นิพนธ์ต้นฉบับ

Detection of the erythrogenic toxin *A*, *B* and *C* genes in group A streptococci isolated from clinical specimens and throat of school children

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Objective : *To detect the erythrogenic toxin or streptococcal pyrogenic exotoxins (spe) genes of group A streptococci (GAS) strains that isolated from the clinical specimens and throat of school children.*

Design : *Descriptive study.*

Setting : *National Streptococcal Reference Center in Thailand, Department of Microbiology, Faculty of Medicine, Chulalongkorn University.*

Materials and Mathods : *Two hundred and one strains of GAS were studied, of 101 strains were isolated from blood, body fluid, skin and soft tissue and 100 strains were from the throat of school children. The gene was amplified by the polymerase chain reaction and spe A spe B and spe C genes were detected.*

Results : *The spe B genes were detected in all GAS strains. The single spe B genes were found in 121 (60%) strains. The combination of spe genes such as spe AB, BC and ABC were detected in 13 (7%), 58 (29%) and 9 (4%) GAS strains. The clinical data were reviewed in 49 of 101 patients and of 16 cases associated with invasive diseases.*

Key words : *Erythrogenic toxin, Clinical specimen, Group A streptococci*

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ผ่องพรรณ นันทาภิสุทธิ์, สุมาณี ศิริเลิศพรรณา, สมใจ เหรียญประยูร, อาชา แทนนา. การตรวจหาอีริโทรเจนิก ท็อกซิน เอ, บี และ ซี ยีนในกรุ๊ปเอ สเตรปโตคอคคัย ที่แยกได้จากสิ่งส่งตรวจ และคอของเด็กนักเรียน. จุฬาลงกรณ์เวชสาร 2540 พ.ค;41(5) : 367-73

วัตถุประสงค์ : *เพื่อตรวจหาอีริโทรเจนิก ท็อกซิน ยีน หรือ สเตรปโตคอคคัล ไพโรเจนิก เอ็กโซ ท็อกซิน (เอสพีอี) ยีน ของกรุ๊ปเอ สเตรปโตคอคคัย ที่แยกได้จากสิ่งส่งตรวจ และคอ ของเด็กนักเรียน*

ชนิดของวิจัย : *เชิงพรรณนา*

ประเภทโรงพยาบาล : *ศูนย์สเตรปโตคอคคัสแห่งชาติ ภาควิชาจุลชีววิทยา คณะแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย*

วัสดุและวิธีการ : *สเตรปโตคอคคัส กรุ๊ปเอ จำนวน 201 สายพันธ์, แยกได้จากสิ่งส่งตรวจ ได้แก่เลือด, น้ำเจาะปอด น้ำในช่องท้อง, น้ำเจาะเข่า, และจากแผลที่ผิวหนัง จำนวน 101 สายพันธ์ แยกได้จากคอของเด็กนักเรียนจำนวน 100 สายพันธ์ นำมาตรวจหาอีริโทรเจนิก ท็อกซิน, ยีน ใช้วิธี โพลิเมอเรส เชน รีแอกชั่น*

ผลการศึกษา : *สเตรปโตคอคคัส กรุ๊ป เอ มี ยีน เอสพีอี บี ทุกสายพันธ์ และมี ยีน เอสพีอี บี อย่างเดียว จำนวน 121 สายพันธ์ คิดเป็น ร้อยละ 60 ที่เหลือมีเอสพีอี ยีนผสม คือ เอสพีอี ยีน เอบี บีซี และเอบีซี จำนวน 13, 58 และ 9 สายพันธ์ตามลำดับ คิดเป็นร้อยละ 7, 29 และ 4 ตามลำดับ ได้รวบรวมข้อมูลทางคลินิกของผู้ป่วยซึ่งแยกได้ สเตรปโตคอคคัส กรุ๊ป เอ และนำมาศึกษานี้ 49 ราย พบว่า 16 ราย มีความสัมพันธ์กับการติดเชื้อสเตรปโต-คอคคัสที่รุนแรง*

Erythrogenic toxins, also known as streptococcal pyrogenic exotoxins (*spe*), cause a skin rash in scarlet fever.[1] They also appear to be involved in the pathogenesis of severe infections.[2] The invasive disease caused by GAS were encountered. Therefore we investigated the detection of *speA*, *speB*, *speC* genes in group A streptococci (GAS) isolated from clinical specimens and in the throats of Thai school children.

Materials and Methods

Bacterial strains: A total of 101 group A streptococci obtained from a collection of the National Streptococcal Reference Center in Thailand were investigated. They were kept in 70°C. These 101 strains were isolated during 1991-1995 from clinical specimens of patients admitted to Chulalongkorn Hospital. Sources were blood, body fluid (pleural fluid, joint fluid, peritoneal fluid), skin and soft tissue, and other specimens. Furthermore, 100 strains of throat GAS isolated from school children in 1995 were also included in the study. Group A strain R80/3210, which is known to contain the *speA*, *speB* and *speC* genes, was used as a positive control and was provided by the Streptococcus Reference Laboratory, Central Public Health Laboratory (CPHL), London, United Kingdom.

Detection of *speA*, *speB* and *speC* genes

The gene was amplified by polymerase chain reaction (PCR). The procedure and use of oligonucleotide primers were followed according to the method used in the Streptococcus Reference Laboratory, CPHL, London. The *speA*, *speB* and *speC* oligonucleotide primers were

5' CTTAAGAACCAAGAGATGGC and
5' ATAGGCTTTGGATACCATCG;
5' TTCTAGGATACTCTACCAGC and
5' ATTTGAGCAGTTGCAGTAGC;
5' CATCTATGGAGGAATTACGC and
5' TGTGCCAATTTCGATTCTGC, respectively.

DNA extraction: One loop full of such tested strain was obtained from an overnight growth colony on sheep blood agar and was emulsified in 1 ml of sterile distilled water in an Ependorf tube, then heated at 100°C for 15 minutes and centrifuged at high speed in a microcentrifuge. The supernatant fluid containing chromosomal DNA was used in PCR amplification.

Amplification of DNA by PCR : The amplification reaction was performed on a total volume of 50 ul containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.1% Triton-X 100, 200 uM each dATP, dCTP, dGTP and dTTP deoxy-nucleoside triphosphates, 0.5 uM (each) primers, and 1 U of *Taq* polymerase (Perkin-Elmer Cetus, Norwalk, Conn.) The test sample

was added in a volume of 10 ul and the reaction mixture was carried out in a DNA thermal cycle (Perkin-Elmer Cetus, Norwalk, Conn.). The temperature of the sample was first raised to 96°C for 2 minutes to denature the DNA. The amplification cycle was performed with a denaturation temperature of 94°C for 30 seconds, an annealing temperature of 50°C for 30 seconds and extension temperature of 72°C for 1 minute. A total of 35 cycles were carried out and the reaction was further carried at 72°C for 10 minutes. The samples were kept at 4°C in the thermal cycle until removed for analysis. The amplified products were recovered by gel electrophoresis.

Results

There were 201 strains used in this study. From these strains, the *speA*, *speB* and *speC* genes were detected as shown in Table 1. Of 100 strains from the throats of school children, 45 strains contained the *speB* gene alone and 43 contained both *speB* and *speC* genes. This result is different from strains isolated from clinical specimens which show a predomonation of the *speB* gene alone by 75% (76 strains out of 101). The *speA* gene was seldom detected in any of these strains.

Table 1. Occurrence of the *speA*, *speB* or *speC* gene of group A streptococci isolated from clinical specimens and throat of school children.

Specimens	Total isolated	No. of isolated detected *spe* genes			
		B	*AB*	*BC*	*ABC*
Blood	14	11	-	3	-
Body fluid	7	6	-	1	-
Skin, soft tissue	67	50	7	10	-
Other	13	9	3	1	-
Throat of school children	100	45	3	43	9
Total	**201**	**121**	**13**	**58**	**9**

Table 2 presents the serological T-type of the strain correlated to the *spe* gene. There was no difference between the strains isolated from clinical specimens and the throats of the school children.

Table 2. Correlation of *spe* genes and serological T-type of strains isolated from clinical specimens (63 strains) and throat of school children (100 strains), (Total 163 strains).

Serological T-type	Total No.	No. of isolated detected toxin genes of Clinical specimen strains *spe* genes				Throat strains *spe* genes			
		B	*AB*	*BC*	*ABC*	*B*	*AB*	*BC*	*ABC*
1	10	4	-	2	-	3	-	1	-
2	1	-	-	-	-	1	-	-	-
3	6	3	-	1	-	1	-	1	-
4	6	1	-	3	-	1	-	1	-
6	3	1	2	-	-	-	-	-	-
8	5	1	3	-	-	-	-	-	1
9	3	1	-	-	-	1	1	-	-
11	7	3	-	-	-	3	-	-	1
12	13	-	-	-	-	1	-	10	2
13	4	4	-	-	-	-	-	-	-
14	3	-	-	-	-	2	-	-	1
18	1	1	-	-	-	-	-	-	-
22	3	2	-	-	-	1	-	-	-
23	1	1	-	-	-	-	-	-	-
28	1	-	-	-	-	1	-	-	-
B/3264	7	3	1	-	-	3	-	-	-
2/4	3	2	-	1	-	-	-	-	-
3/B3264	3	-	-	-	-	2	-	-	1
8/25	5	1	-	-	-	1	-	3	-
8/Imp19	2	-	-	-	-	1	-	1	1
11/12	14	3	-	-	-	1	-	10	-
NT	62	14	3	2	-	23	2	16	2

A clinical data review was carried out for only 49 of 101 patients. Of these 49 patients, 16 had invasive diseases, and 7 of them died. Within these 16 cases, 4 were not recorded as having an underlying disease (Table 3).

Table 3. Correlation of toxin genes of GAS isolated from known cases invasive diseases.

Patient No.	Age/Sex years	Specimens isolated GAS	*spe* genes	Clinical diagnosis	Underlying condition
*1	66/M**	Blood,pus Lt. leg	*B*	Cellulitis Lt. Leg, erysipelas, septic shock	HT; IHD
*2	63/F	Blood, pus	*BC*	Cellulitis Lt. leg, necrotizing fasiitis, septic shock	HT
*3	39/F	Blood, pus	*B*	Cellulitis Rt. thigh, septic shock	Rheumatic heart diseases; CHF
*4	82/F	Blood	*B*	Cardiogenic shock with aspiration	Upper GI bleeding
5	67/F	Blood	*B*	Septic cerebral emboli	Infective endocarditis; acute renal failure
*6	26/M	Blood	*B*	Sepsis with jaundice	-
7	34/F	Blood	*BC*	Sepsis	Hyperthyroidism; GI bleeding
8	56/F	Blood	*B*	Lt. groin ulcer	CA Endometrium stage Ic; gut obstruction
*9	25/F	Ascitic fluid	*B*	End stage CRF	HIV+ve
*10	50/M	Pus Lt. foot	*B*	Cellulitis Lt. leg, necrotizing fasiitis, septic shock	NIDDM
11	50/M	Tissue	*BC*	Necrotizing fasiitis	-
12	56/M	Pus	*B*	Cellulitis Lt. foot, necrotizing fasiitis	DM
13	24/F	Pus, bleb Lt. foot	*B*	Necrotizing fasiitis Rt. foot	Drug addict
14	53/F	Pus Lt. leg	*B*	Necrotizing fasiitis	-
15	21/M	Pus Lt. leg	*B*	Necrotizing fasiitis	Drug addict
16	33/M	Pleural fluid	*BC*	Empyema	-

* patient expired

** M/F = Male / Female

The results of this study are similar to these obtained in other investigations in that all strains of GAS contained the *speB* gene.[1,3] The erythrogenic toxin B is identical to the proteinase precursor and there is no evidence of *speB* phage association as there is with the *speA* and *speC* genes.[1] The *speA* gene was found associated with strains isolated from severe disease patients.[4,5] However, in this study the *speA* gene was not found in any GAS strains isolated from patients with known severe diseases.

We were able to evaluate only the serological T-type and not the M-type of each strain. However, 9 strains were M-typed by the Streptococcus Reference Laboratory, CPHL, London. Five of the 9 strains were isolated from known cases of invasive streptococcal infection. The M-type strains 31, 78, 22, 4931 and the nontype were isolated from patients 2, 3, 6, 11 and 12. Type M 11, 22 (2 strains) and M 4931 were isolated from skin and soft tissue. Although GAS strains with a known M-type were low in number, it seems that type M 22 was found frequently. The clinical data was not available for all patients, and it is possible that some of these cases also had severe infections.

In conclusion, the occurrence of group A streptococci in this study mostly contained *speB* or both *speB* and *speC* genes, and in strains isolated from patients with invasive diseases, the *speA* gene was not detected.

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