Animal Health Research Institute
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ELECTROPHORETIC ANALYSIS AND IMMUNOLOGICAL CHARACTERIZATION OF *STAPHYLOCOCCUS AUREUS* ISOLATED FROM CHICKEN MEAT

(With 4 Tables and 2 Figures)

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التحليل الكهربي والتوصيف المناعي للميكروب العنقودي الذهبي المعزول من لحم الدجاج

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يظهر هذا البحث الكشف عن الميكروب العنقودي الذهبي في لحوم الدجاج المباعة في محلات التريش اليدوي الموجوده في محافظة أسيوط. أجرى التحليل البكتريولوجي على 80 عينة من لحوم الدجاج الطازج لمعرفة مدى تواجد هذا الميكروب. وكانت نسبة تواجده 47.5% بينما كانت النسبة الإيجابية لوجود أنزيم التخثر 27.5%. كما أنه قد تم عد الميكروب حيث وجد بمتوسط $28.5 \times 10^3 \pm 38.8$ مستعمرة بكتيرية/ جرام.ولقد تم إجراء الاختبارات البيوكميائية لهذا الميكروب وأوضحت نتائج الصورة البروتينية والطبع المناعي لأنتجن الميكروب العنقودي الذهبي المعزول من لحوم الدجاج الطازج أهميتها في توصيف وتشخيص الميكروب.

SUMMARY

This work presents an investigation of the *S.aureus* of poultry meat sold on the manual processing shops located in Assiute Governorate. Bacteriological analysis was performed on 80 samples of fresh poultry meat for the presence of *S.aureus*, the isolated percent of the organisms was 47.5, while coagulase positive *S.aureus* was detected in 27.5% of the tested samples. Also the organisms was counted, the mean count was $28.5x10^3 \pm 38.8$ CFU/g. The results obtained demonstrated that the protein profile and immunoblotting of the *S.aureus* antigen isolated from raw chicken meat was characteristic and helpful in the diagnostic procedure.

Key words: *Electrophoretic analysis, staph. auries, chicken meat.*

INTRODUCTION

It in well documented that contamination of food with pathogen is a major public health concern worldwide.

Staphylococcus aureus is a significant cause of avian disease and may thus contaminate food as a result of processed carcasses (Mead and Dodd, 1990). Enterotoxin producing *S.aureus* is the most common cause of food –borne human illness through out the world (Le Loir *et al.*, 2003, Do Carmo *et al.*, 2004). The foods that most frequently cause this type of poisoning are red meat and poultry and their product. (Gearnaras, 1989; Wieneke *et al.*, 1993; Balaban and Rasooly 2000; Kitai *et al.*, 2005).

Staphylococci are one of bacterial groups commonly occur on the skin of poultry during the slaughtering and processing of poultry (Pepe *et al.*, 2006). In poultry processing, live birds enter the abattoir carrying large member of microorganism on their feathers, feet and skin, with the feather being the most contaminated (Kotula and Pandya, 1995). Although the feather are removed during the defeathering stage, the preceding of scalding process and the defeathering process itself allow cross contamination of carcasses skin with bacteria from feather, feet and guts, as well as equipments and environmental sources, such as water and air (Mead, 1989; Kotula and Pandya, 1995).

The majority of Egyptian prefers to use fresh chicken, chicken parts on giblets, the matter that lead to deal with small scale manual poultry processing shops. These shop have not implemented effective hygienic measures or food safety instruction, since most of the recommended hygienic measures in the processing chain in the modern poultry processing plant are not applicable (Mira and Eskandar, 2007).

S.aureus rarely survives a proper heat treatment but in frequently a contaminated from the hands of persons whom handle and package the meats after processing (Elmossalami, 2002).

In the present work, *S.aures* were characterized by SDS PAGE of whole all proteins as well as the immunoblotting technique for detection of specific immunogenic bands of this fractionated protein.

MATERIALS and METHODS

1. Collection of samples:

Eighty samples of fresh whole poultry carcasses were collected from manual processing poultry shops located in Assiute Government. The samples were brought under aseptic condition to the laboratory and analyzed directly.

2. Bacteriological count and isolation:

Twenty five gm from each carcass were blended in a stomacher for 2min (Capita *et al.*, 2001). In 225ml of 0.1% peptone water at 3000 r.p.m. Decimal dilution were carried out using the same diluents the spread plate technique was used to prepare duplicate plates for determination of *S.aureus* on Baird, Parker plates were incubated under aerobic condition at 37ºC for 24h, plates between 25 and 250 colonies were counted, and mean counts were calculated (Capita *et al.*, 2001).

3. Identification:

S.aureus was confirmed using cell morphology, arrangement of the cells, gram reaction (Harrigan 1998a), catalase activity (Cowan 1974), modified oxidase test (Fallller and Schleifer, 1981) coagulase activity (Baron *et al.*, 1994), acid production form maltose and mamitol and aceton production (Baired – Parker, 1980).

4. *S.aureus* strain: Was grown on Columbia agar (Gibco, united kingdom) supplemented with 5% ovine blood, incubated overnight at 37ºC in 5% Co_2 enriched environment and checked for purity (Hermans *et al.*, 2001).

5- Culture supernatant (Sefa *et al.*, 2001):

From each culture, a loopful of overnight growth was suspended in 15ml broth in Brain Heart Infusion Broth and incubated in a rotated incubator for 7h (at 35ºC, 150r.p.m). Samples were then transferred into appends tubes and centrifuged for 3min at 12.000 rpm and the collected cells were washed three times with distilled water. The washed cells were stirred after the addition of 25ml SDS sample buffer (0.06 M Tris, 2.5% glycerol, 0.5% SDS, 1.25% B-mercapto-ethanal) and the proteins were denatured in boiling water for 5min supernatant was then centrifuged again for 3min, at 12.000 rpm collected in eppendorf tube and kept at -40ºC until used in electrophoresis and western blot analysis.

6. Sodium dodecyl sulphate palyacrylamid gel electrophorsis (SDS-PAGE): Antigen in *S.aueus* supernatant were separated by SDS-PAGE according to (Laemmli, 1970). The protein content of the sample was determined by the Lowry *el at.* (1951) and Markwell *et al.* (1978). The protein sample was treated with sample buffer loaded into each lane (10ml/lane). The protein was separated on SDS-polyacylamide slab gel using Mini-Protien II vertical dual slab cell apparatus (Bio-Rad laboratories, Richmond CA). The completed gel used in this study consisted of a stacking and a separating gel. The stacking gel contained final concentration of 4% acrylamide /N methylene bisacrylamide (Sigma), 0.125M Tris.Hcl (PH6-8) and 10% (W/v) SDS. The separating gel contained 12% acrylamide /N methylen-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) Ń, Ń tetramerthylenediamine (TEMED) and 0.05% (W/V) ammonium persuphate (Sigma).

Electrophoresis was performed at room temperature at a constant voltage of 100 V till the bromophenol blue dye reached 1cm from the bottom. The gels was stained with Coomassie blue R 250. Molecular weight of the demonstrated antigen were estimated on the basis of thier migration distance in the polyacylamide gel, by comparison of their localization with the protein bands of the standard molecular weight maker Rainbow protein marker. Was used (Fermentas, U.S.A). It is a mixture of highly purified colored proteins with the apparent molecular weights ranged form 10 KDa to 250KDa.

7. Western blot analysis:

According to (Towbin *et al.,* 1979), the fractionated *S.aureus* antigen using SDS-PAGE was electrically transferred onto nitrocellulose (NC) membrane at 360 mA for 4 hours using trans-Blot cell (Bio-Rad) NC sheets were cut into 0.5cm strips (Towbin *et al.,* 1979) followed by blocking in blocking buffer including 1% (W/V) gelatin in tris-buffered saline (TBS: 20mM Tris. Hcl PH 7.4, 0.5M Nacl). The membranes were rinsed three times with TBS containing 0.05% Tween 20 and then incubated for 3 hours with hyperimmun serum obtained after infection.

Each serum sample was diluted (1: 500) in blocking buffer. Membrane strips were rinsed three times and incubated with rabbit anti chicken IgG peroxidase conjugate (diluted 1: 2, 500 in blocking buffer) for 3 hours (Sigma, st.louis, Mo). Immunoreactivity was detected by incubating blot with TBS containing H_2O_2 and 4-chloro-1-napthol. The reaction visualized by the neked eye.

RESULTS

Table 1: Statistical values of *Staphylococcus aureus* in the examined samples

Minimum	Maximum	Mean	± standard error
$1x10^3$	$56x10^3$	$3x10^4$	$4.8x10^{-1}$

Table 2: Incidence of *Staphylococcus aureus* in the examined samples.

No. of samples	Positive samples	
80	No	%*
	38	47.5%

* The percentage was calculated on the basis of the total number of samples.

Table 3: Incidence of coagulase positive *Staphylococcus aureus* in the examined samples.

No. of samples	Coagulase +ve		Coagulase –ve	
80	No	%*	No	%*
	22	27.5%	16	20%

* The percentage was calculated on the basis of the total number of samples.

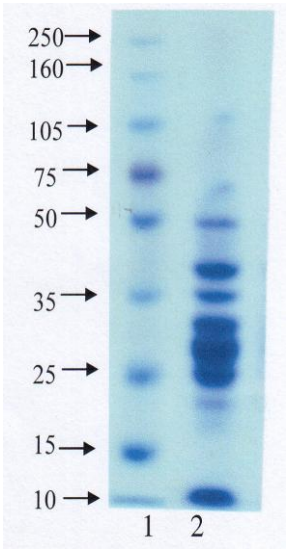

Fig. 1: SDS-PAGE of whole cell protein antigen of *S.aureus*
Lane 1: standard molecular weight marker
Lane 2: *S.aureus* whole cell protein

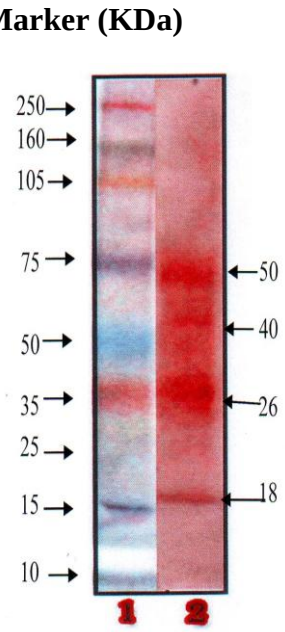

Fig. 2: Immunoblot of whole cell protein antigen of *S.aureus* isalated from chicken meat.
Lane 1: Standard molecular weight maker.
Lane 2: Immunogenic bards from the reaction of *S.aureus* antigen and hyperimmune serum obtained after infection.

The electrophoretic profile of the whole cell protein antigen of *S.aureus* displayed 10 protein bands with molecular weight range 18kDa-72kDa (Fig. 1).

The immunoblot profile of whole cell protein antigen of *S.aureus* show 4 reacted immunogenic bands at 18, 26, 40 and 50 DKa (Fig. 2).

Table 4: Protein analysis of S.aureus isolated from chicken meat.

Band	Marker (Lane 1)		S.aureus (Lane 2)	
	Mol. Wt.	Rf	Mol. Wt.	Rf
1	10	0.966	18	0.868
2	15	0.881	26	0.776
3	25	0.806	28.43	0.722
4	35	0.658	31.67	0.696
5	50	0.571	35.82	0.644
6	75	0.391	40	0.601
7	105	0.278	45.61	0.582
8	160	0.212	50	0.571
9	250	0.125	63.04	0.453
10			72.42	0.406

DISCUSSION

The level of contamination with *Staphylococcus aureus* of the examined poultry samples varied from $1x10^3$ to $5.6x10^4$ with a mean value of $2.8x10^4 \pm 38.8$ CFU/g. (Table 1). This result is slightly higher than the mentioned by Mahmoud and Hamaouda (2006) who obtained that mean count of $2.7x10^3 \pm 1.7 \times 10^2$ CFU/g of breast samples of poultry and also obtained the mean count of $8.9x10^3 \pm 1.3x10^2$ CFU/g of thigh samples of poultry which was higher than that obtained in this study while Al-Dughaym and Altabari (2009) revealed that *Staphylococcus aureus* mean count was less than 10^2CFU/g for all examined samples.

The percent of *S.aureus* in the examined samples was 47.5% (Table 2) which was nearly agreed with that obtained by Lidija *et al.* (2006) who recorded 46.15 of the samples of chicken breast fillets.

The incidence of *S.aureus* in this study is lower than that obtained by Capita *et al.* (2002) which was 91.7%, Kitai *et al.* (2005) which is 65.8%. On the other hand lower results were obtained by Antown (2002), Lidija *et al.* (2006), Aggour *et al.* (2008) who recorded 7.5% of total fresh chicken parts, 28.75% of samples of breasts with skin and 22.5% of chicken meat samples respectively.

Coagulase positive *staphylococcus* was detected in 27.5% of the tested samples as shown in Table (3), this percent was lower than that detected by Mahmoud and Hamaouda (2006) who detected coagulase positive *staphylococcus* in 38.7% and 51.5% of both thigh and breast samples, respectively while Cohen *et al.* (2007) detect lower result 10.4%. A review carried out by Waldroup (1996) showed that *S.aureus* levels in poultry meat are variable and that is depends on the paper consulted. In the American state of Nebraska, a microbiological criterion is used as a reference for poultry meat *S.aureus* coagulase positive must be absent in 19 of meat sample. We could state that the *S.aureus* counts obtained in this study do not fit into this criteria established (Bryan, 1980) showed that if poultry carcasses are left without refrigeration for several hours or cooled slowly in refrigerator, growth of *S.aureus* and enterotoxin formation may occur. Growth of *S.aureus* in the product is favoured by lack of competive bacteria which are destroyed by heat.

The protein play a role in virulence of the strains (Hermans *et al.*, 2001). Virulence factors described is *S.aureus* from humans, chicken and Cattle include several proteins with a molecular mass closely corresponding the one of the band observed in this study. These proteins have been identified as protease (Takeuchi *et al.*, 1999) and enterotoxin B (Jone and Khan, 1986). In this study, the protein profile was important characterization of *S.aureus* organism, as displayed 10 protein bands with molecular weight ranged from 18 KDa-72 KDa. These result, were agreement with that obtained by Aggour, *et al.* (2008).

Rasooly and Rasooly (1998) demonstrated that the Western blot assay is a very sensitive method capable of detecting very small amount of Staphylococcal enterotoxin (0.1 mg/ml). The immunogenic bands in this work were detected at 18, 26, 40 and 50 KDa.

CONCLUSION

Control measures should be taken to reduce the microbial population as using clean utensils, avoid contamination of the carcasses during evisceration using clean water for washing carcasses, avoid excessive handling.

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