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SDS PAGE OF WHOLE CELL PROTEIN, IMMUNOBLOTTING AND PROTEIN A ASSAY FOR TYPING OF *STAPHYLOCOCCUS AUREUS* ISOLATED ON DOGS AND CATS

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Abstract

A total of 36 isolates of *Staphylococcus aureus* from hospitalised and out patient dogs and cats were typed using SDS PAGE of whole cell protein, immunoblotting and protein A assayment by ELISA test. 15/24 and 14/24 profiles were recognised using SDS PAGE and immunoblotting respectively. It is concluded that SDS PAGE of whole cell protein and immunoblotting could be used as a typing methods for the characterisation of *S. aureus* strains. Protein A assayment could be used for the detection of *S. aureus* strains in samples but could not be used to differentiate between different strains.

Key words: *Staphylococcus aureus*, SDS PAGE, Immunoblotting and Protein A.

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INTRODUCTION

Polyacrylamide-gel electrophoresis (SDS PAGE) of bacterial proteins has been used extensively as a taxonomic tool for typing, detection of protein A and to differentiate between cell bounded protein A and extracellular protein A (Tabaqchali *et al*., 1984). The high-resolution of polyacrylamide gel electrophoresis (SDS PAGE) of proteins has been used increasingly in bacterial identification at both, species and below species level, and more recently for determination types (Costas *et al*., 1989). Also Allaker *et al.* (1993) examined *S. intermedius* isolates from healthy dogs and canine pyoderma by three methods: SDS PAGE, immunoblotting and restriction fragment length polymorphism (RFLP).

Immunoblotting of *S. aureus* strains gives patterns that can be used to separate some strains. It has a potential to be developed into an alternative or ancillary typing method for routine use, because immunoblotting allows detection of products present in amounts too small to be detected by conventional staining methods (Fiona *et al*., 1989). Thomson-Corrter and Pennington (1989) found that analysis of immunoblotting by uses of the dice coefficient demonstrated that isolates of methacillin resistant *S. aureus* could be divided into two main groups. They also, found that immunoblots of exported proteins provided a rapid, reproducible and sensitive method for characterization of MRSA. Horacio *et al.* (1996) used SDS-PAGE to compare between cell bounded and extracellular protein A and they used western immunoblots analysis to differentiate antigenic differences between the sae mutants of *S. aureus* and the parental strain RC 46.

Sharifah (1992) and Grana *et al.* (1997) used ELISA for the quantification of protein A. Also anti-staphylococcus IgG could be measured By ELISA (Morales *et al.*, 1994). Chang and Huany (1994) and Wantana (1995) used ELISA for the detection of *S. aureus* in food by detecting protein A.

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The aims of this study are: to characterise *S. aureus* strains by fingerprinting profiles developed by SDS PAGE fingerprinting profiles of whole cell protein and immunoblotting and protein A assayment by ELISA test.

MATERIALS AND METHODS

Bacterial Samples

Thirty-six *S. aureus* isolates using blood agar culture media from outpatient and hospitalised dogs and cats, of different sex, age and breeds were isolated from 408 samples collected from January to September 1997 at Veterinary Hospital, Faculty of Veterinary Medicine, University Putra Malaysia.

Preparation of Protein Samples

From each culture, a loopful of overnight growths from a blood agar plate was suspended in 15 ml of brain heart infusion broth and incubated at 37ºC for 17 hours. Harvested growth was obtained by centrifugation for 1 minute at 10,000 rpm. Suspension was made in 1 ml Tris-EDTA-Saline (TES) to wash the pellet. The lysis was done by incubating the samples in 0.5 ml TES with lysostaphin at the concentration of 40 μg/ml at 37ºC for 1h. The lysate was then heated at 60 C for 5 minutes to stop the action of lysostaphin. The protein content was then estimated by the modified Lowry's method (Jaap *et al.*, 1994). The soluble whole cell proteins were then stored at -20ºC until used.

Electrophoresis was carried out according to Bryan (1994), samples for SDS-PAGE were prepared by diluting 5 μg of protein in 20 μl of sample buffer containing; 0.5 M Tris-HCl pH 8.8, 10% (w/v) SDS, 5% (v/v) B-mercaptoethanol, 0.1% glycerol and 0.1% Bromophenol blue as tracking dye. The protein was disrupted by immersion in boiling water for 5 minutes. The samples were cooled down before loading into the wells of polyacrylamide gel.

Briefly electrophoresis was carried out at 120 volts until the tracking dye reached the

resolving gel. The voltage was then increased to 200 volts and electrophoresis was continued until the dye reached 1 cm from the bottom of the gel. The gel was transferred to staining container where it was stained in 0.25% coomassie brilliant blue prepared in (40% methanol, 10% acetic acid) for 4 hours. Stained gel was distained using a distaining solution containing (40% methanol, 10% acetic acid) until the background was clear. Finally the gel was photographed and the molecular weights of the proteins that appeared in the gels were estimated.

Western blotting

After the SDS PAGE the protein was transferred to the nitrocellulose membrane by the semi dry technique (Trans-Blot Semi-Dry Bio-Rad). The blot was run at a current of 20 volts for 1h. After the end of transfer, the nitrocellulose was quickly rinsed in phosphate buffer saline (pH 7.2), dried and sealed in a container to be kept overnight at -20C°. The gel was stained with coomassie blue to determine the success of transfer.

Immunoblotting

Free protein sites were saturated by incubating the nitrocellulose membrane in blocking buffer containing 1% Bovine Serum Albumin (BSA) in PBS-Tween-20 pH 7.4. The nitrocellulose membrane is then incubated in primary antibodies, IgG rabbit anti-protein A, which is developed in rabbits using protein A purified from *S. aureus* as the immunogen (Sigma). Diluted at 1:32, in PBS-Tween-20 pH 7.4, buffer, for 1.5 hour at room temperature then washed 3 times 5 minutes each in PBS-Tween-20, pH 7.4, buffer. Then the nitrocellulose membrane was incubated in the horsradish peroxidase-linked goat anti-rabbit 1gG serum, diluted 1:14,000 in PBS-Tween-20 pH 7.4, buffer for 45 minutes. Then the nitrocellulose membrane was washed 3 times 5 minutes each in PBS-Tween-20, pH 7.4, buffer and one time in PBS buffer. And stained with diamino-benzidine reagent set (Kirkegard & Perry Laboratories, Inc. USA). [2.5 M Tris-HCl pH 7.6, Hydrogen peroxidase 0.01 % (v\v), and DAB solution concentrate].

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Statistical Analysis

The average similarity between the *S. aureus* strains represented by either Coomassie-stained gels or immunoblotting is calculated according to the formula of Nei and Li (1979).

Quantification of Protein A

We used the method used by Holis *et al.* (1986) and Grana *et al*. (1997), with some modifications. Commercial purified protein A, was prepared in quantities of 1000, 500, 250, 125, 62.5, 31.25, 15.63, 7.8, 3.9 and 1.9 ng/ml. From the OD values obtained from triple-replicate aliquots, an exponential regression analysis was calculated and used to estimate the Protein A content of *S. aureus*.

RESULTS

Protein Profiles

The SDS PAGE analysis of the whole cell protein of 24 *S. aureus* isolates yields 19 profiles (Table 1). Overall patterns were similar. The dendrogram obtained from cluster analysis grouped the 24 *S. aureus* isolates into three main clusters I, II and III (Figures 1 and 2).

Western Blotting

The western blotting of the 24 *S. aureus* isolates yields 13 profiles (Table 1). The dendrogram obtained from cluster analysis grouped the 24 *S. aureus* isolates into three main clusters I, II and III (Figure -).

Quantification of Protein A

By using the ELISA test, all of the tested *S. aureus* isolates, which probed with IgG rabbit anti-protein A were positive to the presence of these antibodies. Table 1 showed the concentration of protein A produced by 24 *S. aureus*, which ranged between 3.8 to 5.88 ng/ml.

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DISCUSSION

The results of cluster analysis of protein patterns by conventionally stained SDS-PAGE were almost similar to those of Costas *et al.* (1989) for an identical set of strains. Isolated from out patient dogs clustered together in clusters I and II except one isolate fell in cluster II and isolates from hospitalised or outpatient cats fell in cluster III. At the cut point of 80% the dendrogram divided the 24 *S. aureus* strains into 19 patterns. By immunoblotting profiles the 24 *S. aureus* isolates were typed into 13 different patterns. The results were in agreement with the finding, which reported by Mullignan *et al.* (1988) and Fiona *et al.* (1989). No specific cluster could be assigned according to the source of isolation.

According to the source of isolation, some isolates that came from the same source, clustered into the same cluster. Heterogeneity of protein profiles within hospital isolates and outpatient isolates of *S. aureus* was observed in dogs only. Differences in the banding profiles could be seen between isolates belonging to the same source, hospital or out patient. Common profiles were found in different sources.

However, all the *S. aureus* isolates tested were positive to the presence of protein A and the concentration of it ranged between 3.8 to 5.99 ng/ml, but no clear difference within the different strains. This is the same as the finding of Takeuci *et al.* (1995). Therefore, ELISA could be used for the diagnosis of *S. aureus* but not for typing.

It is concluded that there is a correlation with other molecular characterization methods utilized in this study. The clusters analysis can define the genetic variability among the isolates in each species. At the cut point of 80% the dendrogram divided the 24 *S. aureus* strains into 19. For the western blotting of *S. aureus* no correlation with protein profiles utilized in this study. The clusters analysis can also, define the genetic variability among the isolates in each species. At the cut point of 90% the dendrogram divided the *S. aureus* strains into 19 profiles. ELISA could be used for the diagnosis of *S. aureus* in blood, milk or food, but could not be used for typing.

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Table 1: Protein profiles, immunoblotting and ELISA of isolates of *S. aureus*

No.	**Source**	**Protein patterns**	Immunobloting[a]	ELISA[b]
1	13DHE	1	1	4.01
2	2DON	1	2	3.98
3	**3DOE**	2	2	5.25
4	10DOS1	3	3	5.76
5	16DOE	4	4	4.03
6	18DOS	5	5	3.85
7	20DON	5	6	3.93
8	24DOS	6	7	4.78
9	25DOS	7	1	3.87
10	27DOE	8	8	3.89
11	29DOE	9	8	4.20
12	5CHS	10	8	4.68

13	29CHE	11	1	3.95
14	31CHE	12	1	4.14
15	33CHN	13	1	3.95
16	35CHE	14	9	4.20
17	50CHN	15	10	5.88
18	61CHN	16	6	5.25
19	1CON	16	6	5.30
20	8COS	17	11	5.70
21	9COE	18	1	5.06
22	10CON	14	13	5.85
23	14COS	14	13	5.20
24	17COE	19	13	5.59

[a] Immunoblotting against protein A antigen.

[b] Enzyme Linked Immunosorbant Assay, concentration of protein A ng/ml.

D= dog, C= cat, H= hospitalized, O= outpatient

S= skin, N= nose, E,= ear

الملخص باللغة العربية

اختبار الرحلان الكهربائي و تفاعل الأضداد للبروتين الكامل للخلويات و مستوي تركيز بروتين (أ) للبكتريا العنقودية الذهبية المعزولة من القطط و الكلاب

تم تصنيف (24) عينة من العنقودية الذهبية معزولة من كلاب و قطط من داخل المستشفى أو العيادة الخارجية. طرق العزل تضـــمنت الرحلان الكهربـائي علي مـادة عديد أكريل أمايد (Polyacrylamide) ، التخطيط المناعي و التحليل المنـاعي المرتبطـة بـالخميرة (ELISA) . تم عزل 24/15 بطريقـة الرحلان الكهربـائي و 24/14 بطريقـة التخطيط المناعي . خلصــت الدراســة إلي أن اســتعمال هاتين الطريقتين يســاعد علي معرفة نوع و ذراري (strain) العنقودية الذهبية بينهما .

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تحليل البروتين (أ) يساعد فقط علي إثبات وجود و التعرف علي بروتين (أ) (prptein A) في العينات و لكن لا يمكن استعماله للتفريق بين الذراري المختلفة .

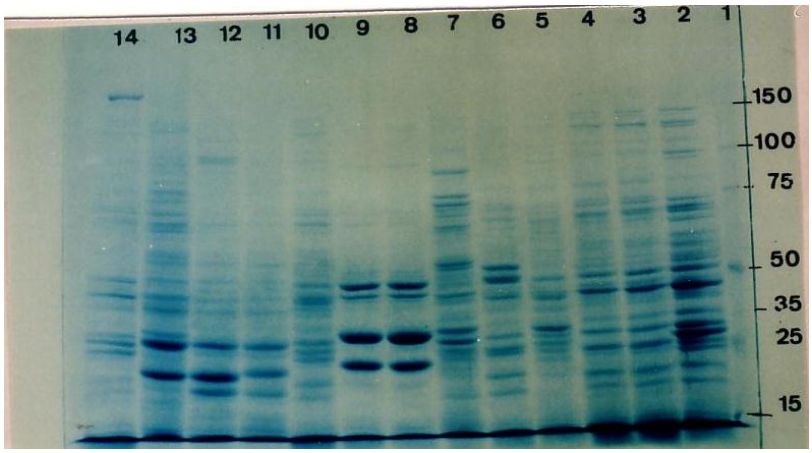

Figure 1: Protein profiles of *S. aureus* obtained with SDS PAGE. Lane: 1, protein marker (kDa). Lane: 2, reference strain. Lane: 3,1; 4,2; 5, 3; 6, 4; 7, 5; 8, 6; 9, 7; 10, 8; 11, 9; 12, 10; 13, 11; 14, 12 correspond to isolate numbers given in table 1.

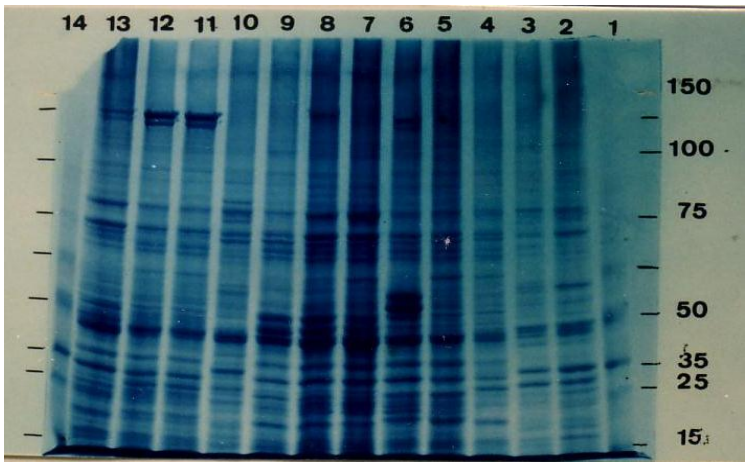

Figure 2: Protein profiles of *S. aureus* obtained with SDS PAGE. Lane: 1, protein marker (kDa). Lane: 2, 13; 3,14; 4, 15; 5, 16; 6, 17; 7, 18; 8, 19; 9, 20; 10, 21; 11, 22; 12, 23; 13, 24 correspond to isolate numbers given in table 1.

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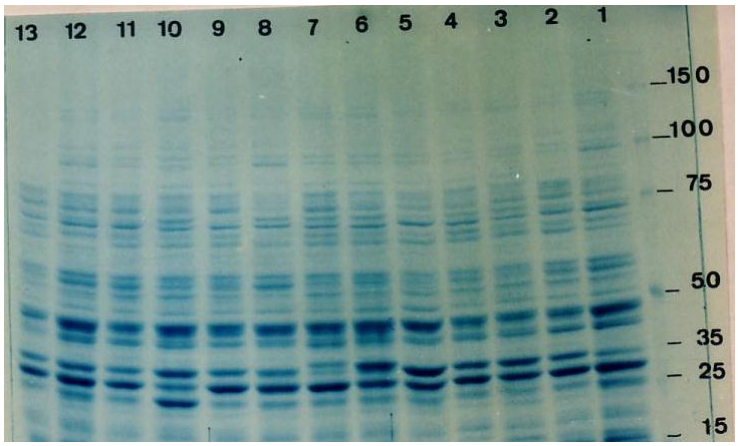

Figure 1: The SDS PAGE of whole cell protein of different isolates of *S. intermedius*. Isolate number refer to the order in which they were isolated. Lane 1, is reference isolate.

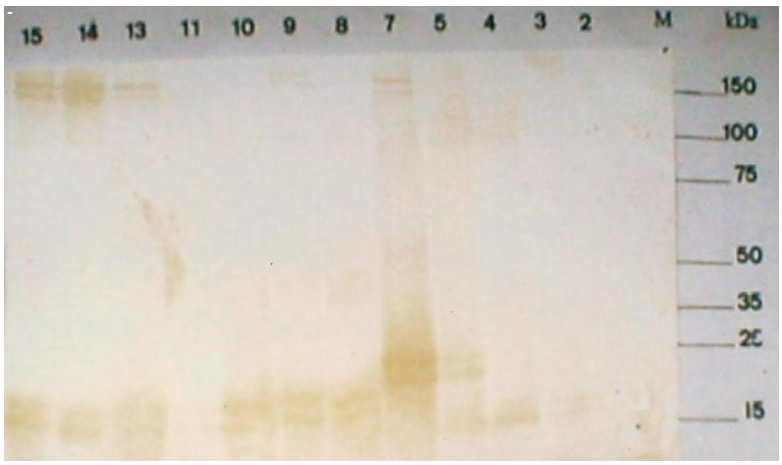

Figure 2: The western blotting of whole cell protein of different isolates of *S. intermedius*. Isolate number refer to the order in which they were isolated. Lane 1, is reference isolate.

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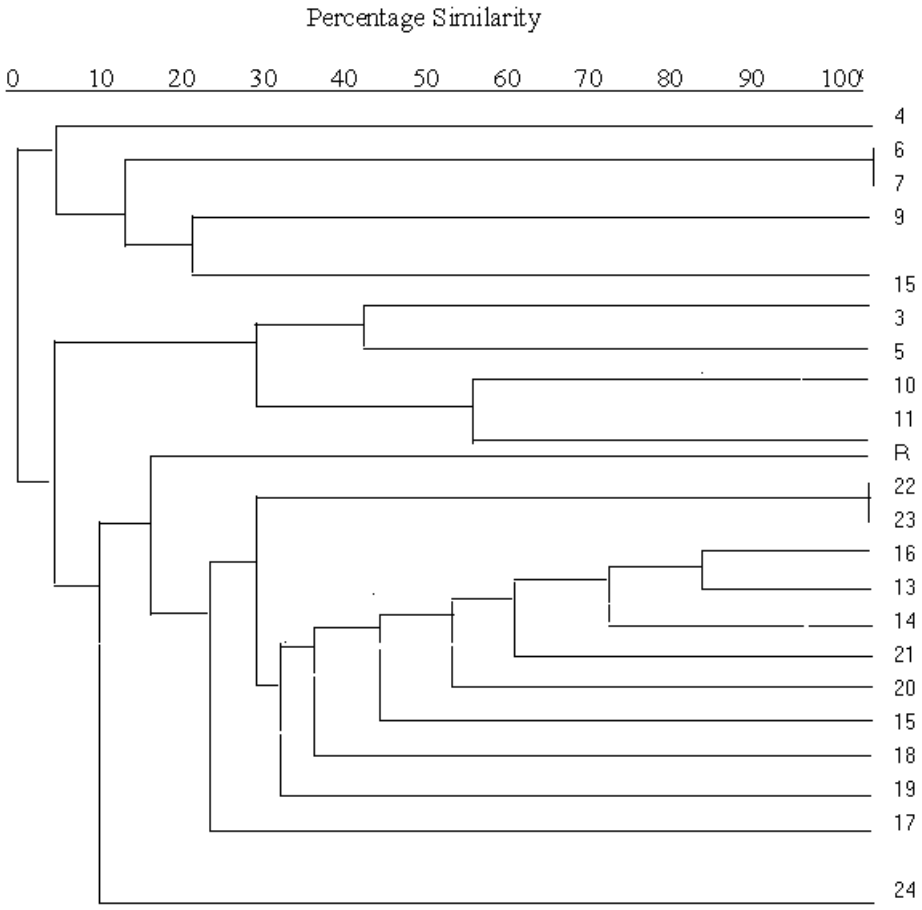

Figure 3: The dendrogram generated from matrix data obtained from SDS PAGE of whole cell protein. The parasitic distance showed the genetic dissimilarity among the *S. aureus* isolates. R, reference. Isolate lane numbers correspond to isolate numbers given in Table 1.

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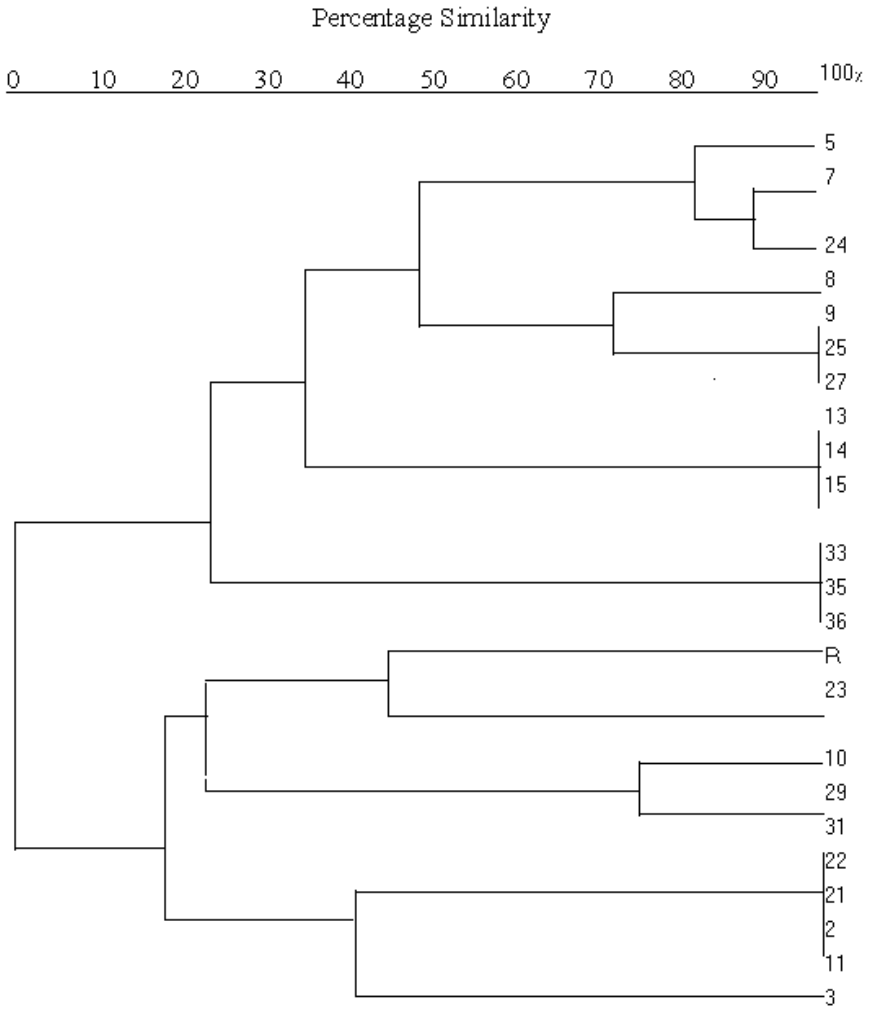

Figure 4: The dendrogram generated from matrix data obtained from western blotting of whole cell protein. The parasitic distance showed the genetic dissimilarity among the *S. aureus* isolates. R, reference. Isolate lane numbers correspond to isolate numbers given in Table 1.