Arab Gulf Journal of Scientific Research, 21 (1), pp 1- 4 (2003)

ORIGINAL PAPER

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Analysis of Methicillin-Resistant *Staphylococcus aureus* Strains Isolated in Palestine by Arbitrarily Primed PCR

Abstract. We report on a study of 35 methicillin-resistant *Staphylococcus aureus* (MRSA) clinical isolates collected between February and May 1998 from 4 different hospitals in Northern and Southern Palestine. The isolates were recovered from infection and carriage sites of patients and hospital environmental samples. The arbitrarily primed PCR (AP-PCR) method with two primers appears to be a useful tool, provides a fast and simple method for genetic analysis of MRSA infections and allows us to differentiate 22 different AP-PCR patterns. The major cluster, however, sharing common AP-PCR as well as a common PFGE pattern, represented 34% of all MRSA isolates. Isolates of this cluster were spread throughout the neonatal and intensive care units of Rafidya hospital during the entire period. In addition, these isolates were distributed in the southern part of Palestine as well.

Keywords: Methicillin-resistant *Staphylococcus aureus*, genetic analysis, geographical distribution, Palestine.

دراسة بكتيريا العنقوديات الذهبية المقاومة للميثيلين في فلسطين

اعتمادا على تفاعل البلمرة المتسلسل باستخدام

نوعين من البادىء العشوائي

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المستخلص: تمت دراسة 35 عزلة بكتيرية من نوع العنقوديات الذهبية المقاومة للميثسلين والتي عزلت من مستشفى رفيديا في شمال فلسطين وثلاثة مستشفيات اخرى في جنوب فلسطين. اظهرت الدراسة وجود 22 مجموعة. المجموعة السائدة والتي تمثل 34% من العزلات، كانت متشابهة، باستخدام كل من تفاعل البلمرة المتسلسل وPulsed field gel electrophoresis. تتميز عزلات هذه المجموعة بقدرتها على الإنتشار الجغرافي حيث وجدت في شمال وجنوب فلسطين.

كلمات مدخلية: تفاعل البلمرةالمتسلسل، عزلات بكتيرية، عنقوديات ذهبية، الإنتشار الجغرافي، فلسطين.

Introduction

Methicillin-resistant *S. aureus* (MRSA) continues to be an important cause of nosocomial infections worldwide (Leski *et al.* 1998; Wang *et al.* 1998; Durmaz *et al.* 1997; Hoefnagels-Schuermas *et al.* 1997; Kumari *et al.* 1997; Ichiyama *et al.* 1991; Zuccarelli *et al.* 1990). Rapid precise typing of MRSA is essential for epidemiological studies and hospital infection control (Hooky *et al.* 1999, Wang *et al.* 1998). Genetic typing based on pulsed-field gel electrophoresis (PFGE) is considered to be one of the most discriminative methods for detecting DNA polymorphism among MRSA. It is, however, time consuming and requires expensive equipment that is not available in all microbiology laboratories. An alternative is one of the PCR-mediated genotyping methods (Hooky *et al.* 1999; Leski *et al.* 1998; Wang *et al.* 1998; Kumari *et al.* 1997). In the present study, an attempt was made to improve the arbitrary primed PCR (AP-PCR) method by the use of two primers. To validate this method, the results of the AP-PCR on chosen isolates were compared with PFGE analysis. The AP-PCR method was then used to study the epidemiology of MRSA isolates obtained from two different geographical areas in Palestine.

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Materials and Methods

MRSA isolates. A total of 35 MRSA isolates, 29 from Rafidya hospital in northern Palestine and 6 from three other hospitals in southern Palestine, were collected between February and May, 1998. Information about the origin of these isolates is presented in Table I. Isolates were recovered from patients with infection (20%), colonized patients (42.9%) and the hospital environment (37.1%). Isolates which caused infections were obtained from the umbilicus (28.6%) and urine (71.4%).

Strain Identification. Isolates were identified as *S. aureus* according to colonial and microscopic morphology, positive catalase and coagulase production by Staphytect plus tests (Oxoid).

Determination of methicillin resistance. All *S. aureus* isolates were tested for methicillin resistance in the microbiology laboratories, An-Najah National University, Palestine. The disk diffusion method outlined by the National Committee for Clinical Laboratory Standards, (NCCLS) was used with a 1 µg oxacillin disk. Zone sizes were read after incubation at 35°C for 24h. Isolates with zone sizes ≤ 10mm were considered as methicillin resistant. A standard strain of *S. aureus* (ATCC 25923) was used as a control.

AP-PCR assay. A description of the three PCR-related procedures is given below.

(i) *DNA extraction.* Three to five colonies, picked from 18-hour culture on trypticase soy agar (Oxoid), were suspended in 100µl of lysis solution, which consists of 125µg lysostaphin (Sigma) per ml and 1mg lysozyme (Sigma) per ml in TE buffer (10mM Tris, 0.1mM EDTA pH 8). The suspension was incubated at 37°C for 60 min. After that, 10µl of proteinase K (10mg/ml) was added and incubation was continued for a further 30 min. The suspension was then heated at 99°C for 10 min and cooled to room temperature.

(ii) *DNA amplification.* PCR was performed in 25-µl vol containing 10mM Tris HCl pH 9.0, 50mM KCl, 1.75mM MgCl2, 0.1mM Triton X-100, 250 µM of each dNTP, 1µM of primer, 1 unit of AmpliTaq DNA polymerase (Promega, USA) and 5µl of the prepared DNA. PCR mixture was overlaid by 50µl of mineral oil. DNA amplification was performed in a Stuart Scientific thermocycler, with the following thermal cycling profile: initial denaturation at 94°C for 5 min followed by 40 cycles of amplification (denaturation at 94°C for 60 sec, annealing at 25°C for 60 sec, and extension at 72°C for 90 sec) ending with a final extension at 72°C for 5 min. The primers used to discriminate MRSA isolates were ERIC2 (E2) (AAGTAAGTGACTGGGGTGAGCG) and 1 (GGTTGGGTGAGAATTGCACG) (Van Belkum *et al.* 1995).

(iii) *Electrophoresis.* PCR products (25µl) were mixed with 2µl of agarose gel loading dye, separated on 2% agarose gels containing 0.25µg ethidium bromide per ml and run at 100V for 1 hr. A 100-bp DNA ladder was used as a molecular size marker. Gels were photographed on a 392-nm-wavelength transilluminator and band patterns were compared visually. Patterns that differed by one or more DNA bands were considered as different types.

Chromosomal PFGE analysis. For pulsed-field gel electrophoresis (PFGE), 5 to 10 colonies of an overnight growth from a Columbia blood agar plate were harvested into 100µl of 1M NaCl-10 mM EDTA (pH 7.5), and 3 µl of lysostaphin per ml was added. The preparation was mixed with an equal volume of 1% low-melting-point agarose at 50°C, allowed to set in a mold, and incubated at 37 °C for 4h in 1ml of 1M NaCl-10 mM EDTA (pH 7.5). The liquid was removed and replaced with 1ml of 0.5mM EDTA (pH 7.5) containing 1mg of proteinase K (Sigma) and 1% N-lauroyl sarcosine (Sigma), and then the mixture was incubated at 50° C for 18h. The plugs were then washed and digested with SmaI according to the manufacturer's recommendations (New England BioLabs). Electrophoresis was performed with Gene-navigator system electrophoresis cell (Pharmacia LKB). The following running conditions were followed (Leski *et al.* 1998): 1% agarose gel; 6V/cm; pulse, 5s for 5h, 10s for 6h, 30s for 6h, and 60s for 5h; 14°C; buffer, 0.5x TBE (Tris-borate-EDTA). The gel was stained with ethidium bromide and visualized as described above.

Results

The AP-PCR assay of 35 MRSA isolates with primer E2 yielded amplicons ranging in size from 160 to 1412 bp, with 3 to 8 fragments being resolved per isolate. For primer 1, amplicons ranged in size from 160 to 800 bp, with 1 to 6 fragments. AP-PCR identified 15 patterns with primer E2 and 13 patterns with primer 1. The combination of the patterns with the two primers resulted in 22 AP-PCR patterns (Table I). The most frequent pattern was 1B (12 of 35 isolates; 34%) as shown in Figure 1A. A single isolate or two isolates represented the remaining types. Repeat testing of 5 strains by AP-PCR using the two primers two months apart showed that the DNA patterns were stable and reproducible. The analysis of the *Sma*I digested chromosomes by PFGE indicated highly homologous fingerprints for all 1B Isolates (Figure 1B).

Table I. AP-PCR patterns of MRSA isolates

Isolate[a]	Area	[b]Hospital designation	Date of isolation	[c]Location	Source	AP-PCR pattern	
						[d]ERIC2	1
271	N	R	02/1998	NU	Umbilicus	1	A
639	N	R	05/1998	NU	Environment	1	B
347	N	R	04/1998	NU	Groin	1	C
655	N	R	05/1998	NU	Nose	1	D
272	N	R	02/1998	NU	Nose	1	B
522	N	R	05/1998	NU	Environment	1	E
442	N	R	03/1998	NU	Environment	1	A
322	N	R	05/1998	ICU	Environment	1	B
315	N	R	03/1998	NU	Environment	1	F
464	N	R	05/1998	NU	Groin	1	B
760	N	R	05/1998	ICU	Nose	1	D
756	N	R	05/1998	NU	Nose	1	G
730	N	R	05/1998	ICU	Nose	1	B
769	N	R	05/1998	NU	Groin	1	B
313	N	R	03/1998	ICU	Environment	1	B
316	N	R	03/1998	NU	Environment	1	B
669	N	R	05/1998	ICU	Environment	1	H
647	N	R	05/1998	ICU	Nose	1	B
386	N	R	04/1998	ICU	Nose	1	B
727	N	R	05/1998	ICU	Nose	1	B
588	N	R	05/1998	ICU	Environment	2	I
731	N	R	05/1998	NU	Groin	3	B
715	N	R	05/1998	ICU	Environment	4	I
668	N	R	05/1998	NU	Environment	5	I
534	N	R	05/1998	NU	Environment	6	J
422	N	R	05/1998	NU	Groin		7
657	N	R	05/1998	NU	Environment	8	K
371	N	R	04/1998	NU	Nose	9	J
663	N	R	05/1998	NU	Nose	10	B
13	S	H	04/1998	Outpatient	Urine	1	B
38	S	H	04/1998	Outpatient	Urine	11	B
14	S	H	05/1998	Outpatient	Urine	12	B
5	S	A	05/1998	Outpatient	Urine	13	B
12	S	A	05/1998	Outpatient	Urine	14	L
37	S	C	05/1998	Outpatient	Umbilicus	15	M

[a] N, northern Palestine; S, southern Palestine
[b] R, Rafidya hospital; H, Hebron; A, Alia; C, Caritas.
[c] NU, Neonatal unit; ICU, Intensive care unit.
[d] ERIC-2, Enterobacterial repetitive intergenic concensus-2

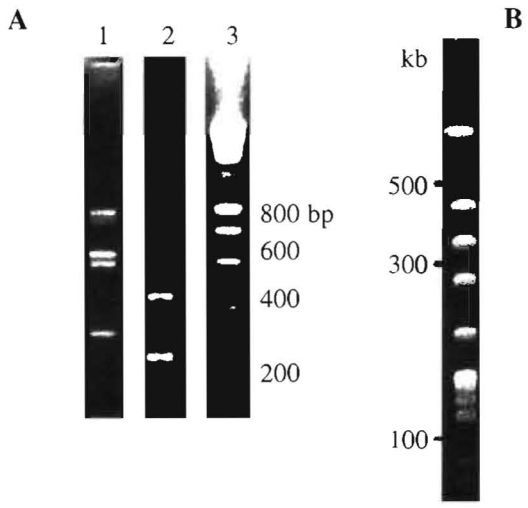

Figure 1.(A)
AP-PCR pattern of MRSA isolates (1A).
Lane 1, with primer ERIC-2
Lane 2 with primer 1
Lane 3, Molecular size marker (bp).

(B) *Sma I* PFGE pattern of MRSA isolates (1B).
The numbers on the left refer to the positions of the lambda ladder pulsed field gel electrophoresis marker (50-1000 Kb).

Discussion

Recently, a large number of reports have described the use of AP-PCR for genetic typing of medically important microorganisms, including MRSA. The procedure is used with increasing frequency, facilitated by general applicability and high speed. However, it was suggested that analysis of isolates from diverse geographic origins should be done for better evaluation of the method (Tambic *et al.* 1997; Alexiou-Daniel *et al.* 1996). Accordingly the AP-PCR method was evaluated by typing 35 isolates of MRSA obtained from different hospitals of two parts of Palestine. The PFGE method was then used to confirm the results of the AP-PCR method on chosen isolates.

In this study arbitrary primers E2 and 1, used in the field of staphylococcal typing (Van Belkum *et al.* 1995), were applied. When primer 1 was used, 13 patterns out of 35 strains could be distinguished. E2 succeeded in distinguishing more patterns than primer 1, because it produced patterns with a greater number of bands, resulting from the amplification of a great number of DNA fragments. Although AP-PCR with primer E2 was more discriminatory than AP-PCR with primer 1, convergent results with the two primers confirmed the results, and some isolates could be discriminated only by the use of primer 1 (AP-PCR types 1A, 1B, 1C, 1D, 1E, 1D, 1F, 1G and 1H). Isolates of the major cluster (1B) were identical with the two primers used for AP-PCR. These isolates were also identical by PFGE. An observation of primary concern is the ability of the major cluster (1B) to spread throughout the neonatal and intensive care units of Rafidya Hospital during the entire period. The recovery of isolates of this cluster in the environmental samples originating in both the neonatal and intensive care unit of the hospital is indicative of the role of the environmental sites in transmitting MRSA. Moreover, detection of cluster (1B) isolates 60 km approximately away in Hebron hospital in southern Palestine suggests that these strains can emerge as a public health problem in Palestine.

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Ref. 2176
Received 15/05/2002.
In revised form 07/12/2002