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Polymerase chain reaction (PCR) for detection superantigenicity of *Staphylococcus aureus* isolated from psoriatic patients

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Enterotoxigenic *Stapylococcus aureus* has an important role in triggering and maintenance psoriasis. This study was done to identify the frequency of the, genetic basis, enterotoxigenc S. *aureus* in psoriatic patient. Polymerase chain reaction (PCR) was performed for detect *S. aureus* enterotoxine *A* and *B genes* of bacteria isolated from psoriatic patients. *S. aureus* of psoriatic skin lesions have the enterotoxin genes (55%) significantly more than those from healthy skin of psoriatic patients (10%). Two genes were examined to be harbored by *S. aureus* isolates, namely *enterotoxin A gene* (22.5%) and *enterotoxin B gene* (10%) from psoriatic lesions, versus 10% of isolates from healthy skin of psoriatic patients that harbored *enterotoxin B gene* only. Enterotoxigenic *S. aureus* (A and B genes*)* isolated from psoriatic patients have an associated role with psoriatic lesions.

Key words: *Stapylococcus aureus*, enterotoxine, superantigen, polymerase chain reaction (PCR), psoriasis.

INTRODUCTION

Psoriasis is a chronic inflammatory skin disease associated with hyper plastic epidermal keratinocytes and infiltrating cells, including CD4+ memory T cells, neutrophiles and macrophages (Hong et al., 1999).

Although psoriasis is considered as an autoimmune disease, increasing evidence suggests an important role for bacteria in its initiation and /or propagation (Travers et al., 1999).

Recent studies have showed a potential role for bacterial superantigens in the induction of the localized inflammatory response that leads to the clinical lesions of psoriasis especially with guttate and chronic plaque psoriasis (Strange et al., 1996; Rodman and Menter, 1999; Stevenson and Zaki, 2002).

Yokote et al. (1995) showed that the toxins of strains of *S. aureus* behave as superantigen, and if present in patients, might play a role in the exacerbation of psoriatic lesions by activating certain V-beta (Vβ) T-lymphocyte subsets. The findings of persistent T-cell clones bearing Vβ 3 or Vβ 13 in skin biopsy specimens from patients with chronic plaque psoriasis suggest the assumption of a superantigen – induced inflammatory response (Chang et al., 1994). In this regard, *S. aureus* has been found on the skin of more than half of patients with chronic plaque psoriasis (Tomi et al., 2005).

The superantigenic staphylococcal enterotoxines are able to induce cutaneous lymphocytes associated antigen on human T- cells (Alber et al., 1990; Leung et al., 1995a; Yamamato et al., 1999). They bind to major histocompatibility complex (MHC) class II molecules with varying affinity (Mollick et al., 1989) and they also bind to T cell receptors with certain variable elements of their ß-chain (Marrack and Kappler, 1990; Kotzin et al., 1993).

MATERIALS AND METHODS

Skin swabs were taken from each psoriatic patient; one from psoriatic lesion and the other from adjacent normal skin by scrubbing 1 cm² of particular area of skin (Hay and Adriaans, 2004). Then skin swab was inoculated firstly into 2 ml of nutrient broth medium and then sub-cultured on agar plate media for identification of isolates (Collee and Marr, 1996). The following media were used

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Table 1. Frequency of *S. aureus* among psoriatic types.

S. aureus growth	Psoriasis types No. (%)			Total
	Plaque	Guttate	Others	
Positive	64 (62.7)	6 (35.3)	2 (66.7)	72 (59)
Negative	38 (37.3)	11 (64.7)	1 (33.3)	50 (41)
Total	102 (83.6)	17 (13.9)	3 (2.5)	122

P = 0.0332; X^2 (plaque and guttate) = 4.5 (Mantel-Haenszel corrected).

for isolation and identification of isolates (Hay and Adriaans, 2004; Baird, 1996).

Blood agar base medium (HIMEDIA M 089), mannitol salt agar medium (HIMEDIA), nutrient agar medium (SCOTT), Brain Heart Infusion Broth (DIFCO), Tryptic Soy Broth (DIFCO), Muller Hinton Agar (11 LABM) and DNase Agar (BECTON DICKINSON).

It must be mentioned that all previous media were prepared and sterilized by autoclave as manufacturer's recommendations. Inoculated media were incubated aerobically at 37°C for 24 to 48 h.

All necessary and valid tests for Identification of isolates were done by using particular diagnostic and biochemical tests (Baird, 1996; Collee et al., 1996; Forbes et al., 1998). Besides that, for rapid and accurate identification, API staph was performed.

Molecular detection of *Staphylococcus aureus* enterotoxin *A* and *B* genes by polymerase chain reaction (PCR)

Deoxyribonucleic acid (DNA) extraction

Genomic DNA was extracted from bacteria according to (Sambrook et al., 1989; Albadran, 2003; Japoni et al., 2004). Briefly the grown bacteria was spun down and washed with PBS. The pellet was mixed with TE buffer, 10% sodium dodecyl sulfate (SDS) and 20 mg/ml and proteinase K (promega) then incubated at 37°C for 1 to 3 h, then 5 M of NaCl and 10% of cetyl trimethylammonium bromide (CTAB) were added and incubated at 65°C for 10 min. The DNA was then extracted with phenol-chlorophorm-isoamylalcohol and precipitated with chilled ethanol. The DNA pellete was dissolved in 50 µℓ of TE buffer.

Genotyping

For the detection of enterotoxin A and B genes, the polymerase chain reaction (PCR) methodology was used with the following oligonucleotide primers:

Enterotoxin A gene

F: 5'- GCA GGG AAC AGC TTT AGG CAA TCT T-3' and R: 5'- GAT TAA TCC CCT CTG AAC CTT CCC A -3'.

Enterotoxin B gene

F: 5'- CAC CCA ACG TTT TAG CAG AGA GTC A -3' and R: 5'- CAG GTA CTC TAT AAG TGC CTG CCT T -3'.

In both genes 50 µℓ of PCR reaction mixture was comprised of genomic DNA samples (100 ng), 200 µm of dNTPs, 1.5 Mm MgCl2, 1 × Taq polymerase buffer, 30 pmol of each primer and 1.25µ of Taq DNA polymerase. The reaction condition used with the thermal cycler (Thermo, USA) was as follows:

The initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 1 min, 55°C for 1 min and 72°C for 1 min. then followed by final extention at 72°C for 10 min.

The PCR products were detected by electrophoresis in 2% agarose gel and stained with ethidium bromide. The presence of bands 499 or 477 bp indicated to A gene or B gene respectively.

RESULTS

Staphylococcus aureus among psoriatic patients

Out of a total 122 psoriatic skin lesions, 72(59%) showed positive culture of *S. aureus* (Table 1). The result shows that patients with plaque type harbored with *S. aureus* (62.7%) more than those with guttate type (35.3%). From data mentioned previously concerning bacterial species from psoriatic lesions, showing that *S. aureus* is the most prevalent bacterial species and for that reason this study was directed toward the role of *S. aureus* in psoriasis rather than other bacterial species.

Molecular basis of *S. aureus* enterotoxigenicity detected by PCR

Out of a total 40 isolates of *S. aureus* from psoriatic skin lesions, 22(55%) have the capacity for harboring enterotoxin gene that was detected by PCR. *S. aureus* isolates which were identified from psoriatic lesions revealed the enterotoxigenic ability more than those isolates from psoriatic healthy skin. This difference was statistically significant (P<0.01).

Table 2. Molecular detection of *enterotoxin A and B* genes of *S. aureus* isolated from lesional and healthy skin of psoriatic patients.

	Sample sites No. (%)		Total
	Skin lesion	Healthy skin	
Enterotoxin A gene	9 (22.5)	0 (0)	9
Enterotoxin B gene	4 (10)	1 (10)	5
Enterotoxin A+B genes	9 (22.5)	0 (0)	9
Others	18 (45)	9 (90)	27
Total	40	10	50

$P < 0.01$; $X^2 = 12.39$.

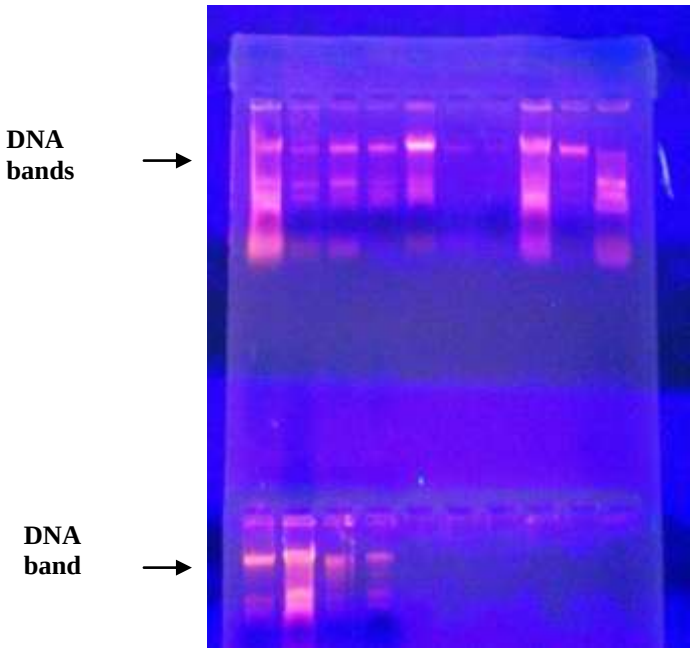

Figure 1. Agarose gel electrophoresis (0.8%) of *S. aureus* DNA bands which extracted from selected isolates.

Two enterotoxin genes were measured in this study. *S. aureus* isolated from psoriatic skin lesions harbored *enterotoxin A* gene (22.5%) compared to those isolates from psoriatic healthy skin which never revealed this capacity. In addition *enterotoxin B* gene also was detected in 10% of *S. aureus* isolated from psoriatic skin lesions while just one isolate from psoriatic healthy skin harbored this gene.

Obviously as it is shown in Table 2, nine out of 40 isolates from psoriatic skin lesions demonstrated their ability of harboring both *enterotoxin A gene* and *B gene*. The remaining isolates (Collee et al., 1996; Forbes et al., 1998) from skin lesions and healthy skin of psoriatic patients respectively, never revealed any capacity for harboring any of the examined enterotoxin genes (neither *A* nor *B gene*).

Agarose gel electrophoresis was performed to detect the extracted DNA from *S. aureus* isolates (Figure 1). Then extracted DNA was subjected to PCR for amplifying the enterotoxine genes. Two primers were set for amplifying as mentioned previously. Amplified genes then subjected again to gel electrophoresis analysis. The individual toxin gene products were characterized by comparison with standard molecular size marker (100 to 1200 bp) (Figures 1, 2 and 3).

DISCUSSION

Many studies have suggested that bacterial infections including staphylococcal and streptococcal colonization are thought to trigger or induce and/or exacerbate psoriatic lesions (Fry et al., 1988; Lewis et al., 1993).

The precise role of focal infection in psoriasis is still not

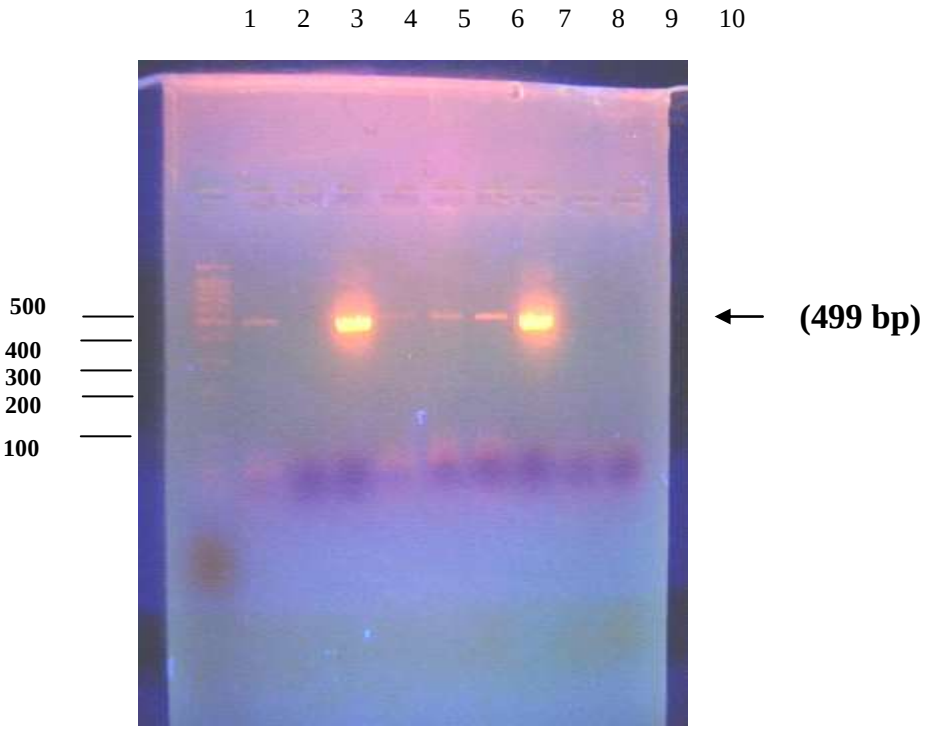

Figure 2. Agarose gel electrophoresis patterns (2%) shows PCR amplified products for *S. aureus* enterotoxin A gene (499 bp).Lane 1- DNA marker (ladder).Lanes (Travers et al., 1999; Rodman and Menter, 1999; Stevenson and Zaki, 2002; Yokote et al., 1995; Chang et al., 1994; Tomi et al., 2005) *S. aureus* amplicons from psoriatic patients. Lanes (Alber et al., 1990; Leung et al., 1995a) *S. aureus* amplicons from control.

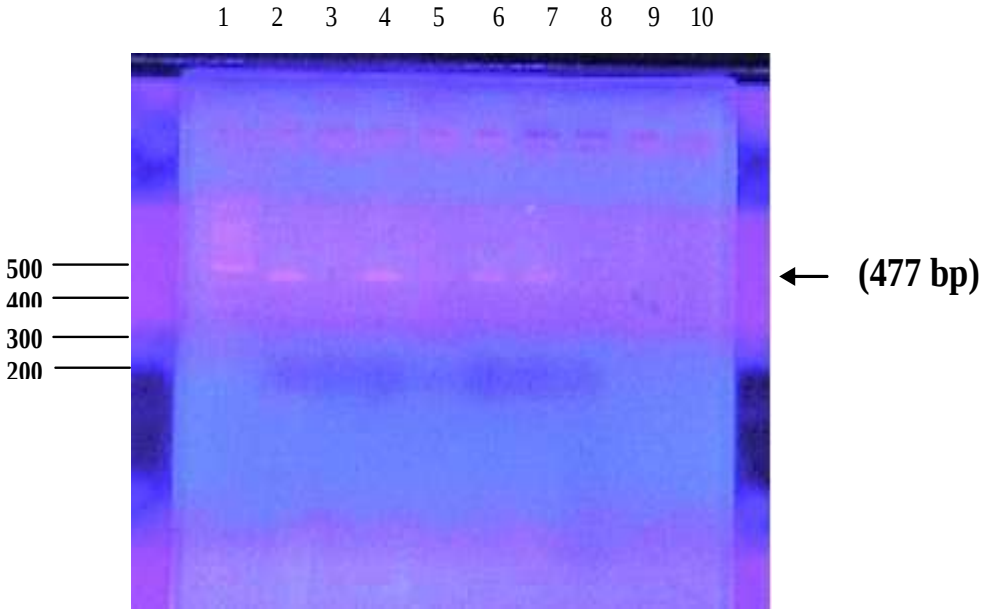

Figure 3. Agarose gel electrophoresis patterns (2%) shows PCR amplified products for *Staph. aureus* enterotoxin B gene (477 bp). Lanes 1- DNA marker (ladder). Lanes (Travers et al., 1999; Rodman and Menter, 1999; Yokote et al., 1995; Chang et al., 1994) *S. aureus* amplicons from psoriatic patients. Lanes (Tomi et al., 2005; Alber et al., 1990; Leung et al., 1995a) *S. aureus* amplicons from healthy skin of patients.

fully understood. Recently *Staphylococcus* superantigens are proposed as a possible antigen in psoriasis (Leung et al., 1993b; Valdimarsson et al., 1995; Norris et al., 1997).

Recent studies suggest that continuing, subclinical staphylococcal and streptococcal infections might be responsible not only for relapse of acute guttate psoriasis but also for a new episode of chronic plaque psoriasis (Bartenjev et al., 2000).

Surprisingly *S. aureus* isolated from psoriatic skin lesions showed a capacity for harboring enterotoxin in 55% compared to 10% of uninvolved psoriatic healthy skin isolates ($P<0.05$) which was adopted by PCR to detect the genetic basis of enterotoxigenicity of *S. aureus* rather than other diagnostic mean.

Obviously, it has been reported that 22 out of 40 isolates of *S. aureus* isolated from psoriatic lesions harbored only the *enterotoxin A* gene and 4 of 40 harbored only the *enterotoxin B* gene. Only one out of 10 isolates from healthy skin harbored *enterotoxin B* gene.

The toxin-producing strains of *S. aureus* behave as superantigen and if present in patients, might play a role in the induction of psoriatic lesions by activating certain Vβ-T lymphocytes subset. Since staphylococcal exotoxin (enterotoxin) has superantigenic properties (Saloga and Knop, 1999) this fact supported our findings because these entorotoxigenic strains isolated from psoriatic lesions are superantigenic that seem to be active in the pathogenesis of several immunologically-mediated diseases such as psoriasis (Boehncke, 1996; Balaban and Rasooly, 2000).

This finding is in agreement with Prodanovich et al. (2000), Zhang et al. (2004), Ajib et al. (2005), Tomi et al. (2005). Such result supports but not confirms the role of superantigen (enterotoxin) in induction of psoriatic lesions in genetically predisposed individuals.

Valdimarsson et al. (1995) proposed that psoriatic skin lesion are initiated by exotoxin- activated T- cells, and persist because of specific T- cells that react both with streptococcal M. Protein and a skin determinant possibly a variant of keratin.

The real role of superantigen in the pathogenesis of psoriasis can be explained as cytokines released by the superantigen stimulated T- cells could induce or enhance the expression of cross reactive autoantigen, leading to the rescue and activation of autoreactive T- cells. In this way the superantigen determined T-cell receptors Vβ phenotype would be maintained by T- cells in psoriatic lesions (Tessier et al., 1998; Torres et al., 2001). Indeed, cytokines prompted keratinocytes to proliferate and the expression of human leukocyte antigen (HLA)-DR, Fas and then apoptosis was followed. That might, more or less, be the reason of maintenance and recurrence of psoriasis (Zhang et al., 2004).

Abe *et al*. (2000) found that 27 out of 48 (56%) clinical isolates of *S. aureus* possessed either staphylococcal enterotoxine G (SEG) or variant *seg* gene. This finding suggests that SEG is one of the most frequently produced superantigenic exotoxins by *S. aureus* participate in inflammatory process of the skin.

In the present study 18 of 40 isolates from psoriatic lesions never harbored neither enterotoxin A nor B gene. This might be explained by the fact that these isolates either have not harbored any gene of enterotoxin or they might have other types regarding *S. aureus* enterotoxins that belongs to the family of nine major serological types of heat stable enterotoxins (Balaban and Rasooly, 2000; Dinges et al., 2000). More recent published report has documented (Baird, 1996) major serological enterotoxin types (Sergeev et al., 2004).

The aforementioned explanation was supported by a study of Paul et al. (2001) that characterized and identified a novel staphylococcal enterotoxin K, SEK. This might clarify the possibility of SEK role with many unexplained cases of psoriasis that were categorized previously as associated with non- enterotoxin producing staphyloccoci at that time (Leung et al., 1993a).

To the best of our knowledge, the enterotoxigenicity of *S. aureus* that was isolated from psoriatic lesions is detected for the first time in Iraq. Such detection is made to clarify the role of these enterotoxigenic *S. aureus* in the pathogenesis of psoriasis.

Notably, the accuracy of this finding depends upon the method that was used in the detection of enterotoxigenicity. Various methods have been developed for the detection of enterotoxin production (Fey et al., 1984; Fujikawa and Igarashi, 1988; Sharma et al., 2000). Out of these, the Reversed Passive Latex Agglutination (RPLA) is the most commonly used method; but cross reaction have been reported (Lee et al., 1980) and toxin production requires long incubation period. More recently oligonucleotides probes have been developed by several workers (Jaulhac et al., 1992; Tsen et al., 1993).

The PCR offers the possibility of detecting gene sequencing by DNA amplification (Forbes et al., 1998) and therefore the bacterial enrichment is not required before a specific gene can be detected.

The specificity of the PCR was shown by a match of actual size of amplified fragments. As it was clearly shown from the result that the *enterotoxin A* gene with (499) bp and *enterotoxin B* gene with (477) bp. The size of PCR products has been determined by comparing it with a DNA ladder, which contains DNA fragments of known size (100 to 1200 bp).

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