26%) from pus, 28 (10·6%) from blood, 25 (9·5%) from respiratory tract specimens, 4 (1·5%) from urine, 7 (2·7%) from tissue, 17 (6·4%) from ear, nose and throat swabs, 8 (3%) from catheter tips, 5 (1·9%) from body fluids and 10 (3·9%) from unknown sources. All isolates were reconfirmed as MRSA by colonial morphology, Gram stain, positive coagulase and deoxyribonuclease tests, and resistance to methicillin by oxacillin disk susceptibility testing following the guidelines of the National Committee for Clinical Laboratory Standards (NCCLS) [5]. Isolates were also tested for susceptibility to erythromycin, sulphamethoxazole-trimethoprim, tetracycline, gentamicin, fusidic acid, rifampicin, ciprofloxacin, chloramphenicol and vancomycin.

Pulsed-field gel electrophoresis

An overnight broth culture of each of the isolates tested was adjusted to a concentration of 1×10^9 c.f.u./ml. After washing in TE1 buffer (10 mM Tris–HCl, 50 mM EDTA; pH 7·5), 200 μl of the bacterial suspension was added to an equal volume of 2% low-melting point agarose. Lysostaphin (1 mg/ml) was added immediately and the suspension mixed well before being allowed to solidify in a plug mould. The gel plugs were incubated overnight at 55 °C in 2 ml ES buffer (1% *N*-laurylsarcosine in 0·5 ml EDTA; pH 8·0) containing proteinase K (10 mg/ml) with gentle shaking. After washing in TE2 buffer (10 mM Tris, 1 mM EDTA; pH 8·0), a 2·5 mm slice of the plug was digested with *Sma*I and incubated overnight. DNA electrophoresis was carried out in 1·2% agarose using CHEF-DRIII (Bio-Rad) with pulse times 5–15 s for 8 h, followed by 15–25 s for 10 h. Gels were stained with ethidium bromide and photographed under UV illumination. DNA fragment patterns that had three or fewer band differences were defined as sharing a common PFGE pattern (capital letter) while subtypes were defined as variants with 1–7 different DNA fragments [6].

RESULTS

Antibiotic susceptibility patterns

The majority of the 264 isolates were resistant to erythromycin, gentamicin, tetracycline and sulphamethoxazole-trimethoprim, with resistance rates of 96–100%. All isolates from HKL, HKB, HUS and HQE were resistant to each of these antibiotics. Erythromycin resistance of 100% was observed for isolates from all hospitals except HUKM, where only 1 of 47 isolates was susceptible. Resistance to ciprofloxacin was 68–96% but less than 20% of isolates in most hospitals were resistant to chloramphenicol. Fusidic acid and rifampicin resistance was 0–30% and 0–23% respectively. The majority of multiresistant isolates were recovered from HQE.

PFGE typing patterns

A total of 31 PFGE patterns was observed among the MRSA isolates and their frequencies are shown in Table 1. Three major pattern types ($\geqslant 5$ isolates) were identified, types A, ZC and K. Type A was present in all the hospitals while ZC and K were confined to HQE and HSE respectively.

Type A accounted for 212 of 264 (80·3%) isolates and represented 60–95% of the isolates from each of the hospitals studied. At least 7–11 subtypes of pattern A were distinguishable in each hospital and the most common, A1, was present in all hospitals. The number of other PFGE types in each of the hospitals ranged from 1 to 7 and 25 patterns were unique. Although HKL and HUKM are situated in the same geographical area, they did not share isolates of the same PFGE type except for type A. The latter was also present in HUS and HQE, which were separated from other hospitals in Peninsular Malaysia by the South China Sea.

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Table 1. *Distribution of PFGE pattern types of MRSA in Malaysian hospitals*

Hospitals (no. of isolates)	PFGE types	No. of subtypes	No. of isolates
HKL (38)	A	10	33
	B	—	1
	C	—	1
	D	—	1
	E	—	1
	F	—	1
HUKM (47)	A	10	41
	G	—	1
	H	2	3
	I	—	1
	J	—	1
HSE (26)	A	7	18
	K	3	7
	L	—	1
HSA (23)	A	10	20
	M	—	1
	N	—	1
	O	—	1
HKB (22)	A	11	21
	ZG	—	1
HAS (28)	A	8	20
	P	—	1
	Q	—	1
	R	—	1
	S	2	2
	T	—	1
	V	—	1
	W	—	1
HUS (40)	A	9	34
	X	—	1
	Y	3	3
	Z	—	1
	ZA	—	1
HQE (40)	A	11	25
	ZB	—	1
	ZC	2	10
	ZD	—	1
	ZE	—	1
	ZF	2	2

Correlation of antibiotic resistance with PFGE types

Isolates of each of the 31 PFGE types were resistant to erythromycin, gentamicin, tetracycline and sulphamethoxazole-trimethoprim. Isolates with additional resistance to ciprofloxacin and chloramphenicol were found in six PFGE types; A, K, W, Y, ZA and ZF. Resistance to both fusidic acid and rifampicin was restricted to type ZC, which was present only in HQE, and types A and I in HUKM (Table 2).

Table 2. *Antimicrobial resistance of MRSA according to PFGE pattern type*

Antibiotic group*	PFGE types	% multiresistant
Ery, Gen, Tet, SXT	A	78
	ZC	4
	K	3
	H, Y	1
	S, ZF	0·8
	Other types	0·4
Ery, Gen, Tet, SXT, Cip	A	63
	ZC	4
	K	3
	H, Y	1
	ZF	0·8
	B, C, F, J, M, X, ZD	0
	Other types	0·4
Ery, Gen, Tet, SXT, Cip, Chl	A	11
	Y	1
	ZF	0·8
	K, W, ZA	0·4
	Other types	0
Ery, Gen, Tet, SXT, Cip, Chl, FA	A	0·8
	Other types	0
FA, Rif	A	1
	ZC	3
	I	0·4
	Other types	0

* Ery, erythromycin; Gen, gentamicin; Tet, tetracycline; SXT, sulphamethoxazole-trimethoprim; Cip, ciprofloxacin; Chl, chloramphenicol; FA, fusidic acid; Rif, rifampicin.

DISCUSSION

A single PFGE type accounted for *c.* 80% of Malaysian MRSA isolates in hospitals in both Peninsular and East Malaysia. The ability to be transmitted over great distances appears to be a defining characteristic of this particular clone of MRSA as other PFGE types were either infrequent or were confined to a single hospital. This finding suggests that inter-hospital spread of MRSA occurs frequently and regularly. The predominance of type A and its subtypes in all the hospitals could be due to increased nosocomial transmission within and between the hospitals and/or may be attributable to a virulence-related property of the particular strain that has yet to be determined. Aathithan et al. [7] found no relationship between methicillin resistance or epidemic type with cell-wall bound protein A content and adherence in highly epidemic strains of MRSA in the UK. Moreover, Enright et al. [8] observed that the most prevalent methicillin-sensitive *S. aureus* (MSSA) clone

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was very closely related to EMRSA-16, a widespread epidemic strain in the UK. They postulated that the success of the latter clone at causing disease in hospital patients may be due to its emergence from a virulent MSSA clone that was already a major cause of invasive disease in both the community and hospital settings.

Seven to 11 subtypes of PFGE type A were identified in the eight hospitals. Some other PFGE types had a maximum of three subtypes but the majority of the different PFGE patterns were uniform and not representative of variant patterns. A well-defined MRSA clone has been shown to generate a number of subtypes as it persists in the hospital *in vivo* environment [9]. This variation may result from genetic events causing point mutations in or around restriction sites or may be related to insertion or deletion of mobile DNA elements [6, 10]. Type A strains and its subtype variants are genetically related and have probably propagated within and between all the eight hospitals. These subtypes arose as the strains persisted and continued to be transmitted in the environment.

Schmitz et al. [11] suggested that the development of higher resistance rates to multiple antibiotics in MRSA is a consequence of the clonal spread of individual multiresistant strains. Here, PFGE type A were consistently multiply antibiotic resistant and clearly had spread among major hospitals in the country. The study of Roberts et al. [2] reported that resistance to gentamicin and sulphamethoxazole-trimethoprim was highest in a clone that was not widely spread, while a lower prevalence of resistance to these antibiotics was found in a widely distributed major clone. Our findings contrast with these as we observed a high resistance rate for these agents in the most widely distributed clone. The common antibiotype pattern was unhelpful in discriminating between any of the strains except those of PFGE type ZC. This type was unique to HQE and comprised strains resistant to both fusidic acid and rifampicin. These latter resistance markers should prove useful to monitor the spread of such clones and alert the detection of new clones as and when they arise.

The increased frequency of transfers of patients between hospitals would be expected to result in an increase in inter-hospital spread of MRSA strains. To this end, information concerning the characteristics of endemic strains present in the individual hospital is of great importance, particularly for the investigation of any MRSA outbreaks. This study shows that a single genotype of MRSA has spread throughout major Malaysian hospitals and its mode of dispersal is most likely through patient transfers. Efforts to control the spread of this clone should therefore be aimed at screening all patients transferred from one hospital to another and this should be implemented as part of infection control in Malaysian hospitals.

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