Drug resistance of *staphylococcus aureus* in locally produced poultry meat

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Abstract
Poultry meat is considered a nutritious food because it contains several important nutrient including protein and fat. Conversely, it can be avehicle for several pathogenic bacteria such as *Staphylococcus aureus*. In this study we isolate *Staphylococcus aureus* 15 (60%) out of 25 broiler in house and 4 (16%) out of 25 in chicken store. The obtained results revealed that the mean value of total *S. aureus* count were $7 \times 10^2 \pm 1.1 \times 10^2$ and $4 \times 10^2 \pm 1.3 \times 10^2$ in broiler and chicken house respectively. Isolates of coagulase positive *S. aureus* resistant to 10 antimicrobial was determined by disc diffusion method. Isolates of coagulase positive *S. aureus* were completely 100% susceptible to cephalexin, ciprofloxacin and gentamycin but showed varying degrees of resistance to ampicillin 100%, erythromycin 100%, ceftrazone 48.27%, ofloxacine 48.27%, clindamycin 34.4%, augmentin 27.5% and cotrimoxazole 34.4%. Staphylococcal enterotoxin determined crystal SEC_2 were tested to toxicity in vivo by I/P injection in rabbit 10ug/kg b.wt. cause emesis and diarrhea.

Key words:
Staphylococcus aureus, Staphylococcal enterotoxin, broiler.

Introduction

Bacterial organisms of the genus Staphylococcus are one of the most prevalent pathogens in both humans and animals ***(Casey et al., 2007)***. *Staphylococcus aureus* is an important agent of food poisoning all over the world ***(Aarestrup et al., 1995; Balaban & Rasooly, 2000)***. It is the most important bacterial agent implicated in toxin production (toxic shock and staphylococcal scalded-skin syndromes), direct invasion and systemic disseminating (bacteremia, septic shock syndrome, skin infections and abscesses) ***(Hatakka et al., 2000; Martineau et al, 2000)***. Although it is found in water, dust, and air, *S. aureus* primarily colonises the mucosa of nasopharynx and skin of humans and animals ***(Songer & Post, 2005)***. The bacterium is considered to be a normal flora of the chicken, isolated from the skin and feathers as well as in the respiratory and intestinal tracts ***(Sleekes, 1997; Casey et al., 2007)***. However, some of the common forms of *S. aureus* associated poultry infections include tenosynovitis ***(Butterworth, 1999)***, omphalitis ***(Hill et al., 1989)***, femoral head necrosis, infected hock and stifle joints secondary to coccidiosis or vaccine reactions ***(McNamee and Smyth, 2000)*** and "bumblefoot" ***(Skeeles, 1997)***, In Nigeria and many other parts of the developing world, chickens are extensively reared in close proximity to human dwellings and therefore play an important role in environmental contamination, in addition to serving as significant vehicles for the transfer of such pathogens to humans by way of handling of live birds or consumption of contaminated meat and other poultry products. Bacterial antimicrobial resistance is a global concern that has been aggra-

vated by the fact that only few new antimicrobial agents come out from the pharmaceutical channels in recent times ***(Talbot et al., 2006, Okonko et al., 2009 and Soliman et al., 2013)***. Selective increase in resistant bacteria has been reported to be as a result of widespread use of antibiotics in animals for growth promotion and prevention of colonization by pathogenic bacteria ***(Munoz et al., 1993; Davies, 1994)***.

Even though poultry industry in Egypt contributes substantially to the Nation's Food Security and Gross Domestic Product, most of the flocks are reared by small scale farmers under limited or no veterinary supervision. In such production systems, antimicrobials are freely used as feed or water additives ***(Adayl, 2008)***. These practices can facilitate the emergence and spread of antibiotic resistant pathogens among birds with possible transmission to humans.

Material and Methods

Sample collection.

Chicken meat were collected from 25 broiler house and 25 from slaughter chickens store in Sharkia Governorate.

Staphylococcus aureus count and isolation according to FDA 2001.

Antimicrobial susceptibility test.

Antimicrobial susceptibility Test (AST) was carried out for the coagulase positive *S. aureus* isolates using disc diffusion method according to Clinical and Laboratory Standards Institute methods **(CLSI, 2010)**. Briefly, each of the *S. aureus* isolates were inoculated into nutrient broth (Oxoid, UK) and incubated at 37°C for 24 hours before they were tested. Turbidity of the growing culture was adjusted to correspond with that of a (0.5 MacFarland) standard. About 0.1 ml of the nutrient broth culture was subsequently inoculated onto Mueller Hinton agar plates and spread over the surface with sterile cotton swabs. Antimicrobial discs were then placed on the surface of each plate by means of antibiotic disc dispenser and incubated at 37°C for 24 hours. Diameters of inhibition zone were measured using a transparent ruler and the interpretative breakpoints for resistance were determined by comparing zone diameters as recommended by Clinical and Laboratory Standards Institute **(CLSI, 2010)**. The coagulase positive *S. aureus* isolates were tested against a panel of 10 antimicrobials namely; ampicillin (30mg), augmentin (amoxicillin clavulanate) (30mg), cephalexin (30mg), ceftriazone (30mg), ciprofloxacin (5mg), clindamycin (10mg), cotrimoxazole (50mg), erythromycin (10mg), gentamycin (10mg) and ofloxacin (5mg).

Extraction of Staphylococcus aureus from samples according to (Wang *et. al.*, 2008).

Results

A total 50 poultry samples from Sharkia Governorate. *S. aureus* were isolated from broiler house samples 15/25 (60%) and from chicken store 4/25 (16%).

All the coagulase positive *Staphylococcus aureus* isolated in this study were resistant to ampicillin and erythromycin but susceptible to ciprofloxacin, gentamycin and cephalexin. Fourteen (88.27%) of the coagulase positive S. aureus isolates were resistance to both ceftrizone and ofloxacin. This was followed by clindamycin and augmentin (34 – 4%) and cotrimazole (72.5%) (Table 1).

The total Staphylococcus count in live broilers and chicken meat store were $7 \times 10^2 \pm 1.1 \times 10^2$ and $4 \times 10^2 \pm 1.3 \times 10^2$ respectively.

Toxicity of *stophylococcus* refered to the endotoxin which in vivo was *stophylococcusaureus* C_2 (SEC_2) were tested for their ability to induce emesis activity in rabbit. The results showed that all SEC_2 induce fever in rabbits from 1 to 4h.

Table (1). Antimcrobial resistance of *S. aureus* isolates from chickens in Sharkia Governorate

Antimicrobs	Number resistance	% of resistance
Ampicillin	20	100
Erythromycin	20	100
Ceftriazone	14	48.27
Ofloxacine	14	48.27
Clndamycin	10	34.4
Cotrimoxazole	10	34.4
Augmentin	8	27.5
Ceplalexin	0	0
Ciprofloxacin	0	0
Gentamycin	0	0

Table (2). Total bacterial count of *Staphylococcus aureus* count in poultry meat

Samples	Total samples	Positive samples	Staphylococcus aureus count
broiler house	25	15	$7 \times 10^2 \pm 1.1 \times 10^2$
Chicken store	25	4	$4 \times 10^2 \pm 1.3 \times 10^2$

Table (3): Enteric activities of SE2 protein in rabbits dose 10mg/kg^{-1} in PBS after intraperitoneal administration

Toxin	No of rabbit	Fever	Diarrhoea
SEC2 from broiler house	3	2/3	2/3
ESC2 from chicken store	3	3/3	3/3
BPS	2	0/2	0/2

Discussion

In the current study, were isolated *S. aureus* among broiler (in broiler house) samples 15/25 (60%) and from chicken store 4/25 (16%) from Sharkia Governorate. Nearly similar results obtained by **Suliman (2013)** who estimated *S. aureus* among meat and poultry samples in the Netherlands where *S. aureus* is dominant food born sequence type.

All the 19 coagulase positive *S. aureus* isolates recovered were resistant to ampicillin and erythromycin and all isolates were susceptible to ciprofloxacin, gentamycin and cephaloxacin. Similar of antimicrobial susceptibility have been reported in Nigeria ***(*Otalu *et al., 2011*, Waters *et al.,* 2011 and Suliman *et al.,* 2013)**, in United States where occurrence of multi-drug resistant *S. aureus* in poultry is rather frequent. However **Geidam *et al.,* (2012)** reported a low resistance of 53% and 85% for ampicilin and erythromycin respectively in *S. aureus* isolates from commercial poultry farms within of Malaysia. This may be connected to the fact that antimicrobial use in commercial poultry setting is more likely to regulated than in small halder farms.

In addition, veterinarians and poultry farmers generally use these antimicrobials for specific

treatment of staphylococcal infection in Sharkia Governorate **(Adayel, 2008)**. Thus subtherapeutic use of such drugs as prophylaxes growth promoter or in accurate dosages given to sick flocks by unqualified personnel may likely result in plasma concentrations that are in consistent with the desired objectives which might possibly have led to the level of resistance observed in the present study.

The data present in table (2) showed mean values of total bacterial count (cfu/ml) of *Staphylococcus aure*us count (cfu/g) in poultry meat were 7 x 10^2 ± 1.1 x 10^2 and 4 x 10^2 ± 1.3 x 10^2 from broiler and chicken house respectively. Nearly similar result obtained by **Smith and Pearson (2010)**.

Although low dose not establish limit for the amount of *S. aureus* in meat, it is known that the amount of enterotoxins produced by enterotoxigenic strain achieve levels that sufficient enough to bring about symptoms of food borne disease.

Toxicity of endotoxin *S. aureus* in vivo were tested for their ability to induce emesis activity in rabbit. The result nearly similar to **Wang *et al.*, (2009)** which detected the bacterial toxin shock syndrome and food poisoning. Although the previously detrmined crystal structure of SEC_2 revealed in some histidin residues contribute to the binding of zin ions. Further toxicity assay in vivo indicate mutant of SEC_2 were devective and febrile activities.

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المقاومة الدوائية لميكروب المكور العنقودي الذهبي في لحوم الدواجن المنتجة محلياً

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الملخص العربى

تعتبر لحوم الدواجن من المواد الغذائية الهامة أنه يحتوي علي عدد من العناصر الغذائية ومنها البروتين والدهون، وهي وسط غذائي هام لنمو البكتريا مثل ميكروب المكور العنقودي الذهبي. وفي هذه الدراسة تم عزل ١٥/٢٥ بنسبة ٦٠% من دجاج التسمين في المزارع، وعدد ٤/٢٥ بنسبة ١٦% من دجاج التسمين في محلات بيع لحوم الدواجن. وبعمل العدد البكتيري للدجاج المصاب المكور العنقودي الذهبي في دجاج التسمين بالمزارع وجد أنه ٧ × ١٠^{٢} ± ١.١ × ١٠^{٢} في لحوم الدواجن، وفي المحلات ٤ × ١٠^{٢} ± ١.٣ × ١٠^{٢} وبعمل اختبار حساسية للمعزولات من المكور الذهبي العنقودي ضد ١٠ مضادات حيوية وجد أنه جميع الميكروبات حساسة بنسبة ١٠٠% لكلاً من سينالوكزين والسبروفلكساسين والجنتاميسين ولكن بدرجات مختلفة للمقاومة الدوائية بنسبة ١٠٠% للأمبسلين والأرثروميسين، وكانت ٤٨.٠٧% للسيفروزول والأفلاتوكسين وبنسبة ٣٤.٤% لكلاً من الكلندزاميسن والأجمين و ٢٧.٥% للكوتي ميزول.

وعزل الإنتروتوكسين لميكروب المكور العنقودي الذهبي وتجربة سميته في الحقل بحقنه في البروتون بجرعة ١٠مجم/ك م في الأرانب سببت ارتفاع في الحرارة والإسهال بعد ١ ساعة إلي ٤ ساعة.