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With 3 tables and 1 figure in the text.

Protein Analysis of *Staphylococcus intermedius* Isolates using Sodium Dodecyle Sulphate Polyacrylamid Gel Electrophoresis, Immunoblotting and ELISA

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ملخص البحث

تم تصنيف ٤٨ معزولة لبكتريا العنقودية المتوسطة (*Staphylococcus intermedius*) من الكلاب والقطط بواسطة ثلاث طرق للتصنيف الرحلان الكهربائي علي مادة عديد أكريل أمايد. (Polyacrylamide) والتخطيط المناعي(Immunoblotting) و المقايسة المناعية المرتبطة بالإنزيم (ELISA) . تم التعرف على ١٦ مجموعة بطريقة الرحلان الكهربائي و ١٢ مجموعة بطريقة التخطيط المناعي. خلصت الدراسة إلي أن استعمال هاتين الطريقتين يساعد في تصنيف البكتريا العنقودية المتوسطة. بينما المقايسة المناعية المرتبطة بالخميرة للبروتين أ تساعد فقط علي إثبات وجوده و التعرف عليه في العينات و لكن لا يمكن استخدامها للتصنيف.

Summary

Forty-eight *Staphylococcus intermedius* isolates from dogs and cats were characterized using sodium dodecyle sulphate Polyacrylamide Gel electrophoresis (SDS-PAGE) of whole cell proteins, immunoblotting and protein A assay by ELISA. Sixteen and 12 profiles were recognized using SDS-PAGE and immunoblotting, respectively. It is concluded that SDS-PAGE of whole cell proteins and immunoblotting could be used for the characterization and typing of *S. intermedius* isolates. However, protein A assay, could be used for the detection of *S. intermedius* isolates in samples but can not be used to differentiate between different isolates.

Introduction

Sodiun dodecyle sulphate Polyacrylamide-Gel Electrophoresis (SDS-PAGE) for bacterial proteins has extensively been used as a taxonomic tool for typing (Tabaqchali *et al*., 1984; Costas *et al*., 1989; Allaker *et al.,* 1993). It was also used for detection of protein A and to differentiate between cell-bound protein A and extracellular protein A (Tabaqchali *et al*, 1984). The high-resolution power of SDS-PAGE to proteins has increasingly been used in bacterial systematic at both species and sub-Species levels, and more recently for typing (Costas *et al*., 1989).

Immunoblotting of *S. intermedius* isolates gives patterns that can be used to differentiate between some of them. It has a potential to be developed into an alternative or ancillary typing method for routine use. This is because immunoblotting allows detection of products present in amounts that are too small to be detected by conventional methods (Fiona and Pennington, 1989). Sharifah (1994), Grana *et al* (1997) and Morales *et al.* (1994) used ELISA for the quantification of protein A and anti-*Staphylococcus* IgG.

The aim of this study was to characterize *S. intermedius* isolates from dogs and cats by fingerprinting profiles developed by SDS-PAGE of whole cell proteins, immunoblotting, and protein A assay by ELISA.

Materials and Methods

Samples:

The present study was conducted in dogs and cats, which were brought to the Veterinary Hospital, Faculty of Veterinary Medicine, University of Putra, Malaysia, from January to September 1997. Four hundred and four samples were collected from skins, nostrils and ears. Out of these samples 48 isolates of *S. intermedius* were isolated onto Blood Agar. The identification of the isolates was carried out according to Kloos and Schleifer (1986).

Preparation of Protein Samples:

Several colonies from a Blood Agar pure culture of each isolate were suspended in 15 ml of Brain Heart Infusion and incubated at 37ºC for 17 hrs on an orbital shaker. Then each culture was centrifuged at 10,000 rpm for 1 min.; the cell pellet was washed twice with 1 ml Tris-EDTA-Saline (TES). The cells lysis was done by incubating them in 0.5 ml TES-buffer with lysostaphin (40 μg/ml) at 37ºC for 1 h. The lysate was then heated at 60ºC for 5 min. to stop the action of lysostaphin. Protein content of the samples was estimated by the modified Lowry's method (Jaap and Mathews, 1994). The proteins were then stored at -20ºC until used. 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 a tracking dye. Protein samples were disrupted by immersion in boiling water for 5 min. The samples were cooled down before being loaded into the wells of polyacrylamide gel.

SDS-PAGE:

The Bryan (1994) method of discontinuous SDS-PAGE was used for analysis of proteins samples. The required amounts of proteins and

the standard protein molecular weight markers (Bio Rad, USA) were loaded in SDS-PAGE (4.75% stacking gel and 12% resolving gel).

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. Thereafter, the gel was stained for 4 hours with 0.035% solution of Coomassie blue.

Immunoblotting:

The proteins on the gels were transferred to nitrocellulose membranes by the semi-dry techniques (Burnette, 1981). The blots were run at 20 volts for one hour. The nitrocellulose membranes were then incubated with a diluted solution (1:32) of the primary antibody (anti-protein A rabbit IgG, Sigma). After washing, the membranes were incubated with horseradish peroxidase-conjugated with anti-rabbit IgG at 1:14,000 dilution. The nitrocellulose membranes, were then stained with diamino-benzedine reagent set (Kirkegard and Perry Laboratories Inc., USA), in accordance with the manufacture's instructions.

Enzyme Linked Immunosorbant Assay:

The ELISA procedure used in this study was described by Bercovich *et al* (1990).

Quantification of Protein A:

The method described by Hollis *et al* (1986) was adopted. From the OD values obtained from triple-replicate aliquots, an exponential regression analysis was calculated and used to estimate the Protein A contents of *S. intermedius* isolates.

Statistical Analysis:

Bands sizes obtained from SDS-PAGE and immunoblotting for each *S. intermedius* isolate were used to calculate the average similarity between these isolates. For this calculation Nei and Li (1979) formula was used:

$$\text{Average \% similarity (\%S)} = \frac{2NXY}{NX+NY} \times 100$$

Where N = number of bands, X = sample X and Y = sample Y.

Results

SDS-PAGE:

Thirty-six isolates were isolated from dogs and 12 from cats. Whole cell protein profiles of the 48 *S. intermedius* isolates were used for comparison with each other. Overall patterns were similar. About 35

protein bands were visible by Coommassie blue with bands molecular weights that ranged between 15 and 150 Kda. Differences among isolates were seen within bands molecular weights range of 15-35 kDa (Fig. 1). The analysis of SDS-PAGE protein profiles differentiated the 48 *S. intermedius* isolates into 16 different groups (Table 1).

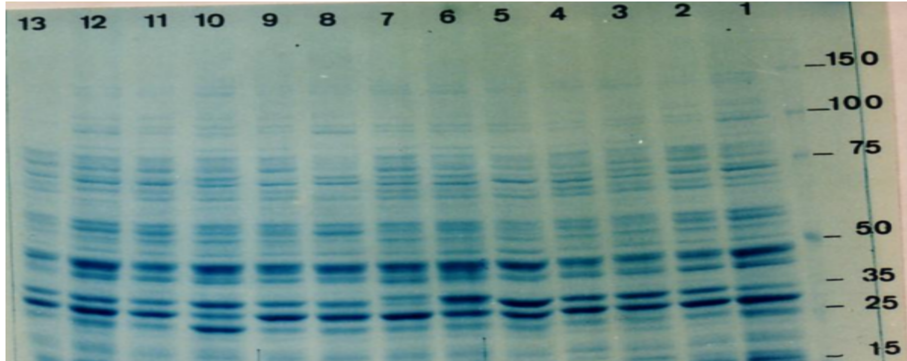

Fig. 1: The SDS-PAGE of whole cell proteins of different isolates of *S. intermedius*. Isolate number refer to the order in which they were isolated. Lane 1 is a reference strain.

Table 1: No. of *S. intermedius* isolates from dogs and cats assigned to each group according to analysis of whole cell proteins using SDS-PAGE

Source. of Isolate	GROUPS No. of *S. intermedius* isolates assigned to each group																Total
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	
Dogs																	
Skin	5	3	1	-	1	-	2	9	-	-	-	-	-	-	-	-	21
Nostril	4	2	-	1	1	-	-	1	-	-	-	-	-	-	-	-	9
Ear	3	1	-	1	-	1	-	-	-	-	-	-	-	-	-	-	6
Total	**12**	**6**	**1**	**2**	**2**	**1**	**2**	**10**	-	-	-	-	-	-	-	-	**36**
Cats																	
Skin	-	-	-	-	-	-	-	-	-	1	-	-	-	1	-	-	2
Nostril	-	-	-	-	-	-	-	-	1	-	2	-	-	-	-	-	3
Ear	-	-	-	-	-	-	-	-	1	-	1	1	1	-	1	1	6
Total	-	-	-	-	-	-	-	-	**2**	**1**	**3**	**1**	**1**	**1**	**1**	**1**	**11**

Immunoblotting:

Eight bands of protein were detected with anti-protein A rabbit IgG. These protein bands were of molecular weights 53, 52, 51, 47, 42,

36, 35, and 29 Kda. Immunoblotting characterized the 48 *S. intermedius* isolates into 12 different groups (Table 2).

Table 2: **The grouping of *S. intermedius* isolates according to immunoblotting analysis.**

Source of isolate	Groups												
	1	2	3	4	5	6	7	8	9	10	11	12	**Total**
Dogs:													
Skin	-	2	1	2	1	-	9	2	1	3	-	-	21
Nostril	1	3	-	-	1	-	4	-	-	-	-	-	9
Ear	-	1	-	2	-	2	1	-	-	-	-	-	6
Total	**1**	**6**	**1**	**4**	**2**	**2**	**14**	**2**	**1**	**3**	-	-	**36**
Cats:													
Skin	-	-	-	-	-	-	1	1	-	-	-	1	3
Nostril	-	-	-	-	-	-	1	3	-	-	-	-	4
Ear	-	-	-	-	-	-	2	2	-	-	1	-	5
Total	-	-	-	-	-	-	**4**	**6**	-	-	**1**	**1**	**12**

Quantification of Protein A:

ELISA test showed that all the 48 *S. intermedius* isolates were capable of producing protein A. The concentration of protein A produced by the 48 *S. intermedius* ranged from 3.8 to 4.7 ng/ml (Table 3).

Discussion

For characterization of *S. intermedius* isolates from dogs and cats, three tests were used, viz SDS-PAGE of the whole cell proteins, Immunoblotting and detection of protein A produced by this bacterium. The patterns of canine isolates protein profiles are generally similar to their feline counterparts. However, minor differences occur.

All canine isolates are assigned to groups 1-8, while all feline isolates to groups 9-16 (Table 1). On the basis of band staining intensity, the present study has shown that the bands of molecular weights of 47, 29 and 35 Kda are considered to be the major proteins present on the whole cell of *S. intermedius*.

In the immunoblotting study, the antibodies used for detection of protein immunogens of *S. intermedius* were raised from a purified *Staphylococcus aureus* protein A. Therefore, the observed resultant bands may only represent the immunogenic proteins shared between *S. intermedius* and *S. aureus* rather than all proteins of *S. intermedius*. Further studies using protein A isolated from *S. intermedius* to develop primary antibodies (anti-protein A rabbit IgG) are required. When comparing the similarity between the isolates, according to their immunoblotting bands, all cats isolates are members of groups 7-12,

while dog isolates are scattered into 10 groups except groups 11 and 12 (Table 2). Our results are different from those of Mullignan *et al* (1988) and Fiona and Pennington (1989).

Table 3: ELISA detectable-protein A concentration of 48 isolates of *S. intermedius* from dogs and cats.

No.	Source	Protein A (ng/ml)	No.	Source	Protein A (ng/ml)
1	1DHN	4.07	25	12DOS	3.89
2	2DHS	3.95	26	13DOS	4.16
3	4DHN	4.29	27	14DOS	4.01
4	6DHN	4.00	28	15DOS	3.91
5	7DHS	3.95	29	16DON	4.03
6	8DHE	3.93	30	17DOS	4.00
7	9DHE	4.33	31	19DOS	3.85
8	11DHS	3.93	32	20DOS	3.93
9	12DHS	4.03	33	21DOS	3.85
10	17DHN	4.61	34	22DOS	3.87
11	19DHE	3.93	35	23DOS	3.93
12	20DHS	3.95	36	26DOS	3.87
13	21DHN	4.43	37	25CHN	3.93
14	22DHN	4.01	38	27CHE	4.18
15	23DHN	4.07	39	33CHS	4.14
16	24DHE	3.87	40	35CHN	3.95
17	25DHE	3.97	41	47CHE	4.38
18	1DOS	3.95	42	57CHE	4.12
19	3DON	3.91	43	2CON	3.95
20	4DOS	3.87	44	6COE	4.05
21	6DOE	4.38	45	7COS	4.73
22	8DOS	4.42	46	7COE	4.47
23	10DOS	3.89	47	16COS	4.20
24	11DOS	4.33	48	18CON	3.91

D= dog; C= cat; H= hospitalized; O= outpatient; S= skin; N= nostril; E= ear.

ELISA results disclose that protein A from *S. intermedius* has affinity to rabbit IgG developed by protein A of *S. aureus.* Our finding agrees with that of Grana *et al* (1997). It appeared that ELISA can be used for the diagnosis of *S. aureus* and *S. intermedius* by their protein A. However, because we used antibodies developed by *S. aureus* protein A, the affinity of *S. intermedius,* in this study, is low (Table 3). This supports the finding that there are some affinity variations within these two species of bacteria to different antibodies (Smith and Godeson, 1996).

In conclusion, the profile of the whole cell proteins appears to be a useful epizootiological tool. However, it does not appear to be related to specific source. It is also useful for the analysis of the *S. intermedius* isolates, since it is reproducible and differentiates isolates into different patterns. ELISA can be used for the diagnosis of *S. intermedius* in blood, milk or food, but it could not be used for typing.

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References

Allaker, R. P.; Garrett, N.; Kent, L.; Noble, W. C. and Lloyd, D. H. (1993). *J. Med. Microbiol.*, **39:** 429-433

Bercovich, Z.; Taaijke, R. and Bokhout, B. A. (1990). *Vet. Microbiol.,* **21**: 255-262.

Bryan, J. S. (1994). SDS Polyacrylamide gel electrophoresis of proteins. In: J. M. Walker. (ed.). *Basic Protein and Peptide Protocol.* Methods in Molecular Biology. Vol. 32. Humana press. Totowa, New Jersey, USA. Pp. 1-6.

Burnette, W.N. (1981). *Anal. Biochem.*, **112**: 195-203.

Costas, M.; Cookson, B. D.; Talsania, H. G. and Owen, R. J. (1989). *J. Clin. Microbiol.,* **27**: 2574-2581.

Fiona, M. T. C. and Pennington, T. H. (1989). *J. Med. Microbiol.,* **28:** 25-32.

Grana, R.; Fajado, E. M.; Ruiz, N.; Silva, E. and Perez, M. T. (1997). *Rev. Salud Anim.*, **19**: 23-26.

Hollis, U. C.; Schmeer, N. and Newman, S. S. (1986). *Am. J. Vet. Res.*, **47**(9):1881-1884.

Jaap, H. W. and Mathews, H. R. (1994). The Lowery method for protein quantitation. *Basic Protein and Peptide Protocol.* In: J. M. Walker. (ed.) *Methods in Molecular Biology*. Vol. 32. Humana press. Totowa, New Jersey, USA. Pp. 7-13.

Kloos, W. E. and Schleifer, K. H. (1986). *J. Clin. Microbiol.,* **1**:82-83.

Morales, C. A.; Shuttz, K. T. and de Boer, D. J. (1994). *Vet. Immunol. Immunopathol.*, **42:** 137-147.

Mullignan, M. E.; Kwok, R. Y. Y.; Citron, D. M.; John, J. F. and Smith, P. B. (1988). *J. Clin. Microbiol.*, **26:** 2395-2401.

Nei, M. and Li, W. H. (1979). *Proc. Natl. Acad. Sci.* USA, **76**: 5269-5273.

Sharifah, T. (1994). Production of extracellular protein A from a methicillin resistant strains of *S. aureus.* MSc Thesis, University of Putra, Malaysia.

Smith, J. C. G. and Godeson, D. L. (1996). *J. WildLife Dis*., **32**: 358-361.

Tabaqchali, S.; Holland, D.; O'Farrell, S. and Silman, R. (1984). *Lancet*, **1**: 935-938.