

Bacteriological Status Of Some Egyptian Chicken Meat Products

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ABSTRACT

Twenty five (25) samples from each type of selected chicken meat product (filets, nuggets, shawrma and kofta) were collected from different supermarkets in Egypt and subjected to bacteriological examination. The results revealed that the mean value of total aerobic and *Staphylococcus aureus* bacterial count was the highest in Kofta (2.5×10^6 , 5.4×10^4 respectively), enterobacteriaceae while the highest count of enterobacteriaceae recorded in showarma (6.2×10^3).

Regarding the isolation percentages of which were 40%, 44%, 60% and 68% in chicken filets, nuggets, shawrma and kofta, respectively. Meanwhile the frequency distribution of *Salmonellae spp.* were 4%, 12% and 16% for nuggets, shawrma and kofta respectively. *Cl. perfringens* were 12%, 16%, 24% and 32% respectively, *Cl. perfringens* typing revealed that type (A) was the most predominant than type (D)..

Antibiogram test revealed *Staph. aureus* were highly sensitive to tetracycline, erythromycin and penicillin G, while all tested isolates of *C. perfringens* were highly sensitive to chloramphenicol, tetracycline and penicillin G. Also *Enterobacteriaceae spp.* were highly sensitive to tetracycline, ampicillin, chloramphenicol and oxytetracycline, While *Salmonella spp.* were highly sensitive to chloramphenicol and ampicillin.

PCR is a rapid direct diagnostic assay of *Staph. aureus* enterotoxins SEA, SEE & SED genes of enterotoxins could be amplified .

Recommendations for production of high quality chicken meat product were discussed.

INTRODUCTION

During the last decade, the demand of meat and chicken ready to eat products has increased in Egyptian food markets and receive a real consumer preferability because they represent quick easily prepared meat meals and solve the problem of shortage in fresh meat of high price (1).

Chicken meat constitutes an excellent source of high quality, easily prepared and digested protein of the first class which contains all essential amino acids, high proportion of unsaturated fatty acids and less cholesterol, also it characterized by versatility in menu planning, (2,3).

The processing, handling, distribution and storage as well as marketing of most chicken products constitute a public health hazards either due to the presence of spoilage bacteria responsible for unfavorable changes or

pathogenic bacteria leading to harmful effects as food infection or intoxication among consumers (4). Such contamination may render the product of inferior quality or even unfit for human consumption (5, 6). Heavy bacterial loads enter the processing operations with the raw material or living birds and these bacteria tend to disseminated throughout the plant during processing (7).

During processing most of the microflora changes from in general gram positive rods and micrococci to most frequently gram negative bacteria in final products including *Pseudomonas*, *Actinobacter* and *Enterobacteriaceae*, further more pathogens as *Salmonellae*, *Campylobacter*, *Clostridium perfringens* and *Staphylococcus aureus* also contaminate the final product (8,9).

Staph. aureus is the most prevalent contagious pathogens, which rapidly and easily

transmitted, as well as it cause a zoonotic disease which transmitted to human being, due to the permanent interchange of *Staph. aureus* from humans to animals and the reverse occurs as a result of the close ecological relations between man, environment and animal. This is the characteristic of the modern way of production in the food sector (10). Also *Staph. aureus* produces powerful enterotoxins which causes human food poisoning (11,12).

Broilers are considerable reservoir for *Salmonella* infections in man due to the ability of salmonella to proliferate in the gastrointestinal tract of chickens (13). The contamination of food by *Coliforms* leads to clinical signs including fever, nausea, vomiting, diarrhea and abdominal cramps (14). While consumption of food containing spores of anaerobic bacteria grown in the intestine and released toxins caused illness in human with high mortality rate (15,16).

It is cleared that the global importance of food safety is not fully appreciated by many public health authorities despite to the contact increase in the prevalence of food borne illness. In Egypt, it is considered that, the main cause of high mortality rate among infants and young children up to 400 deaths and 5 million illness each year is caused by contaminated meat and poultry products (17,18).

The hygienic condition of chicken meat product can be assured by the application of hazard analysis critical control point (HACCP) which is a well accepted systematic program for identification and control of microbiological hazards is associated with poultry processing (19,20).

Isolation and identification of *Staph. aureus* enterotoxins genes was time consuming and the cultures need to be handled with care because of the zoonotic potential. The biological activity of toxins remains unchanged even after thermal processing of food (21). Using PCR assay as an alternative method in routine diagnosis for rapid, sensitive, and specific simultaneous detection of *Staph. aureus* enterotoxins genes in clinical isolates (22,23).

The aim of the present work was to study the bacteriological profile of some Egyptian chicken meat products with special reference to food poisoning. In addition, using polymerase chain reaction (PCR) test to diagnosis of *staph aureus* enterotoxins directly in chicken meat product and antibiogram of bacterial isolates.

MATERIAL AND METHODS

Samples

Twenty five samples from each of selected chicken meat products (filets, nuggets, shawrma and kofta) were purchased from different supermarkets in Egypt. The collected samples were transferred to the laboratory in an ice bag without delay for bacteriological examination APHA (24).

Bacteriological examination

The collected samples were prepared according to the technique recommended by ICMSF (25) for aerobic and anaerobic bacteriological examination.

A. Bacterial count

1-Aerobic count

The total bacterial count /gm was done according to APHA (24) the standard plate count agar in duplicate plates and incubation was done at 30°C for 48 hour.

2-Staphylococcus aureus count

The technique recommended by FAO (26) was applied by using Baird parker's agar, the plates were incubated at 37°C for 24 hours, the presumptive colonies were tested for coagulase activity.

3- Enterobacteriaceae count

The technique recommended by APHA (24) using violet red bile dextrose agar at 45°C was carried out the plates were incubated at 37°C for 24 -48 hours.

B. Isolation of some food pathogens

1-Aerobic isolation

Cultural methods were made from the collected samples by sterilizing the exposed

surface of the sample and cutting down small piece from the deeper parts, and inoculated directly into nutrient broth, and aerobically incubated at 37°C for 24 hours, then subcultured onto nutrient agar, blood agar, MacConky bile salt lactose agar, crystal violet blood agar and Baird parker's agar media for isolation of *Staph. aureus*, and Eosin Methylene blue agar media for isolation of Enterobacteriaceae, all inoculated plates were incubated aerobically at 37°C for 24-48 hours. Suspected colonies onto the surface of these media were identified by studying characters of the colonies as well as Gram's stain, then identified morphologically according to the previously method described by (27, 28).

One single colony showed typical colonial appearance and morphological characters was picked up and streaked into semisolid agar media and incubated at 37°C for 24 hours, for further identification.

The pure colonies were biochemically identified (29,30). The Gram negative bacteria included Enterobacteriaceae family were typed (31).

2-Isolation of *Clostridia perfringens*

A loopful from a small piece of the deeper parts of the sample was inoculated into tubes of freshly prepared Robertson's cooked meat medium at 37°C for 24 hours. Loopful from each tubes was streaked onto the surface of 10% sheep blood agar, then incubated anaerobically at 37°C for 24 hours. The plates were examined for characteristic colonies of genus *Clostridium*. Subcultures from suspected colonies in cooked meat broth were made for further biochemical identification (32-34).

Typing of *C.perfringens* isolates was done by the intradermal inoculation test in guinea pigs (35).

Susceptibility of isolates to chemotherapeutic agents

Antibiogram of antibiotic sensitivity test on the pure subculture of the prevalent isolates was carried out to detect the drug of choice

against different types of bacteria by using disc agar diffusion methods (33, 36).

Extraction of *Staph. aureus* enterotoxins DNA and PCR determination

Isolated *Staph. aureus* strains were incubated overnight in 10 ml brain heart infusion broth (Oxoid), centrifuged at 500g, for 15 min the sediment was discarded and supernant was subject to toxin determination and resuspended in 0.5 ml TE buffer (10 mM Tris, 1 mM EDTA - pH 8).

Total cellular DNA was extracted using Qiagen DNA extraction kit (Qiagen, Germany) according to manufacturer's protocol for gram-positive bacteria. The extracted DNA from chicken product samples (4 positive samples for *Staph aureus*) was dissolved in 25 µl sterile distilled water and stored at -20°C until further use.

Multiplex PCR was performed on the extracted DNA of the isolated bacteria to clarify the presence of enterotoxins genes type A, B, C, D and E as previously described in detail (37).

Primers for *Staph aureus* enterotoxins

Specific oligonucleotide multiplex primer assay as shown in Table 1 (synthesized by MWG-Biotech AG, Holle & Huttner GmbH, Germany), designated (37) was used for rapid diagnosis and typing of staph aureus enterotoxins.

Staph. aureus enterotoxins DNA amplification

The PCR was performed according to the previously described technique (37), in a touchdown thermocycler in a total reaction volume of 30 ul containing 2.5µl of extracted DNA, 1 µl of each primer (10 pmol/µl), 0.6µl of deoxynucleoside triphosphate (10 mmol/L), 3 µl of 10 X thermophilic buffer (Promega), 1.8µl of MgCl₂ (25 mmol/L), 0.1 µl of Taq DNA polymerase (5 U/µl), and complete the reaction volume using distilled water in 0.2-ml reaction tube. The presence of PCR products was determined by electrophoresis of 10 µl of the DNA product in a 1.5 % agarose gel with 1

X TAE buffer (40 mM Tris-HCl, 1 mM EDTA/L, 1.14 ml/L glacial acetic acid, pH 7.8) at a voltage of 4 volts /cm and stained with 0.5 mg/ml ethidium bromide and the Fluorescent bands were visualized with a UV transilluminator and photographed. A 100-bp DNA ladder (Gibco BRL) was used as a

molecular marker. Amplification was obtained with 35 cycles. Each cycle involved initial denaturation at 93°C for 3 minutes, denaturation at 92°C for 1 minutes, annealing at 52°C for 1 minutes , and extension at 72°C for 1 minutes. The final extension was performed at 72°C for 7 minutes .

Table 1. Oligonucleotide primers used for amplification of various type of *staph. aureus* enterotoxins

Genes	Oligonucleotide sequence	Size (bp)
Se A F Se A R	(5'-TTGGAAACGGTAAAACGAA-3') (5' GAACCTCCCATCAAAAACA -3)	120
Se B F Se B R	(5'- TCGCATCAAACGACAAACG -3') (5'- GCAGGTACTCTATAAGTGCC -3')	478
Se CF Se C R	(5'- GACATAAAAGCTAGGAATTT -3') (5'- AAATCGGATTAACATTATCC -3')	257
Se D F Se D R	(5'- CTAGTTGGTAATATCTCCT -3') (5'-TAATGCTATATCTTATAGGG -3')	317
Se EF Se E R	(5'-TAGATAAAGTTAAAACAAGC -3') (5'- TAACTTACCGTGGACCCTTC -3')	170

The presence of PCR products was determined by electrophoresis of 10 µl of the DNA product in a 1.5 % agarose gel with 1 X TAE buffer (40 mM Tris-HCl, 1 mM EDTA/L, 1.14 ml/L glacial acetic acid, pH 7.8) at a voltage of 4 volts /cm and stained with 0.5 mg/ml ethidium bromide and the Fluorescent bands were visualized with a UV transilluminator and photographed. A 100-bp DNA ladder (Gibco BRL) was used as a molecular marker.

RESULTS AND DISCUSSION

Unfortunately, chicken meat products offer ideal medium for microbial growth as they are highly nutritious, have a favorable pH and are normally lightly salted or not salted at all (38). The results in Table 2, illustrates the microbiological profile of some Egyptian chicken products, several previous (18, 39, 40) illustrated nearly the same quality . In addition to pathogenic bacteria, special attention in the hygienic production and storage of chicken meat

is paid to the total count of aerobic bacteria (Table 2) which are considered indicators of microbiological quality (12,39).

If the total count of aerobic bacteria is high, the risk of spoilage consequently is high (41).

Similar studies were carried out in South Africa (42,43). Because of the inability of some enteric pathogens to ferment lactose, enumeration of all enterobacteriaceae family instead of only enumerating coliforms or fecal coliforms in food is advocated as this family included many species that are enteric pathogens and the enumeration of the whole group could be used as a good indicator of the level of sanitation, fecal contamination and possible presence of enteric pathogens (44).

Mean total staphylococcal counts which considered as an important indicator for the hygienic condition of food (45) is depicted in Table 2.

Poultry and poultry products ranks first or second in food associated with diseases in most

of the countries all over the world which in the USA ranked third of the reported food-borne diseases out breaks (46). Epidemiological reports suggest that poultry meat is still the primary cause of human food poisoning (47), because the microflora of poultry itransferred from the primary production sites to the production lines (9).

Processed raw poultry meat naturally harbors bacteria. most of them are responsible for the spoilage of poultry meat. However, poultry products can harbor food borne pathogens from which *salmonella*, *C. perfringens* and *Staph. aureus* (48).

Table 2. Mean counts of bacteria isolated from some Egyptian chicken meat product

Sample	Aerobic plate count	Enterobacteriaceae count	<i>Staphylococcus aureus</i> count
Filets	1.3×10^6	2.6×10	2.0×10^3
Nuggets	7.1×10^6	5.0×10^2	3.1×10^3
Shawrma	5.4×10^7	6.2×10^3	1.7×10^4
Kofta	2.5×10^8	3.3×10^3	5.4×10^4

The results in Tables 3,4, showed the frequency distribution of *C. perfringens* and typing which revealed that type A was the most predominant than type D. Previous study has been found that type A superior to type D which act as the main cause of food poisoning in man. *C. perfringens* is highly proteolytic microorganism and considered as one of the major cause of spoilage of meat and poultry products, the strains implicated in outbreaks of food poisoning particularly of products have spores surviving heating at 100°C (ICMSF, 45).

Salmonellae is one of the microorganisms mostly frequently associated with outbreaks of illness spread by food. Meat in general and poultry in particular are the commonest source of food poisoning by salmonellae (49). It is failed to be detect *salmonellae spp* in samples of

chicken filets. Similar results were reported (40,50,51).

The annual cost of medical treatment of salmonellosis, in addition to loss of productivity, imposes a significant financial burden on many countries (52).

Staph. aureus is one of the most specific microorganisms responsible for food poisoning among human being due to production of enterotoxins (12). Contamination of food with *Staph. aureus* is important in the evaluation of safety and hygienic quality of chicken meat powerful and investigation of the aetiology of food poisoning. Food poisoning with *C. perfringens* and *Staph. aureus* enterotoxins can occur when chicken meat contaminated by large number of bacteria during processing and preserved at temperature higher than 14°C (53,54).

Table 3. Incidence of food borne pathogens isolated from some Egyptian chicken meat product

Samples	<i>C. Perfringens</i>		<i>Salmonellae</i>		<i>Staph. aureus</i>	
	No. of samples	%	No. of samples	%	No. of samples	%
Filets	3	12	-	-	10	40
Nuggets	4	16	1	4	11	44
Shawrma	6	24	3	12	15	60
Kofta	8	32	4	16	17	68

Table 4. Typing of *C. perfringens* isolated from some Egyptian chicken meat product

Samples	Toxigenic isolates				Total
	Type A		Type D		
	No. of samples	%	No. of samples	%	
Filets	2	66.7	1	33.3	3
Nuggets	3	75	1	25	4
Shawrma	4	66.7	2	33.2	6
Kofta	6	75	2	25	8

The wide use of chemotherapeutic drugs may produce a new resistant strain for this reason the first steps in treatment of aerobic and anaerobic infection is the use of appropriate chemotherapeutic agent. So the kind of antibiotic should be selected on the basis of its sensitivity which could be detected by laboratory examinations as shown in Table 5. *Staph. aureus* were highly sensitive to tetracycline, erythromycin and penicillin G, while most of these isolated were highly resistant to chloramphenicol, ampicillin and nitrofurantoin which support previous study (55). While all tested isolates of *C. perfringens* were highly sensitive to chloramphenicol, tetracycline and penicillin G while most of these isolates were highly resistant to neomycin, erythromycin and ampicillin. Similar sensitivity of the organism has been reported from cases bovine mastitis (56).

Enterobacteriaceae spp. were highly sensitive to tetracycline, ampicillin, chloramphenicol, and oxytetracycline while highly resistant to erythromycin, nitrofurantoin and moderate resistant to neomycin. While *Salmonella* spp. were highly sensitive to chloramphenicol and ampicillin and has moderate resistant to penicillin G, tetracycline, oxytetracycline and neomycin.

The result of *Staph. aureus* investigation enforced us for advanced study us for further by application of PCR technique where rapid pathogen identification is vital to the food industry and increased public health protection. PCR is highly sensitive specific and rapid

method and substitute biochemical and serological characterization of the pathogen (57). PCR showed the same results of traditional methods for *Staph. aureus* isolation from samples.

Fig.1, show four chicken product samples representative for positive *Staph. aureus* isolates selected and subjected to PCR enterotoxins analysis. The specificity of the oligonucleotide primer was confirmed by the positive amplification of Lane1SEA & D (120bp and 257bp) fragments respectively, while Lane 2 and 4 SEE (170bp) fragments and Lane 3 SEA (120bp) fragments from the extracted DNA of *Staph. aureus*.

These results suggest that the PCR assay is a rapid and extremely sensitive procedure, for the detection of enterotoxins genes in clinical isolates of *Staph. aureus* (22,23).

Results of our study are indicative for contamination and inadequate hygienic conditions in the production and processing of chicken meat products.

Finally to improve the hygienic quality of Egyptian chicken meat products to be safe for human consumption the contamination must be reduced by implementing satisfactory manufacturing practices and effectively training plant workers in hygiene, safety and quality assurance; application of strict hygienic measures during handling preparation and serving the products.

Table 5. Antibiogram of antibiotic sensitivity to the prevalent isolates

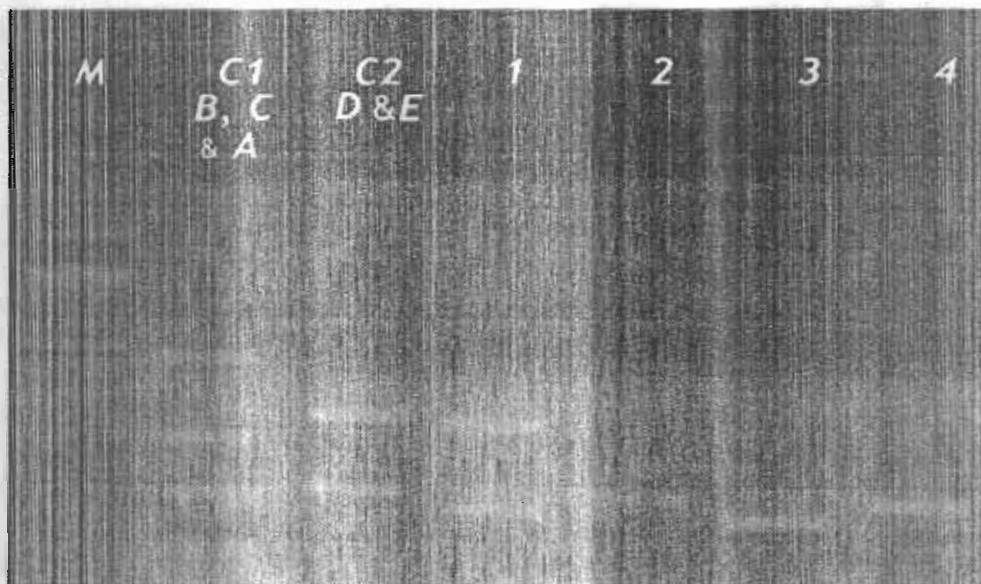
Antimicrobial agent	<i>Staph. aureus</i> N=10			<i>C. perfringes</i> N=8			<i>Enterobacteriace</i> N=8			<i>Salmonellae</i> N=7		
	R	I	S	R	I	S	R	I	S	R	I	S
Chloramphenicol	8	2	-	0	0	8	1	1	6	1	-	6
	80	20	-	0	0	100	12.5	12.5	75	14.2	-	83
Tetracycline	1	1	8	0	0	8	-	1	7	3	2	3
	10	10	80	0	0	100	-	12.5	87.5	42.8	28.5	42.8
Penicillin G	2	1	7	0	0	8	2	2	4	1	1	4
	20	10	70	0	0	100	25	25	50	14.2	14.2	57.1
Nitrofurantion	6	2	2	4	2	3	4	1	3	2	1	4
	60	20	20	50	25	37.5	50	12.5	37.5	28.5	14.2	57.1
Oxytetracyclin	2	2	6	3	2	3	1	1	6	3	3	1
	20	20	60	37.5	25	37.5	12.5	12.5	75	42.8	42.8	14.2
Neomycin	4	2	4	5	2	2	3	1	5	2	2	3
	40	20	40	62.5	25	25	37.5	12.5	62.5	28.5	28.5	42.8
Erythromycin	1	1	8	6	2	0	4	2	3	1	4	2
	10	10	80	75	25	0	50	25	37.5	14.2	57.1	28.5
Ampicillin	8	1	2	5	2	1	2	-	7	1	1	5
	80	10	20	62.5	25	12.5	25	-	87.5	14.2	14.2	71

R. Resistant

I. Intermediate

S. Sensitive

N= No. of isolates

Fig.1. Gel analysis of PCR- amplified *Staph. aureus* enterotoxins gene sequences

M : 100bp marker. C1: Control positive for SEB,C & A. C2: Control positive for SED & E, Lane 1 SEA & D (120bp and 257bp), Lane 2 and 4 SEE (170bp), Lane 3 SEA (120bp)

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الملخص العربي

الحالة البكتريولوجية لبعض منتجات لحوم الدواجن المصرية

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أجريت هذه الدراسة على (١٠٠) عينة من أربع أنواع (كل نوع ٢٥ عينة) مختلفة من منتجات لحوم الدجاج المصنعة محليا؛ والتي تم تجميعها من السوبر ماركت المختلفة بمصر؛ وكانت أنواع هذه المنتجات هي صدور الفيلية وقطع الدجاج (ناجت) وشاورمة الدجاج وكفتة الدجاج وذلك للفحص البكتريولوجي.

أثبتت التحاليل البكتريولوجية أن متوسط العدد الكلي للبكتيريا الهوائية كان 1.3×10^6 , 7.1×10^6 , 5.4×10^7 and 2.5×10^8 من صدور الفيلية وقطع الدجاج (ناجت) وشاورمة الدجاج وكفتة الدجاج علي الترتيب. بينما كان العدد الكلي للأنتريوباكترياسي 2.6×10^3 , 5.1×10^2 , 6.2×10^3 and 3.3×10^3 بينما كان العدد الكلي لميكروب المكور الذهبي العنقودي 2×10^3 , 3.1×10^3 , 1.7×10^4 and 5.4×10^4 للمنتجات السابق ذكرها علي الترتيب.

وقد تم عزل الميكروب الذهبي العنقودي بنسبة 40% , 44% , 60% and 68% من صدور الفيلية وقطع الدجاج (ناجت) وشاورمة الدجاج وكفتة الدجاج علي الترتيب. بينما تم عزل الكلوسترديم بيرفرينجينز بنسبة 12% , 16% , 24% and 32% من صدور الفيلية وقطع الدجاج (ناجت) وشاورمة الدجاج وكفتة الدجاج علي الترتيب وبتصنيف الكلوسترديم بيرفرينجينز وجد انها نوعين هما (أ) و(د) فقط. كما تم عزل السالمونيلا بنسبة 4% , 12% and 16% من قطع الدجاج (ناجت) وشاورمة الدجاج وكفتة الدجاج علي الترتيب.

وتم أيضا دراسة مدى حساسية العترات البكتيرية المعزولة للمضادات الحيوية فكانت معظم العترات المعزولة من الميكروب المكور الذهبي العنقودي حساسية للتراسيكلين والأريثروميسين والبنسيلين ولكن العترات المعزولة من الكلوسترديم بيرفرينجينز كانت اكثر حساسية للكلورامفينيكول والتراسيكلين والبنسيلين؛ بينما العترات المعزولة من الأنتيروبيكتريسي كانت اكثر حساسية للتراسيكلين والأميسيلين والكلورامفينيكول والأوكسي تتراسيكلين بعكس السالمونيلا كانت اكثر حساسية للكلورامفينيكول والأميسيلين

وقد تم استخدام اختبار تفاعل البلمرة المتسلسل كطريقة حديثة وسريعة لتشخيص الميكروب المكور العنقودي الذهبي المسبب للتسمم الغذائي باستخدام البريمر المخصص له. وقد أثبتت النتائج سرعة ودقة اختبار تفاعل البلمرة المتسلسل في التشخيص المعملية السريع للميكروب المكور العنقودي الذهبي العنقودي مع تحديد نوع الجين المسبب للتسمم الغذائي من منتجات لحوم الدجاج مباشرة وهما SEA, SEE & SED (120bp, 170bp and 257bp) وقد أثبتت النتائج حساسية وأهمية استخدام تفاعل البلمرة المتسلسل في تصنيف سموم الميكروب المكور العنقودي الذهبي بدقة وسرعة مقارنة بالطرق التقليدية المستخدمة في الكشف عن هذه السموم. وقد تم مناقشة النتائج والتوصيات لإنتاج لحوم دواجن ذات قيمة عالية.