

Occurrence of Enterotoxigenic *S. aureus* in Half-Cooked Chicken Products.

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ABSTRACT

Sixty samples of half-cooked chicken (chicken wings, chicken drumsticks and chicken pane) (20 each) were collected from Menofia governorate and were examined for the incidence of *Staphylococcus aureus* and their enterotoxins. The percentage of positive samples for *S. aureus* was 40%, 40% and 25% with mean count $0.4 \times 10^2 \pm 0.15 \times 10^2$, $0.28 \times 10^2 \pm 0.2 \times 10^2$ and $0.19 \times 10^2 \pm 0.2 \times 10^2$ for chicken wings, chicken drumsticks and chicken panes, respectively. All isolated strains of *S. aureus* showed positive Catalase, Coagulase and Mannitol Fermentation test and 100% of isolates had hemolytic activity on sheep blood agar and 9.52% had hemolytic activity on human blood agar. The incidence of enterotoxin A produced by *S. aureus* was 50% and 37.5% in chicken wings and chicken drumsticks, respectively.

INTRODUCTION

Food is a chemically complex matrix, and predicting whether, or how fast, microorganisms will grow in any given food is difficult. Most foods contain sufficient nutrients to support microbial growth. Several factors encourage, prevent, or limit the growth of microorganisms in foods, the most important are water availability, pH, and temperature (1-3).

Poultry meat has become the second most popular eaten meat due to good source of protein and many nutrients and is relatively low in fat.

The increasing price of meat has encouraged the food process on to products available as either fresh or precooked (i.e. fried) in different shapes, easily handled, stored and rapidly used with low costs as luncheon and nuggets. Deep fat frying is a popular cooking method because it generates flavorful products having crispy exteriors with moist and juicy interiors (4).

The presence of a microbial hazard, such as pathogenic bacteria or a microbial toxin, in ready to eat poultry products is one basis on which these products may be found (5).

Poultry meat has desirable nutritional such as low lipid content and relatively high concentration of polyunsaturated fatty acids which can be further increased by specific dietary strategies (6).

Nuggets and fajitas were manufactured from equal portions of breast and thigh chicken meat from chicken fed either a supplemented level of vitamin E.

Chicken nuggets are a partially cooked retail product that may be perceived by consumers as fully cooked and therefore be handled in an unsafe manner in domestic environment (7-9).

The concept of "ready-cooked food to go" is as old as cities themselves. In Egypt as many other African countries, fast foods are commonly vended on streets and at market sites in urban area where there is constant and heavy movement of people. Although there are no readily obtainable epidemiological data about the risks of food borne diseases resulting from these foods, the laboratory data show that street vended foods frequently have high microbial populations and occasionally high population of pathogenic bacteria (10-12).

Consumption of fast food in the world has been associated with obesity leading to many diseases (18).

Furthermore, it was reported the prevalence of *Campylobacter* spp., *Staphylococcus* spp., *Escherichia coli*, *Salmonella* spp., *Yersinia* Spp. and *Listeria* on meat, sea foods, vegetable ingredients, chicken shawarmas, raw and cooked foods, raw chicken, beef burger sandwiches, ready-to eat salad vegetables, commercial mayonnaise, frozen chicken, poultry products and on the hands of food workers (8,15,13).

Fast foods sold in a restaurant or store with low quality preparation and served to the customer in a packaged form for takeout/take away. In most fast food operations, menu items are generally made from processed ingredients prepared at a central supply facility and then shipped to individual outlets where they are reheated, cooked (usually by microwave or deep frying) or assembled in a short amount of time, fast food are often very high in calories, saturated fat and sodium that can make us fatter, clog our arteries and send our blood pressure soaring (19).

Microorganisms in fast and traditional fast foods are responsible for many human diseases. e.g *Salmonella* bacteria is a common cause of food borne illness, particularly in undercooked chicken and chicken eggs (14-17).

Staphylococcus aureus widely distributed in nature in many species of warm-blood animals. There is an increasing evidence that strains carried by particular natural host have evolved sufficiently so that specific ectotypes or biotypes can be considered as characteristic of particular host (20).

Staphylococcal food poisoning resulting from the growth of enterotoxigenic strains of *S. aureus* in feed leading to the production of enterotoxins which is considered one of the major causes of food borne disease all over the world (21).

Pathogenic *Staphylococci* may produce certain toxins and enzymes causing different

diseases in man, animal or bird. Enterotoxins are groups of single chain protein (poly peptides) with molecular weight 28.000-35.000. Daltons resistant to high temperature (heat stable) and proteolytic enzymes. The enterotoxigenic strains of *S. aureus* produce several types of enterotoxins (A,B,C,D and E) which can cause symptoms of intoxications such as vomiting, diarrhoea and abdominal cramping (22). Enterotoxin A (SEA) is responsible for a majority of staphylococcal food poisoning whereas enterotoxin B (SEB) is rarely involved (23).

In the gastrointestinal tract, activate an emetic reflex and cause nausea, emesis, abdominal cramps and diarrhea (24). SEs are resistant to inactivation by gastrointestinal proteases such as pepsin. Heat stability is one of the most important physical and chemical properties of SEs in terms of food safety (25-27).

The ability of *S. aureus* to grow and produce SEs under a wide range of conditions is evident by the variants of foods that have been unlikable in SEP.

In addition most outbreaks resulted from the combined effect of contamination of the food with *S. aureus* often through unsanitary handling, and holding the food at the wrong temperature thus allowing growth and synthesis of enterotoxins (28). However, the enterotoxination generally is not lethal and the elderly are more susceptible than younger individuals. The amount of enterotoxin necessary to cause intoxication is very small about 94-184ng (29).

Therefore, the purpose of such investigation is to recorded the occurrence of *S. aureus* in some different half-cooked chicken and also detection and typing of their enterotoxins.

MATERIAL AND METHODS

Samples

A total of 60 samples from half-cooked chicken products (chicken wings (20), chicken drum sticks (20) and chicken pane (20) were collected from different supermarkets in Menofia governorate .

Preparation of the samples

Ten grams of each meat product sample only without bread were put into the stomacher bag , to which 90 ml of sterile physiological saline (0.9%) were aseptically added to provide dilution of 1/10, then the content of the bag was stomached for 60 sec, using the stomacher, then 10 fold serial dilutions were prepared.

Determination of *S. aureus* count

S. aureus count/gram was determined using surface plating technique (30). One tenth ml from each of the previously prepared decimal dilutions was transferred onto duplicate plates of Baird-parker media, supplemented with egg yolk tellurite and incubated at 37°C for 24-48 hrs. Then the black and shiny colonies greater than 1mm in diameter with narrow white margin surrounded by clear zone extending into opaque medium were counted and recorded.

Isolation and Identification of *S. aureus*

Suspected colonies *S. aureus* were subcultured on slants of sheep blood agar and brain heart infusion agar (Oxoid) (30) and incubated at 37°C for 24hrs. Isolated purified strains were identified microscopically, culturally and biochemically (31,32) as follow

Identification and characterization of *S. aureus*

The isolates were identified by using the following tests

Catalase test

Loopfuls of organism were suspended into drops of 3% H₂O₂ (V/V) on a glass slide to detect the presence of catalase enzyme (+ve reaction).

Coagulase test

Coagulation of rabbit plasma was tested with tube method because of its high sensitivity to presence of coagulase enzyme. A single colony from the 24 hours growth on sheep blood agar was suspended in a 1:10 dilution of fresh rabbit plasma in physiological saline. Coagulation was evaluated after 4 hours incubation at 37°C and additional evaluation after 20 hours incubation at room temperature.

Mannitol fermentation activity of *S. aureus* on Mannitol salt agar (aerobic Mannitol fermentation) while (glucose was anaerobic fermentation).

Production of haemolysin

The production of alpha and beta haemolysins was determined by using washed human erythrocytes which were added to sterile nutrient agar that had been melted and cooled at 50°C, to obtain a final concentration of 3%.

The interpretation of haemolysin production test was made after 48 hours incubation at 37°C.

Crystal violet agar growth type

Crystal violet agar plates were prepared by adding 6 or 8 ug/ml of crystal violet to trypose agar (oxoid). Few colonies of the isolates were spot inoculated on plates of both concentrations, incubated at 37°C, and examined after 24 hours (31).

Production of staphylococcal enterotoxins from isolated strains

The isolated strains of *S. aureus* were examined for their ability to produce enterotoxins using Sac culture method (33).

Detection and typing of enterotoxins

Detection and typing of enterotoxins were done using serological test by reversed passive latex agglutination technique using Oxoid SET-RPLA (kit used for the detection of staphylococcal enterotoxins A,B,C and D) (34,35).

RESULTS

Table 1. Incidence of *S. aureus* isolated from half cooked chicken product samples (n =20)

Half –cooked chicken products	No. of positive sample	
	No.	%
Chicken wings	8	40
Chicken drumsticks	8	40
Chicken pane	5	25
60	21	35

Table 2. Statistical results of total *Staphylococcal aureus* count of the examined half cooked chicken product samples (n=20)

Half –cooked chicken products	Minimum	Maximum	Mean ± SE
Chicken wings	<10 ²	1.9X10 ²	0.4X10 ² ± 0.15X10 ²
Chicken drumsticks	<10 ²	1.4X10 ²	0.28X10 ² ± 0.2X10 ²
Chicken pane	< 10 ²	1.3X10 ²	0.19X10 ² ± 0.2X10 ²

Table 3. Percentage of accepted and rejected half-cooked chicken product samples

Half –cooked chicken products	Accepted samples		Rejected samples	
	No.	%	No.	%
Chicken wings (20)	12	60	8	40
Chicken drumsticks (20)	12	60	8	40
Chicken pane (20)	15	75	5	25
Total (60)	39	65	21	35

Table 4. Pigment produced from isolated *S. aureus* from different samples on blood agar

Samples	Colour of colony		
	White	Creamy	Golden Yellow
Chicken wings (8)	-	2(25)	6(75)
Chicken drumsticks (8)	-	2(25)	6(75)
Chicken pane (5)	1(20%)	1(20%)	3(60%)
Total (21)	1(4.76%)	5(23.80)	15(71.42%)

Biochemical characterization of *S. aureus* found that all the isolated strains were catalase, coagulase test and Mannitol fermentation test positive, with an incidence 100%

Table 5. Results of hemolytic activity of isolated *S. aureus*

Hemolytic activity on	Total	Type of samples		
		Chicken wing(8)	Chicken drumstick(8)	Chicken pane(8)
Sheep Blood	21(100)	8(100)	8(100)	5(100)
Human Blood	2 (9.52)	1(12.5)	1(12.5)	-

Table 6. Pigment production of *S. aureus* isolated from different samples on crystal violet medium (% according to total No. of samples)

Samples	Yellow	Violet
Chicken wing(8)	-	8(100)
Chicken drumstick(8)	1(12.5%)	7(87.5)
Chicken pane(5)	2 (40%)	3(60%)
Total (21)	3 (14.28%)	18(85.71%)

Table 7. Distribution of enterotoxigenic *S. aureus* isolates from half-cooked chicken products

Chicken products	No. of isolates of <i>S. aureus</i>	No. of enterotoxigenic strains type(A)	
Chicken wings	8	4	50%
Chicken drumsticks	8	3	37.5%
Chicken pane	5	-	-
Total	21	7	33.3%

DISCUSSION

As the effect of microorganisms on human health has been reported, the present study was performed to give information about the presence of pathogenic *Staphylococcus* in traditional fast foods and fast foods from different supermarkets, that important to human and to discuss their role in the food poisoning and also the causation of many human diseases.

Staphylococcus food poisoning bacteria of greatest important to human pathology are the most common causes of human infection and extensively widespread in the environment using fast foods. Our results are in agreement

with the above studies and are supported by many researches (36).

Bacteriological examination revealed that 21 samples of (60) (chicken wings, chicken drumstick and chicken pane) were positive bacteriologically for *S. aureus* organism with an incidence (35%).

It is obvious from such incidence that the level of contamination by *S. aureus* in the examined samples was relatively high. In this respect isolated *S. aureus* (32-36%) from cooked food that *S. aureus* strains isolated from human resembles those cooked food (37) while, It has been stated that stated the outbreaks of *S. aureus* food poisoning are most often associated with processed meats and

most outbreaks resulted from the combined effects of contamination of the food with *S. aureus*, often through unsanitary handling and holding the food at the wrong temperature thus allowing growth and synthesis of enterotoxins by the pathogen (37). The cooked foods (20%), *S. aureus* was found in both cooks and suspect foods (38). It was also found high percentage of the cooks associated with food borne incidents had no health certificates.

Regarding the incidence of *S. aureus* recorded in Table 1, the obtained results were less than those obtained by different investigators (38,39) and higher than those obtained by others (40-44). On other hand *S. aureus* cannot be counted from ready to eat meat sandwiches (45-47). Suggested that the high level of contamination with *S. aureus* resulted from cross-contamination reflecting excessive hand contact with food stuffs (48).

As shown in Table 2 the mean values of *S. aureus* count in the examined chicken wings samples were $0.4 \times 10^2 \pm 0.15 \times 10^2$, chicken drumsticks $0.28 \times 10^2 \pm 0.2 \times 10^2$ and chicken pane $0.19 \times 10^2 \pm 0.2 \times 10^2$. This results agree with (41) who isolated *S. aureus* from food ready eat with a level of 10^2 cfu/G while (49) detected *S. aureus* in fast food in count $< 10^2$ to 10^4 cfu/gm. The growth of *S. aureus* in cooked or sterilized food better than in raw foods (50).

Outbreaks could be resulted when processed or cooked meat has been improperly handled and mistreated in food processing plants and/or catering and food service establishment (3). The increase in the bacteria count in meat meals during distribution in aluminum dishes could be attributed to the holding temperature. (51).

The presence of *S. aureus* may be due to contamination of food, equipments, human and animals are primary reservoirs (52).

The maximum levels of *S. aureus* of both ready to eat chicken wings, drumsticks and pane were 1.9×10^2 and 1.4×10^2 cell/g respectively, these values were exceed the permissible limit (10^2 cell/g) as recommended

EOSQC (Egyptian organization specification and Quality Control) (53) these samples were rejected.

Table 3 revealed the percentage of accepted and rejected half cooked chicken samples. It was found that percentage of accepted samples count were 12(60)% in chicken wings, chicken drumsticks (12)60% and chicken pane (15)75% and the rejected percentage was 8(40%),8(40%)and 5(25)% from chicken wings, chicken drumsticks and chicken pane respectively, this results agree with that of Edris et al. (54).

The handling and preparation of food when in correct manner can reduce the level of microbial contamination thereby enhancing shelf life and he added that, certain foods carry a higher risk of microbial contamination than other (55).

It is important to handle food in such a way that the microorganisms present do not have a chance to multiply and to prevent food from becoming contaminated with other microorganisms (56).

The characterization of isolated *S. aureus* were gram positive, non motile appear as grapes produce golden yellow pigment 15(71.42%) as shown in Table 4, this results near from that recorded which shoed that of 175 strains of *S. aureus*(66.4%) produced golden yellow pigment (57).

On the other hand the biochemical characterization of isolated *S. aureus* were catalase positive, ferment Mannitol and appeared as yellow colour, also coagulase positive .

The coagulation of rabbit plasma by all isolates of *S. aureus* gave further support to the significance of coagulase test with the efficiency of citrated rabbit plasma in species identity of staphylococci.

The high specificity and sensitivity of the coagulase test (58) has been made it a standard method for the identification of *S. aureus* strains. Only half of the *S. aureus* isolates are positive in this test after a 4 hours incubation, and an overnight incubation

is necessary to obtain reliable results (59). This long incubation time represents an important drawback for diagnostic applications, and quicker methods would be preferable.

Regarding to hemolytic activity of *S. aureus* isolates on human and sheep blood agar, as shown in Table 5, was clear that 21 isolates (100%) had hemolytic activity on sheep blood agar. Meanwhile 2 isolates only (9.52%) had hemolytic activity on human blood agar. Testing for the presence and type of hemolysis on blood agar plates represents a first simple and rapid method (59).

Other test like the production of pigmentation crystal violet as shown in Table 6, showed the ability of *S. aureus* to produce violet colour with an incidence of 18 (85.71). Most of *S. aureus* produce violet colour on crystal violet medium (60).

In food microbiology, coagulase positive and haemolytic activity on sheep blood agar, *S. aureus* are considered to be potentially enterotoxigenic as shown in Table 7, the results illustrated that the incidence of enterotoxin A produced by *S. aureus* in chicken wings were 4 (50%), in chicken drumsticks 3 (37.5%). These Enterotoxins were from ready-to-eat beef *S. aureus* isolates (43.3%) (61). Beef isolates of *S. aureus* were the most frequently toxic than other food products and may pose a health hazard with high incidence of enterotoxin type A (61). *S. aureus* causes food poisoning by the production of one or more heat stable extracellular toxins, responsible for the symptoms of disease, time of onset and severity of symptoms depend on the amount of toxin consumed and individual susceptibility (62). The enterotoxins produced at detectable levels (>0.1 ng/mg) in foods occurs only when growth reaches approximately 10^6 cfu/gm (23).

The summarized results recorded in Table 7 nearly agree with those obtained recorded author (42,61,63-65). Lower findings were achieved (39,44). It was found also that enterotoxin type A (SEA) is the most frequently Staphylococcal enterotoxin A (SEA) being the most responsible for the majority of Staphylococcus food poisoning

(21,29,66). However, there is no relation between the enterotoxigenicity and the other biochemical characteristics (67). On the other hand, the direct extraction of Staphylococcal enterotoxins from the food samples revealed negative results (not detectable). The enterotoxins causing food poisoning are produced by about one-third (1/3) of coagulase positive strains of *S. aureus*. (28).

Considering the importance and public health hazard of *S. aureus* organism recovered from fast food, reported that preparing and served food to the public is a very important obligation that can only be fulfilled if everyone in the establishment understand food hygiene, applying sanitary measures at every stage of the operation (68-71). Furthermore, cooked food should not be touched by hands or by equipments that have come in contact with raw food equipments that have come in contact with raw meat, raw products should be separated from cooked products to avoid cross-contamination (3).

It is important to handle food in such a way that the microorganisms present do not have a chance to multiply and to prevent food from becoming contaminated with other microorganisms by: (1) Wash and dry hands before preparing any food and after handling raw foods (meat, poultry, vegetables or fruits). (2) Ensure that food preparation areas and equipment are clean. (3) Protect kitchen areas or restaurants and food from insects, pests and other animals. (4) People with gastrointestinal illness, such as vomiting or diarrhoea, should not handle food intended for consumption by others (56).

Staphylococcal food poisoning is caused by the ingestion of food containing pre-formed toxins secreted by the Bacteria. These are known as staphylococcal enterotoxins, and serologically distinct types (A, B, C₁, C₂, C₃, D, E).

The toxins of *S. aureus* are produced during active growth of the bacteria in foods, often during storage. Each toxin is a single polypeptide chain which is resistant to many proteolytic enzymes and generally withstands

boiling for up to 30 minutes. The type of enterotoxin most frequently involved in food poisoning is staphylococcal enterotoxin A (SEA) and is the most important cause of food poisoning outbreaks (72).

S. aureus food poisoning is one of the most common types of food borne illness and results from the ingestion of enterotoxins produced during growth of enterotoxigenic strains of *S. aureus* in food, hence the detection of preformed staphylococcal enterotoxins (SETs) in food is very important in epidemiological studies of outbreaks of food poisoning (73).

Generally, approximately 15-20% of *S. aureus* isolated from humans are enterotoxigenic, this explains the importance of the food-handler in transmission of this organism. Food handlers is a significant factor in cases of *S. aureus* food poisoning (74).

Staphylococcal food poisoning symptoms are characterized by nausea, vomiting, abdominal pain and prostration often with diarrhea but without fever, approximately one to six hours after ingestion of contaminated food.

One of the complicating features associated with staphylococcal food poisoning is that when the organisms grow in foods they produce no pronounced odour or taste; and a food having hundreds of millions of staphylococci per gram may be taste, smell and appear to be little different from that in which none of these organisms have grown (75).

The need for good hygienic practices, proper handling and storage in clean environment and at refrigeration temperature cannot be over emphasized to ensure good quality and safe salads. Prevention of mycotoxins must become a cooperative effort on the part of all involved in food production.

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المخلص العربي

مدى تواجد سموم الميكروب العنقودي الذهبي في الدجاج النصف مطهى

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معمل فرعى شبين الكوم و قسم البكتريولوجى بمعهد بحوث صحة الحيوان بالدقى

اجريت هذه الدراسة لتحديد مدى تواجد ميكروب العنقودي الذهبي كأحد مسببات التسمم الغذائى فى عينات الدجاج النصف مطهى المجمعة من الاسواق بمحافظة المنوفية (أجنحة الدجاج- دبابيس الدجاج- بانيه الدجاج) وبالتالي تحديد مدى قدرته على افراز السموم حيث دلت النتائج على ان نسبة تواجده بلغت ٤٠% ، ٤٠% و ٢٥% بمتوسط ٤٠% ± ١٥% ، ٢٨% ، ٢٠% ± ١٠% ، ١٩% و ٢٠% ± ١٠% ، ٢٠% لكل من أجنحة الدجاج و دبابيس الدجاج و بانيه الدجاج على التوالى .

و كانت نسبة العينات التى لم تتطابق مع المواصفة القياسية المصرية ٤٠% و ٤٠% و ٢٥% لهذه المنتجات على التوالى. كما اظهر المعزول من هذا الميكروب ايجابية بلغت ١٠٠% لاختبارات , Catalase , Mannitol fermentation , Coagulase و نسبة ١٠٠% من المعزول اظهرت قدرة على تكسير كرات دم الاغنام و ٩,٥٢% منه لها قدرة على تكسير دم الانسان و كان معدل قدرة الميكروب على افراز enterotoxin A بلغت ٥٠% و ٣٧,٥% فى منتجات اجنحة الدجاج و دبابيس الدجاج على التوالى.