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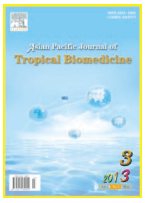

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Clonal distribution and possible microevolution of methicillin-resistant *Staphylococcus aureus* strains in a teaching hospital in Malaysia

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PEER REVIEW

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Comments

This is good piece of work. The authors studied the distribution of MRSA strains in a particular hospital. It is interesting to find one single MRSA strain endemic in the hospital under study. Such intervention measures should be recommended in specific intervals to keep a vigil on hospital infections and to control the same.

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ABSTRACT

Objective: To genotypically characterize methicillin-resistant *Staphylococcus aureus* (MRSA) strains isolated from medical and surgical wards in Universiti Kebangsaan Malaysia Medical Centre (UKMMC) in 2009. **Methods:** MRSA strains were collected and molecularly typed by pulsed-field gel electrophoresis (PFGE). **Results:** PFGE typing on 180 MRSA isolated in UKMMC identified 5 pulsotypes (A-E) and 6 singletons, where pulsotypes B and C were suspected to be divergent clones originating from a single ancestor. This study also showed that most MRSA strains were isolated from swab (119 isolates), followed by blood (22 isolates), tracheal aspirate (11 isolates) and sputum (10 isolates). On the other hand, urine and bone isolates were less, which were 4 and 1 isolates, respectively. The distribution of different pulsotypes of MRSA among wards suggested that MRSA was communicated in surgical and medical wards in UKMMC, with pulsotype B MRSA as the dominant strain. Besides, it was found that most deceased patients were infected by pulsotype B MRSA, however, no particular pulsotype could be associated with patient age, underlying disease, or ward of admittance. **Conclusions:** Five pulsotypes of MRSA and 6 singletons were identified, with pulsotype B MRSA as the endemic strains circulating in these wards, which is useful in establishment of preventive measures against MRSA transmission.

KEYWORDS

Hospital infection, Microevolution, MRSA, PFGE typing

1. Introduction

Escalation of methicillin-resistant *Staphylococcus aureus* (*S. aureus*) (MRSA) infections have been noted in Malaysia with a high incidence rate observable in surgical or orthopedic wards, pediatric wards and intensive care units[1,2]. The National Surveillance on Antibiotic Resistance 2008 recorded a total of 6 022 MRSA out of 23 176 *S. aureus* isolated in 2008. Furthermore, *S. aureus*-associated bacteremia were found to increase from 2 260 cases in 2007 to 2 389 cases in 2008, with 21% of the cases attributed to MRSA[3]. Universiti Kebangsaan Malaysia Medical Centre (UKMMC), which started operating in 1997, is a relatively new university hospital in Malaysia. The focus on microbiology in UKMMC was previously mainly for diagnostic purposes, except for one study by Alfizah *et al*. in 2002. Beginning 2009, as

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the medical center has become more established, we started the initiative to periodically type MRSA isolated in the hospital. This is partly a long term effort for incorporation of molecular biology into diagnostic microbiology in future, as well as for infection control and prevention of MRSA infection.

As a start, we typed strains isolated from medical and surgical wards, owing that these wards reported a higher incidence rate as compared to other wards in UKMMC. It is hoped that, with this research, the relationships between different strains can be determined and their patterns of infection can be identified. This will ultimately help in establishing appropriate preventive measures against the transmission of MRSA infections.

2. Materials and methods

2.1. Bacterial strains

A total of 180 MRSA strains were collected from patients in medical and surgical wards, UKMMC, in 2009. These isolates were obtained from various clinical specimens in different wards. The isolation of these MRSA strains and their identification were carried out and the strains were stocked at −80 °C.

2.2. Genotyping by pulsed-field gel electrophoresis (PFGE)

Chromosomal DNA of each MRSA isolate was digested with *Sma* I restriction enzyme and separated by PFGE with the CHEF-DR III system (Bio-Rad Laboratories, Inc. California, USA). Settings for the PFGE were fixed at: initial switch time, 5 seconds; final switch time, 40 seconds; included angle, 120°; voltage, 6 V/cm or 200 V and total running time, 22 h. The running buffer temperature was maintained at 14 °C and the variable speed pump was set at 80[4]. Banding patterns were visualized by gel documentation system fluor chem model FC2 and analyzed with Fingerprinting II software (version 1.0; Bio-Rad Laboratories, Inc. California, USA). The patterns were identified on unweighted pair group method with averages dendrogram based on dice coefficients, where optimization was set at 1% while band position tolerance was at 2.3%[5]. An 80% relatedness cut off value was selected for defining pulsotypes[6].

2.3. Interpretation of patients' medical history

Corresponding patients' medical history was reviewed using UKMMC on-line hospital system (Integrated Laboratory Management System, ILMS, version 5.4.23.4.).

3. Results

3.1. Types of specimens

In this study, 180 MRSA strains were collected from a variety of clinical specimens, including swab, blood, tracheal aspirate, sputum, fluid, urine, tip, tissue, cranium and bone. Among these, majority of the isolates were collected from swabs (66.11%). The remaining MRSA strains were isolated from blood (12.22%), tracheal aspirates (6.11%) and sputum (5.55%). In contrast, urine (2.22%), cranium (0.56%) and bone (0.56%) specimens were rarely obtained.

3.2. PFGE assay

PFGE with *Sma* I restriction enzyme revealed 5 main pulsotypes of MRSA (A, B, C, D and E) and 6 singletons. The banding patterns of different pulsotypes of MRSA and singletons are shown in Figure 1. Frequencies of each pulsotype were as follows: A, 5 isolates (2.78%); B, 140 isolates (77.78%); C, 21 isolates (11.66%); D, 3 isolates (1.67%) and E, 5 isolates (2.78%), respectively. Among the 180 strains being analyzed, it was clearly shown that pulsotype B MRSA had the highest prevalence in medical and surgical wards in UKMMC in 2009, indicating its endemicity in this hospital.

Pulsotype B and C MRSA were widely spread among medical wards (61 isolates of pulsotype B and 9 isolates of pulsotype C) and surgical wards (79 isolates of pulsotype B and 12 isolates of pulsotype C) in UKMMC. However, the distribution of other pulsotypes in these wards was less. Three isolates each of pulsotype A, pulsotype D and 1 isolate of pulsotype E were identified in medical wards whilst surgical wards were found to contain 2 isolates of pulsotype A and 4 isolates of pulsotype E. Singletons were mostly isolated from medical wards (5 isolates) as compared to surgical wards (1 isolate) (Table 1).

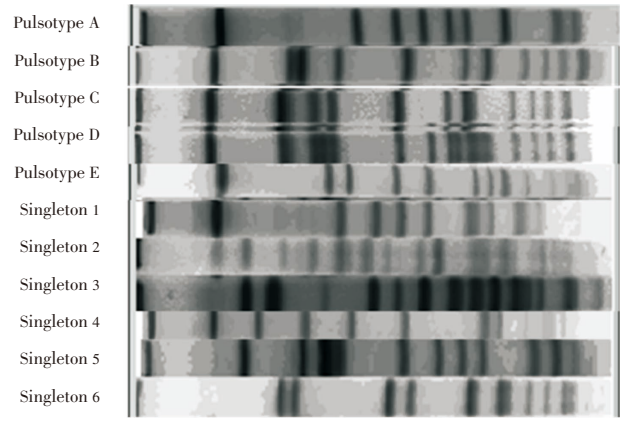

Figure 1. The comparison of banding patterns between different clusters of MRSA and singletons.

Table 1
Prevalence of different pulsotypes of MRSA in accordance with wards.

Wards	Pulsotype						Total
	A	B	C	D	E	Singleton	
Medical 1		8	4			3	15
Medical 2		6	2	2			10
Medical 3		8	3	1		1	13
Medical 4		13				1	14
Medical 5	1	22					23
Medical 6	2	4			1		7
Surgical 1	1	25	1			1	28
Surgical 2	1	8			3		12
Surgical 3		10					10
Surgical 4		15	6				21
Surgical 5		3	1				4
Surgical 6		10	1				11
Surgical 7		4	1		1		6
Surgical 8		4	2				6
Total	5	140	21	3	5	6	180

Based on the pulsotypes of these strains, it was found that pulsotype B shared similar banding patterns with pulsotype C, with a difference of only 7%. In fact, pulsotype C also had a higher prevalence in the hospital compared to pulsotypes A, D or E though not as high as pulsotype B.

3.3. Interpretation of patients' medical history

Analysis of the patients' data showed that the majority of MRSA-infected patients that had passed away were infected with pulsotype B MRSA, while the cause of death is mostly related to ventilator- or healthcare-associated pneumonia as a result of MRSA septicemias. In addition, many of our MRSA patients were elderly (aged above 50 years old) and were found to be suffering from various underlying diseases such as diabetes mellitus, hypertension and end-stage renal failure.

4. Discussion

MRSA is a pathogen mainly found in skin and soft tissues infections, for instances cellulitis, necrotizing soft tissue infections, wound infections, ulcers, septic bursitis or abscesses[7]. Infections of the bloodstream, lower respiratory system and pneumonia are also the common pathological conditions caused by MRSA. On the other hand, urinary tract infections and infections at the bone do not frequently occur[8]. This may explains for the abundance of clinical isolates collected from swabs, and to a lesser extent, from blood, tracheal aspirates and sputum, whereas isolates from urine and bones are very few.

In this study, pulsotype B MRSA was identified as an endemic strain circulating in this hospital. The emergence of a particular endemic strain of MRSA in a hospital setting is a common finding as shown in other researches. Alfizah *et al*. also reported the findings of endemic strains in Universiti Kebangsaan Malaysia Hospital in the analysis of 71 MRSA strains isolated from different wards from January to March in 2000[9]. Studies conducted by Bosch *et al*. and Said *et al*. in other hospitals also showed similar findings[10,11].

Generally, distinct pulsotypes of MRSA are widely distributed among the medical and surgical wards in UKMMC. This signified the transmission of MRSA infection within this hospital, which might have occurred by several ways. One of the factors might have been cross-infection between patients due to contamination of the wards surroundings by other MRSA-infected patients. It has been shown that the surfaces (bed frames, mattresses, and so on) and air in the ward could function as reservoirs of MRSA from patients[12,13]. Moreover, healthcare personnel is the responsible source and medium for the transmission of MRSA infections in healthcare centers[14]. The failure of compliance to proper hand washing in patient contact and after handling blood, body fluids, secretions and excretions contaminated by them will probably contribute to cross-infections among patients[15]. In fact, Beggs *et al*. clearly shown, in the research conducted, that proper hand hygiene will reduce the frequency of MRSA infections[16].

In our study, PFGE typing revealed possible occurrence of evolution of MRSA strains in the hospital environment with the identification of highly similar MRSA pulsotypes (B and C). Factors which predispose to evolution include the natural selection caused by overuse of broad spectrum antibiotics where subsequent increased nasal carriage of MRSA and altered infection rate of MRSA in hospital were observed[17]. Similarly, MRSA strains will be exposed to natural selection during invasive infection when they encounter phagocytes and other components of the innate immune system in deep tissues and bloodstream[18]. As this is the first initiative to molecularly type the MRSA strains in our medical centre, it is difficult to determine if pulsotype B was the original dominant MRSA where some of its progeny has started to evolve into pulsotype C, or whether if a short term of less than 15 years (UKMMC was founded in 1997) was enough for the original dominant pulsotype (in this case, pulsotype C) to be replaced by pulsotype B. Subsequent and periodic molecular studies will provide the answer to this.

By interpreting the patients' medical history, the cause of death for the majority of deceased MRSA-infected patients were found to be related to ventilator- or healthcare-associated pneumonia as a result of MRSA septicemias. In agreement with our findings, MRSA-related pneumonia has been reported to cause high mortality rates[19,20]. Furthermore, these patients were mostly aged and presented with various underlying diseases such as diabetes mellitus, hypertension and end-stage renal failure. The elderly

had been reported to have worse prognosis when infected due to immobility, poor nutrition and prior exposure to antibiotics[21,22]. Studies have also shown that patients with these underlying diseases were prone to MRSA infections[23-25]. Diabetic patients possess higher risk because it is likely for them to develop complications, such as foot ulcers, that predispose them to infections[26]. Besides, *in vitro* studies have proven that diabetic patients will have compromised neutrophil functions[27,28] and their antioxidant as well as humoral immune systems will be suppressed[29]. Meanwhile, the usage of invasive devices for hemodialysis in patients with chronic renal failure might also be the causative factor for MRSA infections since the use of intravascular devices is one of the contributing factors to staphylococcal bacteremia[30].

As a conclusion, molecular typing of MRSA strains isolated from medical and surgical wards in UKMMC in 2009 revealed five main pulsotypes (A-E) and six singletons with pulsotype B as the endemic strains circulating in these wards. It would be interesting to determine whether MRSA of this particular pulsotype is ubiquitously present in the hospital environment; and if so, what are the locations that serve as reservoirs for the transmission. We are currently planning a study to investigate this, along with a molecular study on carrier MRSA isolates from hospital personnel. It is also in the plan to periodically type the medical centre's MRSA isolates to monitor the dynamics of the pulsotypes, which in turn will be useful for the establishment of preventive measures against MRSA transmission.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

Diseases from *S. aureus* are a major problem world wide. The ability of the organism to cause a multitude of infections is probably due to the expression of different toxins, virulence factors and also cell wall adhesion proteins and staphylococcal super antigen like proteins involved in immune-evasion. Virulence factors and antibiotic resistant increase the severity of the infection. Recent studies show that 55%-70% of MRSA are endemic in many hospitals. Vancomycin resistance is also reported. Few hospitals are interested in typing *S. aureus*.

Research frontiers

Six MRSA strain variations were found (Clonal variation A-E and singleton) in the present study. About 140/180 MRSA isolates were of single strain, clonal B type. From the year 2000 and later the culprit strain was identified to be MRSA type Clonal -"B" endemic in the particular hospital. It resulted in an increase in the mortality of immuno-compromised and elderly patients. The significant out come is the realization of the need to revise the hospital management including handling of catheters and patient hygiene

Related reports

Mexico and European countires conducted similar studies in different time points (1993-2000). The studies revealed the Brazilian MRSA strain's strong capacity to cause epidemics and also the capacity to spread. The origin of the strains was Portugal.

Innovations and breakthroughs

This study emphasizes that each hospital should characterize the prevalence of hospital/geographical specific pathogenic strains which cause nosocomial infections, and it is important to reduce infectivity rate and burden of patient health care.

Applications

The awareness can be passed onto similar hospitals anywhere to keep a vigil of the situation.

Peer review

This is good piece of work. The authors studied the distribution of MRSA strains in a particular hospital. It is interesting to find one single MRSA strain endemic in the hospital under study. Such intervention measures should be recommended in specific intervals to keep a vigil on hospital infections and to control the same.

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