

Pulsed-field gel electrophoresis of chromosomal DNA of methicillin-resistant *Staphylococcus aureus* associated with nosocomial infections

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Abstract

Methicillin-resistant *Staphylococcus aureus* (MRSA) infection has been endemic in the University Hospital, Kuala Lumpur since the late 1970s. Fifty isolates of MRSA obtained from clinical specimens of patients with nosocomial infections associated with this organism have been studied by pulsed-field gel electrophoresis (PFGE) of its chromosomal DNA fragments to discriminate between strains and to identify the predominant strain. Twenty-one chromosomal patterns were observed which could be further grouped into nine types. The predominant strain was Type 9-b (40% of isolates) found mainly in the Orthopaedic and Surgical Units. Outbreak strains found in the Special Care Nursery were of Type 1, entirely different from those of the surgical ward S2, which were of Type 9-b. Type 8 strains were found mainly at one end of the hospital building where the maternity, paediatric and orthopaedic units were situated. Genomic DNA fingerprinting by PFGE is recommended as a useful and effective tool for the purpose of epidemiological studies of MRSA infections, particularly for nosocomial infections.

Key words: Methicillin-resistant *Staphylococcus aureus*, nosocomial infection, pulsed-field gel electrophoresis.

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) is an important pathogen associated with nosocomial infections in the past three decades. The international spread of this organism has led to many clinical problems due to its multiple antibiotic resistance.^{1,2}

In the University Hospital Kuala Lumpur, MRSA has been isolated since 1978. This organism has established itself in the hospital environment and have caused increasing problems therapeutically and economically to the patients and hospital.

Outbreak strains have been suspected on the basis of antibiograms by antibiotic disc diffusion sensitivity and minimum inhibitory concentration testing. This method has been proven unsatisfactory for differentiating isolates as the current MRSA are already resistant to numerous other antibiotics besides beta-lactams, namely chloramphenicol, tetracycline, erythromycin and gentamicin.

Various other techniques, such as capsular typing, phage typing, coagulase typing, plasmid typing and ribotyping have been used for epidemiological studies.^{3,4,5} Phage typing has some

limitations such as instability of the phages over a period of time leading to poor reproducibility and the need to perform numerous standardisation tests. Plasmid typing has been reported to be useful^{3,4} but this method also has its limitations, especially in relation to isolates with one or no plasmids.

This study describes the usefulness of genomic DNA fingerprinting of fifty MRSA isolates by pulsed-field gel electrophoresis (PFGE) as one of the epidemiological markers in studying the characteristics of MRSA causing nosocomial infections. These isolates had earlier been characterised by coagulase, phage and plasmid typing.³ PFGE is more specific and enables us to analyse patterns produced by large chromosomal DNA fragments, and is particularly useful for discrimination of isolates in outbreak situations.^{5,6,7,8,9}

MATERIALS AND METHODS

Bacterial isolates

Fifty isolates of MRSA, numbered chronologically, were obtained randomly from clinical specimens of infected patients in the University Hospital over a two-year period from 1987 to 1989

with the inclusion of some isolates from out-breaks in the SCN and surgical Ward S2.

The clinical sources of the infecting strains were from wound, blood, tracheal secretions, throat, nose, umbilical, ear, urethral and skin swabs. The first five isolates were isolated in 1987 from the Special Care Nursery (SCN) and the last strain (number 50) was isolated in November 1989. They had been identified as MRSA by gram stain, a positive tube coagulase test and antibiotic disc sensitivity testing to methicillin (10 ug disc - Mast Laboratories Ltd, UK) with incubation at 30°C overnight on Mueller-Hinton agar. An inhibition zone of less than 9 mm diameter was considered to indicate resistance to methicillin. Their minimum inhibitory concentrations to methicillin were later performed.

Determination of minimum inhibitory concentration to methicillin

The minimum inhibitory concentration (MIC) values to methicillin were determined by the recommended method of the Japanese Society of Chemotherapy by the plate agar dilution method with Mueller-Hinton agar (Difco Laboratories). Antibiotic concentrations of 0.013-100 mg/ml and a 1:100 dilution of an overnight culture of the isolate (about 10⁶ cfu/ml) were used. The antibiotic plates were incubated at 37°C for 24 hours before being read.

Chromosomal DNA analysis by PGFE

All the 50 isolates were subjected to chromosomal DNA analyses. The DNA was prepared in agarose blocks as described by Wada *et al*¹⁰ as follows: MRSA cells were embedded in 30 ul of 0.5% low temperature melting agarose blocks (SeaPlaque, FMC) measuring 4.5 x 5 x 1.2 mm. The samples were incubated in 0.5M EDTA, 1% lauroylsarcosine and 100 ug/ml lysostaphin (Sigma) with gentle shaking at 37°C for 24 hours, after which they were incubated in 1 mg/ml proteinase K (sigma), 1% lauroylsarcosine and 0.5M EDTA for another 48 hours at 50°C. The agarose blocks were rinsed with TE (10mM Tris-Cl, pH8.0, 1mM EDTA) with 1mM phenylmethyl sulfonyl fluoride (Sigma) for 30 minutes at 50°C. The blocks were washed three times with TE buffer (10mM Tris-Cl, pH 8.0, 1mM EDTA), and digested with 40 units of *Sma*I (Toyoba) at 25°C for 18 hours. 100 ug/ml proteinase K in TE was added and incubated at 37°C for 1 hour. The agarose blocks were rinsed three times in TE buffer. The samples were subjected to PFGE in 1.2% agarose gel (BioRad

High Strength Analytical Grade Agarose) in 0.5 x TBE running buffer at 10°C using CHEF DR II (Bio-Rad). The conditions for electrophoresis were 200V with switching time of 20 seconds. Lambda DNA concatamers were used as molecular weight markers for the size standards. The gel was finally stained with 1 ug/ml ethidium bromide, washed with distilled water and photographed.

Restriction enzyme

*Sma*I was chosen for digestion of chromosomal DNA to differentiate these isolates as it produced a reasonable number of fragments.

RESULTS

All isolates, except for three (numbers 10, 14 and 19), were methicillin-resistant by minimum inhibitory concentration testing. *Sma*I restriction enzyme cut the chromosomal DNA into 12-15 bands ranging from 30kb to 500kb.

Twenty-one distinguishable restriction patterns were identified from the 50 isolates. Based on close resemblance of some of the patterns, differing slightly in their migration rates or the presence or absence of one band, these twenty-one strains could further be grouped into nine types, Types 1-9, of which Type 9 was the commonest, comprising of 26 (52%) isolates of all those tested (Table 1). Twenty (40%) of these were classified as Type 9-B (chromosomal pattern 18). This strain 9-B was found in the Orthopaedic (8 isolates), Surgical (8 isolates), Medical (1 isolate), Intensive Care Unit (1 isolate) and Paediatric (1 isolate) units.

Two isolates from the ICU were found to be different. Both these isolates were associated with respiratory tract infections. Isolate number 6 was chloramphenicol sensitive but isolate number 40 was chloramphenicol resistant.

Isolate number 6 was Type 3-a, isolated in February 1988 and the other, isolate number 40 was Type 9-b, isolated in August 1989.

There were 15 isolates from patients of the Orthopaedic Unit. Ten (67%) isolates were of Type 9, of which Type 9-b was commonest, occurring in 8 (53%) isolates. Types 8, 3 and 6 were also seen occasionally.

Type 9 was also commonly seen in the Surgical Unit. Of sixteen isolates tested, there were 11 (69%) Type 9 isolates, Type 9-b (9 isolates) being commonest. There were five burns patients (isolate numbers 33, 34, 35, 36 and 37) and four of these patients were infected in their burns wounds by MRSA Type 9-b (isolates numbers

TABLE 1: Characterisation of MRSA strains by methicillin resistance, chromosomal pattern and type

Isolate number	Ward*	MIC _{net} (ug/ml)	Chromosomal pattern	Type
1	SCN	25.0	1	1-a
2	SCN	12.5	2	1-b
3	SCN	12.5	3	2
4	SCN	100.0	1	1-a
5	SCN	6.25	1	1-a
6	ICU	>100.0	4	3-a
7	O1	50.0	12	8-a
8	O1	50.0	5	3-b
9	G	100.0	5	3-b
+10	S1	1.56	17	9-a
11	S3	12.5	6	4-a
12	M1	>100.0	21	9-e
13	S3	50.0	8	5
+14	O3	1.56	9	6
15	S3	100.0	10	7-a
16	SCN	25.0	13	8-b
17	S4	>100.0	14	8-C
18	SCN	50.0	14	8-C
+19	P3	1.56	18	9-b
20	O2	25.0	18	9-b
21	M3	12.5	7	4-b
22	O3	25.0	18	9-b
23	O3	100.0	18	9-b
24	O3	50.0	18	9-b
25	O3	50.0	18	9-b
26	P3	50.0	14	8-C
27	SCN	>100.0	14	8-C
28	Mat	>100.0	16	8-e
29	O3	50.0	18	9-b
30	P4	50.0	19	9-c
31	O3	>100.0	14	8-C
32	M1	100.0	11	7-b
33	S1	50.0	18	9-b
34	S1	50.0	18	9-b
35	S3	100.0	15	8-d
36	S4	50.0	18	9-b
37	BURN	100.0	18	9-b
38	S2	50.0	20	9-d
39	B	100.0	21	9-e
40	ICU	25.0	18	9-b
41	O1	50.0	18	9-b
42	M2	100.0	18	9-b
43	O1	25.0	18	9-b
44	S2	100.0	18	9-b
45	S2	100.0	18	9-b
46	S2	25.0	18	9-b
47	O4	25.0	21	9-e
48	S2	>100.0	18	9-b
49	S1	>100.0	18	9-b
50	O4	>100.0	14	8-c

* Special Care Nursery (SCN); Intensive Care Unit (ICU); Surgery wards S1, S2, S3, S4 and Burns; Orthopaedic wards O1, O2, O3 and O4; Medicine Wards M1, M2 and M3; Paediatric wards P3 and P4; Obstetric & Gynaecology wards G and Mat; B, Orthopaedic clinic.

+ Strains that had lost methicillin resistance phenotype.

33, 34, 36 and 37). Although the 150kb band of isolates number 34 was very faint, the chromosomal pattern was similar (Figure 1).

Four of five isolates obtained from one surgical Ward S2 (isolate numbers 44, 45, 46 and 48) over a 3-month period from September till November 1989 were all Type 9-B MRSA. In comparison, four isolates from another surgical Ward S3, over an eight-month period from July 1988 to March 1989, were all non-identical.

It was interesting to note the contrasting types found amongst the 11 isolates from the Paediatric Unit, which were Types 1-a (3 isolates), Type 1-b (1 isolate), Type 2 (1 isolate), Type 8-b (1 isolate), Type 8-c (3 isolates), Type 9-B (1 isolate) and Type 9-c (1 isolate).

The first five isolates obtained were from an outbreak of infection in the Special Care Nursery and they consisted of three types, Type 1-a (3 isolates), Type 1-b (1 isolate) and Type 2 (1 isolate). MRSA isolate number 3, which was Type 2, showed a very similar pattern to Type 1-a but gave slightly different migration rates and the absence of one 150kb band (Figure 1). These four Type 1 isolates were all obtained from the respiratory tract of babies in the SCN. The other three isolates of MRSA from the SCN obtained more than a year after this outbreak were of Type 8 (isolate numbers 16, 18 and 27). The six isolates from the Medical and Obstetric and Gynaecology Units showed varying types. One isolate number 28 from a maternity ward from a patient with post-caesarian section wound infection was very similar to isolate numbers 26 and 27 from the paediatric wards, although it produced only a slight difference in migration rates in two of its bands (bands 4 and 5). However, these were classified as Type B as they were related.

DISCUSSION

Three isolates, which were methicillin-resistant by disc-diffusion sensitivity testing, were found to have lost their methicillin resistance phenotype on MIC testing. This loss of resistance from isolates numbers 10, 14 and 19 could be explained by the loss of the resistance gene, *mecA*, during propagation in antibiotic-free medium, and this was confirmed by polymerase chain reaction and by Southern hybridisation using clonal *mecA* gene, pMR111, as a probe.^{3,11} The technique of pulse-field gel electrophoresis has been recently used to separate chromosomes of bacteria, yeast and also parasites, which was not possible before with conventional electrophoretic

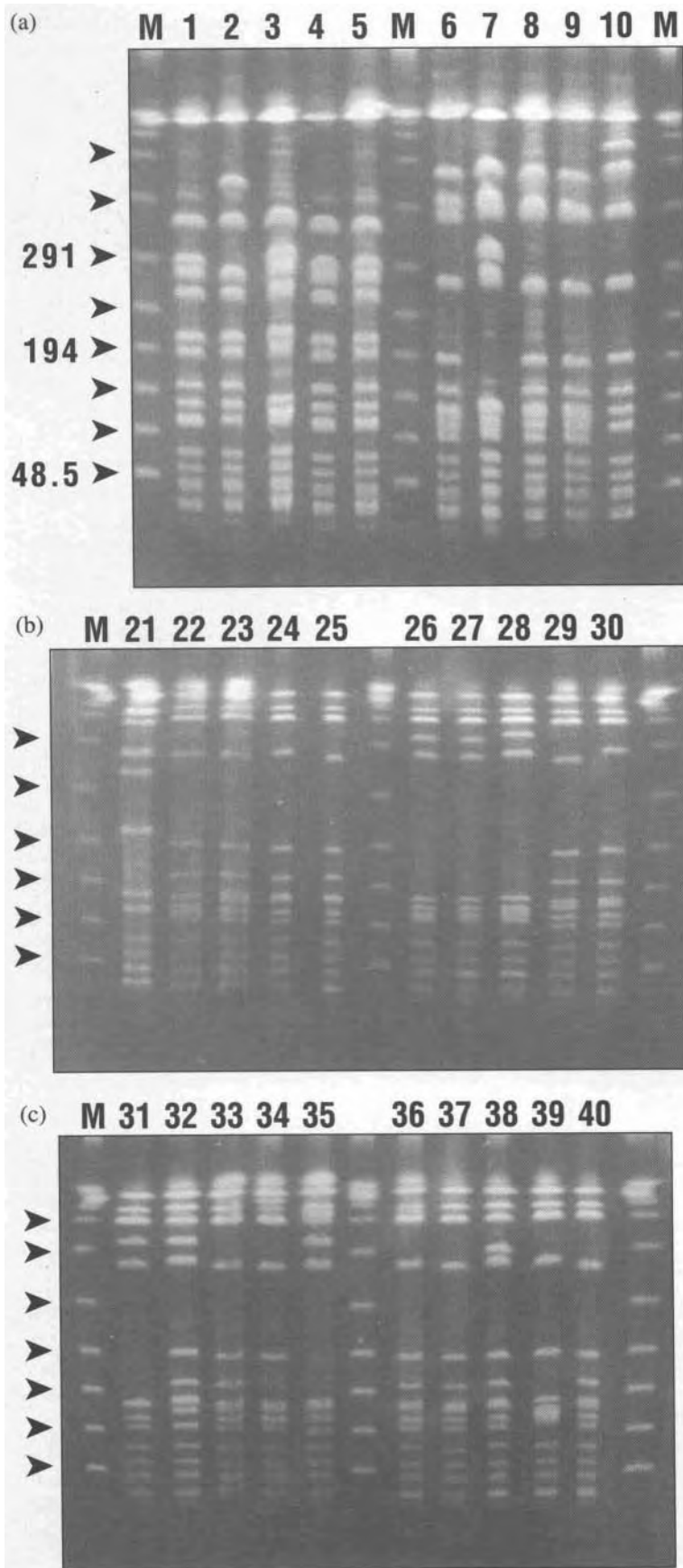


FIG. 1: (a), (b) and (c) shows chromosomal DNA patterns of some isolates of methicillin-resistant *Staphylococcus aureus* numbered 1-10, 21-30 and 31-40. There were 12-15 bands produced by *SmaI* digestion ranging from 30 kb to 500kb. Molecular weight markers (M) were lambda DNA concatamers in wells 1, 7 and 13. The size of the lambda ladder starts at 48.5 kb and increases 48.5 kb with each successively larger band, as shown by arrows. The isolate numbers 1-5 as shown in (a) were from the SCN. Isolates 1, 4 and 5 were Type 1-a. Isolate numbers 22, 23, 24, 25, 29, 33, 36, 37 and 40 were Type 9-b. Type 8 is represented by isolate numbers 7, 26, 27, 28, 31 and 35.

methods due to their large DNA size.¹²

In this study, we applied this method to distinguish the strains isolated from various units and wards of the University Hospital. Twenty-one chromosomal restriction patterns were observed from the 50 isolates studied, which could further be grouped into nine types based on similarities in their patterns, which suggest clonal relationships. Type 9-b was shown to be the most common type of MRSA and they were found mainly in the Orthopaedic and Surgical Units, which often showed high incidence of MRSA infections and frequent outbreaks of infections in wounds, particularly post-operative and bums wounds. The predominant strain found in an outbreak of infection in the surgical ward S2 was Type 9-b, indicating that cross-infection had occurred amongst the patients. These patients, being in a neuro-surgical ward, tend to be long-staying and those who are infected become potential sources of infections to other patients, if control measures become ineffective.

In comparison, MRSA infections rarely occur in the Special Care Nursery where the handwashing requirement is very strict. However, occasional outbreaks of MRSA infections have been reported and from this period we studied five isolates, which were noted to be mainly Type 1. Further isolates from the SCN more than a year after the outbreak did not reveal any Type 1 MRSA, but were Type 8 instead.

There were altogether ten isolates of Type 8, found in Orthopaedic (3 isolates), paediatric (4 isolates), maternity (1 isolate) and surgical (2 isolates) wards. Except for two of the four surgical wards, it is of interest to note here that this Type 8 MRSA appeared to cluster around the orthopaedic, paediatric (including the SCN) and the maternity wards, which were all closely situated at one end of the hospital, away from the fourteen-floor tower block consisting of other units.

Types 3,4,5,6 and 7 were found intermittently throughout the hospital but on a smaller scale. These varied strains of MRSA may be transiently transmitted by the movement of hospital staff, patients, nursing and medical students between wards leading to cross-infections.

Epidemiological surveillance of nosocomial infections by MRSA is important and the method of pulsed-field gel electrophoresis on the chromosome DNA of this bacteria has been shown to be a useful and effective tool in strain discrimination, particularly in an outbreak of infections within a limited area in the hospital. DNA fingerprinting by PFGE have been demonstrated

to be stable and reproducible even after repeated subcultures of the isolates and this method has been highly recommended by many authors for epidemiological studies of MRSA infections.^{5,7,8,9}

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