DNA Fingerprinting of Methicillin-Resistant Staphylococcus Aureus by Pulsed-Field Gel Electrophoresis (PFGE): Comparison of Strains from 2 Malaysian Hospitals

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ABSTRACT

Aim of Study: To determine and compare the pulsed-field gel electrophoresis (PFGE) patterns of endemic MRSA strains in 2 major Malaysian hospitals and to compare the PFGE patterns with antibiotypes of the strains studied.

Methods: Fifty-six MRSA strains selected randomly between September 1997 and July 1998 from Hospital Queen Elizabeth (HQE) and Hospital Umum Sarawak (HUS) were tested for antimicrobial resistance and DNA fingerprinting was carried out by pulsed-field gel electrophoresis (PFGE) technique.

Results: Seven PFGE types were recognised (A, B, C, D, E and F). All 7 PFGE types were observed in HQE while only 2 PFGE types (B, C) were noted in HUS strains. There is a predominance of a single PFGE pattern (type B) in both hospitals, as seen in 46% of HQE strains and 89% of HUS strains. Subtype B2 was the commonest subtype in HQE while subtype B1 predominated in HUS. Strains resistant to fusidic acid and rifampicin exhibited PFGE type F that is unique to HQE. All strains were resistant to penicillin, erythromycin, cotrimoxazole, tetracycline and gentamicin. Strains with the same antibiotic susceptibility pattern can be different PFGE types.

Conclusion: Molecular typing of the MRSA by PFGE is a useful tool in the study of endemic strains present in an institution. Strains in HQE were found to be more heterogeneous than HUS strains. Common PFGE types can also be seen in both hospitals suggesting that some of the strains was genetically related and has propagated within and between the 2 hospitals. Our findings also indicate that the relationship between antibiotic susceptibility and PFGE patterns was not close and antibiograms should not be relied upon for typing strains in epidemiological studies. By knowing the DNA fingerprints of the isolates endemic in each hospital, the spread of MRSA with a particular PFGE type can be monitored within and between hospitals.

Keywords: DNA fingerprinting, MRSA, PFGE, Malaysia

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INTRODUCTION

Methicillin-resistant Staphylococcus aureus (MRSA) causing endemic and epidemic nosocomial infection represents an increasing problem in many parts of the world[1,2].

An increasing occurrence of methicillin-resistant Staphylococcus aureus infections is also noted in Malaysian hospitals. In 1988 methicillin resistance rates of Staphylococcus aureus in Malaysian hospitals were reported to be between 10-25%[3]. A high prevalence of MRSA were observed in surgical/orthopaedic wards, paediatric wards and the special care units[4]. Data obtained from the National Surveillance on Antibiotic Resistance programme showed an increase in the percentage of MRSA from 28% in 1992 to 36% in 1996[5].

The increased prevalence of MRSA in a hospital could be due to increased transmission of various strains among the inpatients and/or admission of patients already colonised from other hospitals. Therefore the knowledge of strains endemic in each hospital will be valuable in the epidemiological investigation of sporadic or epidemic outbreaks. DNA-based typing techniques have been applied for the epidemiological studies of MRSA and is based on the principle that epidemiologically related isolates share the same genetic features[6,7].

In this study we describe the use of DNA fingerprinting technique by pulsed-field gel electrophoresis to determine and compare the PFGE patterns of endemic MRSA strains in 2 major hospitals and also to compare the PFGE patterns with antibiotypes of the strains studied.

MATERIALS AND METHODS

Strains

A total of fifty-six methicillin-resistant Staphylococcus aureus (MRSA) strains were selected randomly from a collection of strains obtained from 2 general hospitals (Hospital Queen Elizabeth and Hospital Umum Sarawak). These hospitals were tertiary referral hospitals located in East Malaysia, approximately 800 km apart. The cultures were collected during the duration of September 1997 until July 1998. Twenty-

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eight strains were taken from each hospital. None of these strains were repeated isolates from the same patient. The isolates were from pus, tracheal aspirates, eye discharges, sputa, intravenous catheter tips, skin and wound swabs.

Determination of methicillin resistance and antibiotic susceptibility testing

MRSA was reconfirmed by Gram stain, tube coagulase test and resistance to 1 μg oxacillin disc on Mueller-Hinton agar incubated at 35°C. Antibiotic susceptibility was carried out by disc diffusion test following guidelines by National Committee for Clinical Laboratory Standards[8]. The antibiotics tested were penicillin, gentamicin, erythromycin, fusidic acid, cotrimoxazole, tetracycline, rifampicin, ciprofloxacin, chloramphenicol and vancomycin.

Pulsed-field gel electrophoresis

For each strain tested, a well isolated colony was inoculated into 5 ml tryptic soya broth and incubated overnight at 37°C. The broth culture was adjusted to a concentration of 1 X 10^9 cfu/ml. After washing in TE buffer (l0mM Tris-HCl, 50mM EDTA; pH7.5), 200 μl of the bacterial suspension was added with an equal volume of 2% low-melting point agarose and 6 ul of lysostaphin added and mixed well before being allowed to solidify in a plug mold (Bio-Rad Laboratories). The gel plugs were incubated overnight at 55°C in 2 ml ES buffer (1% N-Laurylsarcosine in 0.5M EDTA pH8.0) containing proteinase K (10 mg/ml) with gentle shaking. The plugs were washed 3 times with TE2 for 20 minutes on ice. A slice of the plug (2.5 mm) were cut and digested with 40 U of *Sma*l in recommended restriction enzyme buffer and incubated at 30°C overnight.

The DNA were then electrophoresed in 1.2% agarose (Bio-Rad Laboratories) using a contour-clamped homogeneous electric field (CHEF-DRIII) apparatus from Bio-Rad Laboratories. The pulse times used were 5 s to 15 s for 8 hours followed by 15 s to 25 s for 10 hrs. The gel was then stained with ethidium bromide and photographed under ultraviolet light using Gel Doc 1000 (Bio-Rad Laboratories). Differences between isolates were determined by visual comparison of DNA fragments.

RESULTS

The DNA fragment patterns generated by PFGE that differed up to three bands were defined as sharing a common PFGE type (capital letter) while the subtypes were defined as variants with one to three different DNA fragments[9,10]. By these criteria, 7 major PFGE macrorestriction patterns or PFGE types (A to G) were observed among the 56 MRSA strains examined. Based on the difference of one to three fragments Type A, B and C can be further categorized into subtypes A1 to A4, B1 to B5 and C1 to C3 respectively.

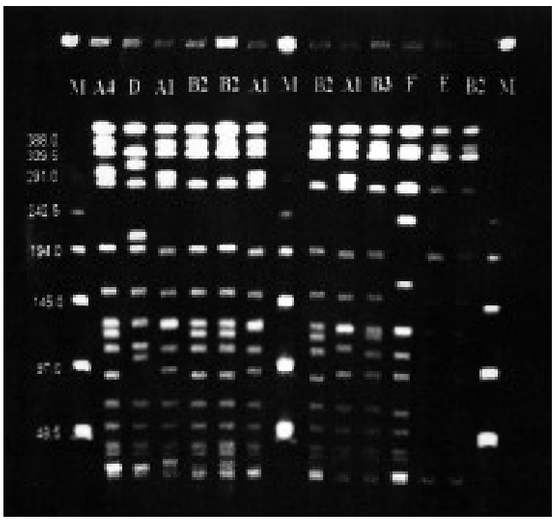

Fig.1 PFGE patterns observed in MRSA strains from Hospital Queen Elizabeth. Lanes M, marker.

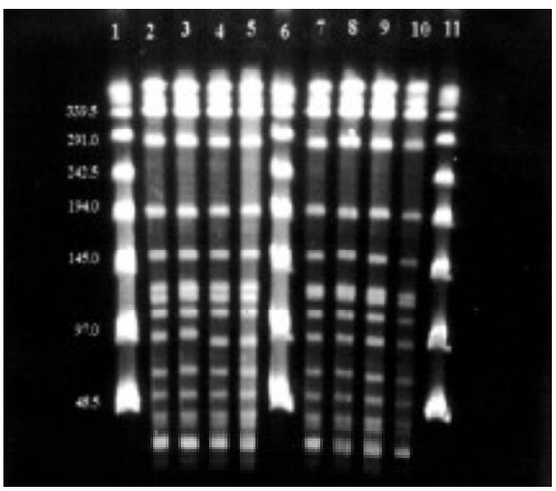

Fig.2 PFGE patterns most commonly observed in HQE and HUS. Lanes 2 and 3, pattern B1 from HUS; lanes 4 and 5, pattern B2 from HQE; lanes 7 and 8, pattern B3 from HUS; lanes 9 and 10, pattern B3 from HQE. Lanes 1, 6 and 11, molecular weight marker.

PFGE types A, B, C, D, E, F and G were observed in MRSA strains from HQE (Fig. 1) while strains from HUS exhibited type B and C macrorestriction patterns. PFGE type B was the most common pattern seen in both hospitals, as seen in 46% of HQE strains and 89% of HUS strains. Of the type B pattern observed subtype B2 was the most common subtype in HQE strains (8 out of 13) while subtype B1 was the commonest subtype observed in HUS strains as seen in 17 out of 25 strains (Fig. 2). While subtype B2 was seen only in HQE strains, subtype B1 and B3 were noted in both hospitals.

Of the MRSA isolated from Burns's unit in HQE, 4 out of 5 exhibited subtype B2 while 58% of MRSA

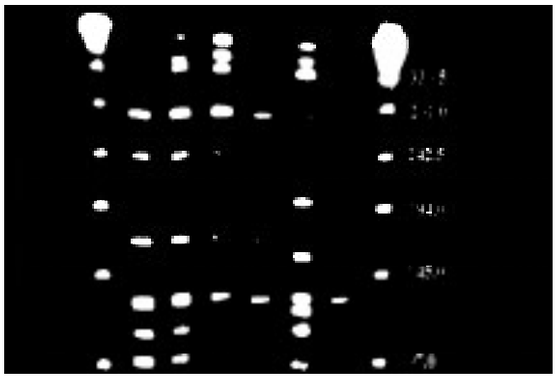

Fig.3 PFGE pattern of MRSA strains with different susceptibility patterns to fusidic acid and rifampicin. Lanes 2 to 5, resistant to both fusidic acid and rifampicin (pattern F); lane 6 resistant to fusidic acid only (pattern B2); lane 7, resistant to fusidic acid and intermediately resistant to rifampicin (pattern A1). Lanes 1 and 8, marker.

from orthopaedic ward in HUS exhibited subtype B1. Beside orthopaedic ward, this subtype was also recovered from surgical, obstetric and gynaecology, medical and paediatric wards.

The second most common pattern in the HQE strains is PFGE type A as seen in 25% of the isolates. This pattern beside type D, E, F and G was not seen in HUS strains.

All the strains examined were resistant to penicillin, methicillin, erythromycin, cotrimoxazole, tetracycline and gentamicin (Table I). Four strains were also resistant to fusidic acid and rifampicin and only sensitive to chloramphenicol and vancomycin. They exhibited PFGE type F pattern which is unique to HQE. Three of these strains were isolated from the intensive care unit. One strain was intermediately resistant to rifampicin and resistant to all other tested antibiotics except vancomycin. This strain exhibited subtype A1 and was isolated from HQE (Fig. 3). Resistance to all tested antibiotics except fusidic acid, rifampicin and vancomycin was seen in nine strains. Five of these strains were of PFGE type A, 3 were type B and 1 was type E. The type B strains were from HUS.

Table I. Antimicrobial resistance patterns of methicillin-resistant isolates of S. aureus from 2 Malaysian hospitals.

Antibiotic resistant pattern*	Hospital Queen Elizabeth No. of MRSA	PFGE patterns (no.)	Hospital Umum Sarawak No. of MRSA	PFGE patterns (no.)
Pn, Gm, Er, Sxt, Tet	28	A1(4)	28	B1(17)
		A2(1)		B3(4)
		A3(1)		B5(2)
		A4(1)		C1 (1)
		B1 (1)		C2 (1)
		B2 (8)		
		B3 (4)		
		C3 (1)		
		D(1)		
		E (1)		
		F(4)		
		G (1)		
Pn, Gm, Er, Sxt, Tet, Cip, Chl	6	A1 (3)	3	B4 (1)
		A2(1)		B3 (2)
		A3(1)		
		E (1)		
Pn, Gm, Er, Sxt, Tet, Cip, Fu, Rif	4	F (4)	O	Nil
Pn, Gm, Er, Sxt, Tet, Cip, Fu, Chl, Rif (intermediate)	1	A1	O	Nil

* Pn, Penicillin; Gm, Gentamicin; Er, Erythromycin; Sxt, Cotrimoxazole; Tet, Tetracycline; Cip, Ciprofloxacin; Chl, Chloramphenicol; Fu, Fusidic acid; Rif, Rifampicin.

DISCUSSION

Many typing systems have been used to explore the epidemiology of MRSA. This has often assisted in the interpretation of the success or otherwise of control measures. PFGE technique has been proven useful in determining the relatedness of isolates and investigating nosocomial transmission[11-13].

Increased frequency of patient transfers from hospital to hospital would result in increase in hospital-to-hospital spread of multiresistant MRSA strains. Information concerning the endemic strains present in the individual hospital is important particularly during the tracking of the origin of an outbreak. Outbreak caused by a new clone can also be recognised and its spread controlled.

In this study, 7 PFGE types or macrorestriction patterns were identified in MRSA strains from HQE while from HUS only 2 PFGE types were observed. This would suggest that MRSA strains in HQE are more heterogeneous than HUS strains. PFGE type B is the commonest pattern and appeared to be widespread in both hospitals, with 5 subtypes identified. The generation of subtypes is due to the alterations in SmaI macrorestriction patterns resulting from insertions or deletions of DNA or the gain or loss of restriction sites. A well defined MRSA clone have been shown to generate a number of subtypes as it persist in the in-vivo environment[14]. These could result from genetic events causing point mutations in restriction sites or may be related to insertion or deletion of mobile DNA elements[9,10]. However in-vitro, profiles generated by this technique appear stable and reproducible, even after 40 subcultures[15]. These suggest that the PFGE type B strains was genetically related and has propagated and spread within and between the two hospitals.

Differences in the distribution of subtypes was noted in which subtype B2 is the commonest subtype in HQE but not detected in HUS, while in HUS

subtype B1 is most common. Sharing of pattern were observed in the strains exhibiting subtype B3. The subtypes arise as the strains persisted and continued to be transmitted in the environment.

The predominance of certain subtypes in the hospitals could be due to the increase in nosocomial transmission of the particular strains within the hospital. The reason why certain PFGE pattern predominate in an institution is unknown but could be attributed to a virulence-related property in the particular strain.

Strains from other government hospitals in Malaysia have not been characterised. There could be the possibility of PFGE type B being the predominant PFGE pattern in other hospitals.

All the MRSA examined here were multiresistant. Strains with the same antibiotic susceptibility pattern can be different PFGE types, for example, if the strains were broadly categorized as resistant to penicillin, methicillin, erythromycin, co-trimoxazole, tetracycline and gentamicin, 7 PFGE types will be observed. Conversely clinical isolates of the same PFGE pattern were found to exhibit different antibiotic susceptibility patterns. Thus the relationship between antibiotic susceptibility and PFGE patterns was not close and antibiograms should not be relied upon for typing strains in epidemiological studies. Three different PFGE types were noted in strains that were resistant to all antibiotics except fusidic acid, rifampicin and vancomycin. In this study most strain exhibited a common antibiogram pattern which did not help in the discrimination of any of the strains except strains with PFGE pattern F. These strains which is unique to HQE were resistant to all antibiotics except chloramphenicol and vancomycin. The spread of these particular strains, which are mostly isolated from intensive care unit, should be closely monitored and controlled. A strain with intermediate resistance to rifampicin was shown to share the same PFGE pattern A1 with 3 other strains. The possibility of the strains with the same banding pattern becoming resistant in future should be kept in mind and surveillance should be carried out this strain. This is of special concern because the nosocomial spread of strains with resistance to a particular group of antibiotics will reduce the usefulness of that antibiotic in the hospital.

The identification of new or unusual patterns of antibiotic resistance among bacteria isolated from various patients may raise the suspicion of an outbreak. However, the high incidence of endemic multiresistant patterns as shown in this study may make outbreaks of MRSA difficult to evaluate. This report showed that strains with different PFGE patterns had similar antibiotic susceptibility patterns and clinical isolates of the same PFGE pattern exhibited different antibiotic susceptibility patterns. Strains that carry the resistance to particular group of antibiotics can be monitored therefore the monitored and the factors that promote the spread of these strains among patients and across wards identified and infection control measures implemented so that further transmission can be prevented.

The rapid increase of MRSA infections particularly in the intensive care setting and in immunocompromised patients, requires quick and reliable characterization of strains and identification of clonal spread within hospitals. DNA fingerprinting by PFGE is a useful method for investigating the source, transmission and spread of nosocomial MRSA infections. By knowing the DNA profile of the infecting strain, the spread of the strains can be tracked and further transmission prevented by isolating the affected patients and applying appropriate barrier nursing techniques.

The information gained from typing endemic MRSA strains can help the infection control teams to understand the epidemiology of this organism in their institution. By knowing the DNA fingerprints of the isolates endemic in each hospital, the spread of MRSA can be monitored within the hospital and between hospitals. Once the epidemiology of MRSA is established, effective control strategies can be devised and the restricted resources for control can be utilised optimally.

This study is part of a nation wide study to determine the clonality of endemic MRSA in Malaysia hospitals. HUS and HQE were chosen as part of this study to represent government hospitals in Malaysia. In this study PFGE type B were shown to be widespread in both hospitals. Our study also has produced evidence that within one hospital, its endemic MRSA strains can be more heterogenous than another hospital and the common PFGE patterns present in both hospitals showed the ability of certain MRSA clones to spread over enormous distances. The PFGE patterns of these strains will be compared with the PFGE patterns from other hospitals in Peninsular Malaysia to determine whether certain multiresistant clones are widely spread in Malaysian hospitals. Monitoring the geographic expansion of such epidemic clones is important for understanding why certain MRSA clones are spread over considerable distances, whereas others are limited to a single country, city or hospital. The virulence properties of these strains will be further characterised for better understanding why such clones are able to persist in the hospital environment.

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