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ELECTROPHORETIC ANALYSIS FOR PROTEIN OF SOME FOOD POISONING MICROORGANISMS ISOLATED FROM CHICKEN MEAT

(With 2 Tables and One Figure)

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التحليل الكهربى لبروتين بعض الميكروبات المسببة للتسمم الغذائى والمعزولة من لحوم الدواجن

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فى هذا البحث تم التعرف على الصورة البروتينية لبعض المعزولات المسببة للتسمم الغذائى والمعزولة من لحوم الدواجن حيث تم عمل التحليل الكهربى باستخدام طريقة SDS-Page لتحديد الصورة البروتينية والوزن الجزيئى الخاص بكل معزولة على حدة. وقد تم عزل ٣ ميكروبات وهى: ميكروب السالمونيلا تيفيميوريم , الميكروب القولونى ١٥٧- اتش ٧ و الميكروب العنقودى الذهبى ، حيث وجد اختلافات بينهم فى الصورة البروتينية فكانت حلقات عديد البيتيدات عددها ١١ , ٩ , ١٠ فى الثلاثة معزولات على التوالى وكان الوزن الجزيئى للبروتين الكلى يتراوح بين ١٨,٤٦-١٠٢,٧٠ , ١٨,٤٦-٦٣,٦٩ , ١٨,٩٥-٧٢,٤٢ كيلو دالتون على التوالى. وهذه النتائج تعتبر ذات أهمية تطبيقية من الناحية التشخيصية لهذه الميكروبات لدقتها عن الطرق التقليدية.

SUMMARY

In this study, SDS-PAGE analysis of major polypeptides from three strains: *Salmonella typhimurium, Escherichia coli* O157 H7 and *Staphylococcus aureus* isolated from chicken meat were determined. The electrophoretic profile of the three strains expressed 11, 9 and 10 proteins bands respectively. The molecular weight of the whole cell protein also showed some difference between strains which ranged from 18.48-102.70, 18.46-63.69 and 18.95-72.42 respectively. These results demonstrated that the protein profile of the three organisms isolated

from chicken meat was characteristic for each organism which helpful in their diagnostic procedures due to its accuracy than traditional methods.

Key words: *Chicken meat, electrophoresis, S. typhimurium, S. aureus, E. coli*

INTRODUCTION

Bacterial pathogens found in chicken flesh can cause illness and death in humans. The most common pathogens in chicken flesh are: *Salmonella, Escherichia coli and Staph* organisms which are the main causes of human food poisoning. Also, chicken flesh from industrial poultry production operations has a high incidence of contamination with these pathogens.

Salmonella is among the most common causes of foodborne infections disease in the world (D' Aoust, 1989; Baird-Parker, 1990). A characteristic feature of this organism is its broad host spectrum which comprises most animal species, including mammals, birds and cold-blooded animals, in addition to humans. A variety of food products, especially poultry and other types of meat products are the most important source of human infection. The risk of *Salmonella* infection has been heightened by the globalization of trade in food, feed and live animals and changes in production, processing and handling of food.

Poultry are a main source of *Salmonella* food poisoning for humans. More than 2.000 serotypes have been identified, mainly as a result of human food poisoning (Turner *et al.,* 1998).

Salmonella (mainly *S. typhimurium*) are usually associated with food poisoning by virtue of their ability to colonize the alimentary tracts of livestock, particularly poultry. This results in considerable contamination of carcasses at slaughter with entry of *Salmonella* into human food.

Escherichia coli, O157: H7 has emerged as a serious, potentially life-threatening, human food-borne pathogen (Jordan *et al.,* 1999).

Staphylococci are one of bacterial groups commonly occur on the skin of poultry during the slaughtering and processing of poultry (Pepe *et al.,* 2006). *Staphylococcus aureus* is a significant cause of avian disease and may thus contaminate foods as result of processed carcases (Mead and Dodd, 1990).

Enterotoxin producing *S. aureus* is the most common cause of food-borne human illness throughout the world (Do Carmo *et al.,* 2004

and Le Loir *et al.*, 2003). The foods that most frequently cause this type of poisoning are red meat and poultry and their products (Balaban and Rasooly, 2000; Genigeorgis, 1989; Kitai *et al.*, 2005 and Wieneke *et al.*, 1993).

Therefore the objective of this study reported here was to examine the sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) profile of *Salmonella typhimurium: O1412 HI 1,2, Escherichia coli*: *O157: H7* and *Staphylococcus aureus* isolated from examined chicken meat.

MATERIALS and METHODS

Forty eviscerated and refrigerated broiler chicken carcasses were purchased from supermarkets at Giza Governorate and brought under refrigeration to the laboratory and analyzed immediately for:

1- Isolation and identification of *Salmonella* according to the method approved by ISO (2002).
2- Isolation and confirmation of *Staphylococcus aureus* according to coagulase positive APHA (1992).
3- Isolation and identification of *Escherichia coli O157* were applied according to APHA (1992).

Bacterial strains and culture conditions:-

Salmonella typhimurium strains (Charles *et al.*, 1994) was grown on S.S. agar plates at 37°C for 18-24 hours. The colonies were incubated on tryptic Soya broth for 18-24 hours at 37°C. The bacteria were harvested in 10 mm HEPES buffer, pH 7.4 and the suspension was centrifuged at 1.7000 xg for 20 minutes (Barenkamp *et al.*, 1981).

Escherichia coli, *O157: H7* (Chart *et al.*, 2000) was grown on MacConkey's bile salt neutral red lactose agar. The bacteria were cultured in nutrient broth and subculture was done on soft agar pH 7.2-7.4. The cells were harvested and washed twice with 0.85% Nacl and suspended in 10 mm HEPES buffer (pH 7.4) and the suspension was centrifuged at 1.7000 xg for 20 minutes and the supernatant were used for electrophoresis.

Staphylococcus aureus strains (Hermans *et al.*, 2001) was grown on Columbia agar (Gibco, UK) supplemented with 5% ovine blood, incubated overnight at 37°C in a 5% Co_2 enriched environment and cheeked for purity. One colony of each strain was inoculated in 5 ml brain heart infusion (BHI) broth (Oxoid, England) and incubated for 16 hours in a 5% Co_2 enriched environment. Bacterial suspension was then

centrifuged at 10.000 xg for 5 minutes and the resulting supernatants were used in electrophoresis.

Sodium dodecyl sulphate polyacrylamide gel electrophoresis:

The whole cell protein extracts of *S. typhimurium, E. coli O157: H7 and S. aureus* were subjected to discontinuous SDS-PAGE according to the methods of Laemmli (1970). Prior to loading onto the gel, the protein extract of bacterial isolates were heated at 100°C for 4 minutes in sample buffer containing 0.06 MTris, 1.2% SDS, 5% B-mercaptoethanol and 11.9% glycerol. The sample containing 15 µg of protein in 50µl of sample buffer was loaded into each lane (10 µl/ lane). The protein content of each sample was determined by the modified Lowry procedure of Lowry *et al.,* (1951) and Markwell *et al.,* (1978). The protein was separated on SDS-polyacrylamid slab gel using Hoefer mini-gel system (SE 250, Mighty small II) with PS 500 XT power supply. The completed gel used in this study consisted of a stacking and a separating gel. The stacking gels contained final concentration of 4% acrylamide / N methylene- bisacrylamide (Sigma), 0.125 M Tris – Hcl (pH 6.8) and 10% (W/V) SDS. The separating gel contained 12% acrylamide / N methylene- bisacrylamide, 0.375 M Tris – Hcl (pH 8.8) and 10% (W/V) SDS. Polymerization was achieved by the addition of 0.05% (V/V) N, N. N', N' tetramethylene diamine (TEMED) and 0.05% (W/V) ammonium persulphate (Sigma). The electrophoresis buffer (pH 8.3) consisted of 0.025 M Tris base, 0.192 M glycine and 0.1% SDS.

Electrophoresis was performed at room temperature at a constant voltage of 100V with the bromophenol blue dye reached 1 cm from the bottom. Gels were stained with Coomassie blue R 250. Molecular weights were estimated using Alpha Innotech, Alpha Ease FC program.

PageRuler prestained protein ladder was used as a protein marker. It is a mixture of 9 recombinant, highly purified colored proteins with the apparent molecular weights from 17 KDa to 170 KDa.

RESULTS

Table 1: Incidence of some food poisoning micro-organisms recovered from chicken meat samples.

Organisms	No. of examined samples	Positive samples		Serotyping
		No	%	
Salmonella.	40	2	5	S. *typhimurium* O1412 HI 1,2
S. aureus	40	9	22.5	*S. aureus*
E. coli.	40	1	2.5	O 157:H7

Table 2: Protein analysis of *S. typhimurium, E. coli O157: H7* and *S. aureus*.

Band	Marker		Lane 1		Lane 2		Lane 3	
	Mol. Wt.	Rf	Mol. Wt.	Rf	Mol. Wt.	Rf	Mol. Wt.	Rf
1	17.00	0.875	18.46	0.868	18.46	0.868	18.95	0.859
2	26.00	0.802	25.52	0.759	25.79	0.755	25.65	0.757
3	34.00	0.688	28.28	0.724	27.85	0.729	28.43	0.722
4	43.00	0.566	31.67	0.686	31.67	0.686	31.67	0.686
5	55.00	0.444	36.01	0.642	36.01	0.642	35.82	0.644
6	72.00	0.372	40.53	0.602	40.32	0.604	40.32	0.604
7	95.00	0.299	46.08	0.559	45.37	0.564	45.61	0.562
8	130.00	0.229	51.33	0.523	51.07	0.524	50.80	0.526
9	170.00	0.167	63.04	0.453	63.69	0.450	63.04	0.453
10			75.07	0.394			72.42	0.406
11			102.70	0.288				

Lane 1: *S. typhimurium* Lane 2: *E. coli O157: H7* Lane 3: *S. aureus*

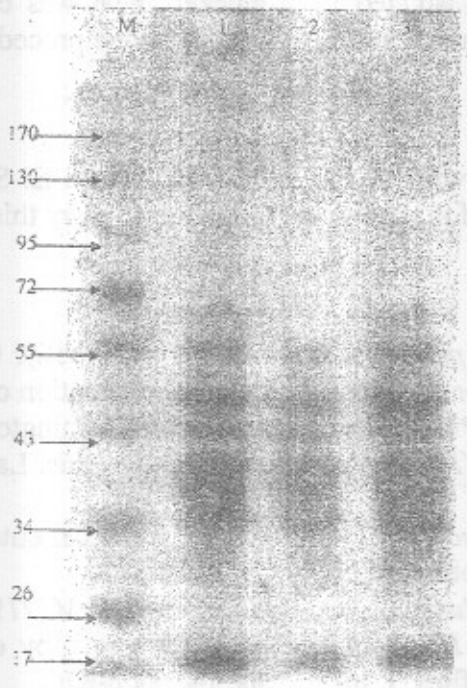

Fig. 1: Electrophoretic protein patterns of three food poisoning micro-organisms isolated from chicken meat.

M: Marker contains protein of molecular weight ranged from 17 KDa to 170 KDa.
Lane 1: Whole cell protein of *S. typhimurium*
Lane 2: Whole cell protein of *E. coli O157: H7*
Lane 3: Whole cell protein of *S. aureus*

DISCUSSION

Table (1) revealed the overall positive rates of *S. typhimurium, S. aureus* and *E. coli* O157 were 5, 22.5 and 2.5% respectively.

SDS-PAGE analysis of major polypeptides from three strains: *S. typhimurium, E. coli O157: H7* and *S. aureus* isolated from chicken meat were visualized by Coomassie staining. The protein profile of each strain was represented in Table (2) and Figure (1).

All strains have a common band with molecular weight of 36 KDa. The electrophoretic profile of whole cell protein of *S. Typhimurium, E. coli O157: H7* and *S. aureus* expressed 11, 9 and 10 protein bands respectively. The electrophoretic protein patterns of the strains showing some degree of heterogeneity in the major band region. Similar results were obtained by Sarasombath *et al.,* (1998); Robin and Catherine, (2000); Santos *et al.,* (2002) and Zang *et al.,* (2002).

In conclusion, our results demonstrated that the protein profile of the three organisms isolated from chicken meat was characteristic for each organism which is helpful in their diagnostic procedures.

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