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Staphylococcus aureus in commercial breeder layer flocks

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Abstract

Four flocks of Layer breeders, consist of 28000 (7000 birds each) at 25 weeks of age, hatchery and one day old chicks were subjected to swab sampling in order to isolate *Staphylococcus aureus* during the period from May till June 2008. Results revealed that more than half of the overall swab samples were positive (52.04%). Different isolation rates were recorded between four flocks, ranging from 62.5 to 79.16%. Hatchery samples revealed that working surfaces were heavily contaminated with *S. aureus* (75%), compared with the relatively low contaminated egg flats and egg shells. One day old chick samples show relatively low percentage of *S. aureus* isolation (29.1%). Antimicrobial sensitivity of 20 S. *aureus* isolates were surveyed for susceptibilities to a panel of 16 antimicrobial agents**.** *S. aureus* were 100% sensitive to five antimicrobials, namely; enrofluxacin; methicillin; trimethoprim with sulfamethoxazol and vancomycin, while in the opposite direction, 100% resistancy were recorded for two antimicrobial, ampicillin and amoxicillin**.** Graded sensitivity was observed in other antimicrobials, include: gentamycin, chloramphinicol, penicillin, erythromycin, ciprofloxacin, colistin, lincomycin, cephaloxin and doxycillin. The importance of the organism in breeder layers health was discussed.

Keywords: *Staphylococcus aureus*, Chicken.
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المكورات العنقودية الذهبية في قطعان امهات الدجاج البياض

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الخلاصة

اخذت مسحات من اربعة حقول لامهات الدجاج البياض تتالف من ٢٨٠٠٠ دجاجة (٧٠٠٠ دجاجة في كل قاعة) بعمر ٢٥ اسبوع و من مفقس الحقل و الافراخ الفاقسة بعمر يوم واحد وذلك لعزل جراثيم المكورات العنقودية الذهبية خلال الفترة من بداية شهر ايار ولنهاية شهر حزيران لسنة ٢٠٠٨ اظهرت النتائج ان (٥٢.٠٤٪) من العينات كانت موجبة تراوحت نسبة عزل هذه الجراثيم من البيوت الاربعة لامهات الدجاج البياض بين ٦٢.٥ و ٧٩.١٦٪. اوضحت نتائج عزل جراثيم المكورات العنقودية ان تواجدها بنسبة (٧٥٪) كان اكبر على اسطح فرز الافراخ في المفقس عند المقارنة مع عينات سطح البيض وصناديق جمعه بلغت نسبة عزل هذه الجراثيم في الافراخ بعمر يوم واحد (٢٩.١٪). تمت دراسة حساسية ٢٠ عزلة لهذه الجراثيم باستخدام ١٦ مضاد حيوي اتضح ان خمسة منها حساس ١٠٠ % لكل من enrofluxacin; methicillin; trimethoprim with sulfamethoxazol vancomycin .

وعلى العكس فقد سجلت هذه الجراثيم مقاومة ١٠٠ % ضد المضادين ampicillin and amoxicillin سجلت حساسية متدرجة للمضادات الاخرى gentamycin, chloramphenicol, penicillin, erythromycin, ciprofloxacin, colistin, lincomycin, cephaloxin, doxycillin. تمت مناقشة اهمية جرثومة *S. aureus* على صحة أمهات الدجاج البياض.

Introduction

Staphylococcus aureus is a Gram positive, coagulase positive coccoid cells in the family Staphylococcaceae (1). *S. aureus* is a ubiquitous organism in the breeder house environment and can be isolated from the litter, dust and feathers. The bacterium is considered to be a normal resident of the chicken, located on the skin and feathers and in the respiratory and intestinal tracts. A staphylococcus infection, or staphylococcosis, refers to a variety of diseases in poultry caused by staphylococci bacteria (2). Approximately 20 species have been isolated, only, *S. aureus*, is of veterinary importance in breeders. *S. aureus* is an important opportunist that can cause superficial to life-threatening illnesses in a variety of animal species. In poultry, the most common form of infection involves tenosynovitis and arthritis (3,4). Staphylococcus infections tend to occur more frequently during the following four periods of a breeder's life: 0 - 2 weeks, Omphalitis and femoral head necrosis are often related to egg or hatchery contamination and minor surgeries; 4 - 6 weeks, Infected hock and stifle joints secondary to coccidiosis or harsh vaccine reactions; 10 - 20 weeks, Infected hock and stifle joints; 24 - 30 weeks, Infected hock and stifle joints and "bumblefoot" (5-7). The milder forms of gangrenous dermatitis is generally caused by *S. aureus*. (8). The organism must enter the circulatory system to cause disease, thus the probability of infection is increased by any injury that provides the bacteria with a route of entry. The most obvious route of infection is through a break in the skin; through the respiratory tract; and through the gut (2,9). In poultry industry and governmental agencies are focused on eradicating staphylococci in live birds and at the processing plants (10). *S. aureus* and other microorganisms are found in poultry environment and regarded as pathogenic to humans and also may be pathogenic to poultry, causing serious infections that may lead to death (7). Poultry and poultry products which are often serve as the vehicles for human pathogens. *S.aureus* is among the predominant bacteria involved in food poisoning and is a leading cause of gastroenteritis resulting from ingestion of enterotoxins performed in contaminated food (11*)*. Whereas most infections can be treated with antibiotics, because of the organism's propensity to acquire antimicrobial resistance, it is important to continually monitor antibiotic susceptibilities of clinical isolates (12). The aim of this work was the possibility of environmental contamination of layer breeder farms with *S. aureus.*

Materials and methods

Samples collection

A total of 144 swab samples were collected from a farm of layer breeder consisted of four houses (7000 layers / each, and at a point of lay, 25 weeks), from a hatchery and from one-day old chicks, from May to June 2008. The integrated commercial breeder layer houses and hatchery were located in AL-Hamdania (Ninevah governorate). Six samples were collected from each of avian feet; feeders and drinkers, egg shells; egg flats; Hatchery working surface and1-day old chick (Table 1).

Table 1: Swab Sampling scheme from layer breeders, hatchery and day old chicks.

Source of swabs	Houses(flocks)				Total number of samples
	House A	House B	House C	House D	
Layers feet	6	6	6	6	24
Feeders	6	6	6	6	24
Drinkers	6	6	6	6	24
Nests	6	6	6	6	24
Egg flats	24				
Egg shells	24				
Hatchery Working surfaces	24				
1-day old chick	24				

Isolation and identification

The method of (1,13) was used for isolation and identification of *S.aureus*. Samples were taken by swabbing various sites with a sterile cotton swabs moistened with sterile peptone water. One day old chicks were sampled by swabing the abdominal and cloacal regions for 30 seconds. Breeder layers were sampled by rubbing their feets vigorously for 30 seconds. Areas of 16 $cm^{2,}$ where possible, from surfaces, feeder and wateres were swabbed for 10s. All cotton swabs from layers, chicks, egg surfaces and environmental fomites and hatchery, were placed in tubes of trypticase soya broth. All tubes were incubated at 37 ^{o}C for 24h. After incubation all tubes were streaked for primary isolation on mannitol salt agar. Typical colonies were isolated from fermented (changed to yellow) (Figure 1) media were selected for inoculating blood agar, performing coagulase test, and preparing Gram stain of these colonies. Inoculated blood agar were incubated at 37^{o}C for 24h. Coagulase test was performed by inoculating small tube of plasma with several loopfuls of the selected colonies and incubated it in a 37^{o}C water bath for several hours. Gram staining was used for identification of selected colonies. For confirmation of *S. aureus* identification API Staph Ident miniaturized test strip system was used (14).

Antimicrobial sensitivity testing

We surveyed 20 *S. aureus* isolates, for susceptibilities to a panel of 16 antimicrobial agents**.**

Diffusion susceptibility testing

The disk diffusion (Kirby –Bauer) method using Muller-Hinton agar was employed. Suspension of *S. aureus* colonies was used within 15 minutes for inoculation Muller-Hinton agar. Within 15 minutes of inoculating the agar surface with the bacterial suspension, a paper disks impregnated with antimicrobial agents were applied to the surface of the agar. After 16-18 hours, the plates were examined and the diameters of the zones are measured to the nearest millimeters. Discs with the following concentration of antibacterial substances (µg/disc) were used: ampicillin (AmP)-10; ampivap (Amv)-10; colistin (Col)-10; ciprofloxacin (Cip)-5; Chloramphenicol (Chl)-30; erythromycin(E)-15; Enrofloxacin (Enr)-5 amoxicillin (AxC)-25; doxycyclin (Dox)-30; Gentamycn (Gen)-10; lincomycin (Ln)-2; Methicillin(Met)-5; penicillin G (Pi)-10 IU; Trimethoprim+sulfamethoxazol (TS)-25; cephaloxin (Cf)-30; Tetracyclin (Tet)-30; Vancomycin (van)-30. The results were defined by measuring the diameter of the zones of inhibition and for interpretation the three-stage system of Kirby-Bauer was used (13).

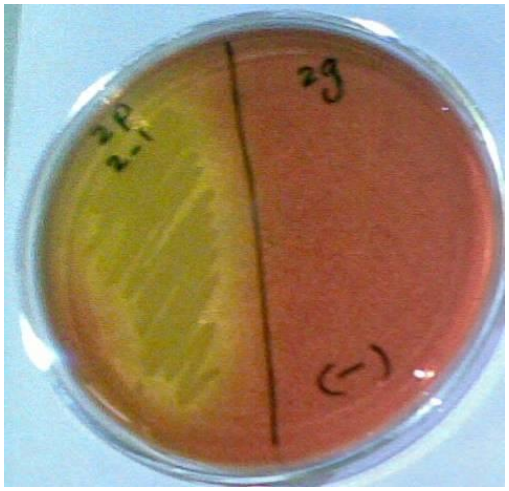


Figure 1: Negative (red) and Positive (change to yellow) discoloration of Staphylococcus isolate on mannitol salt agar (MSA).

Results

Prevalence of *S. aureus*

Figure 2 shows the overall percentage of *S. aureus* positive and negative samples from breeder layer flocks, from hatchery and from hatched 1-day old chicks. More than half were positive (52.04%). In details, table 2 and figure 3 and 4, show that breeder flock A and D had the highest percentage of positive samples being (79.16%), followed by flock C with (75%), while flock B had the lowest percentage of (62. 5%).

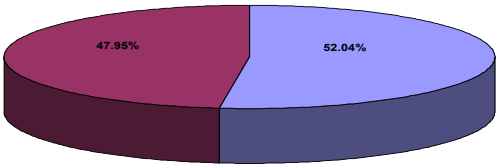


Figure 2: Overall positive and negative Percentages of *S. aureus* isolation from houses of breeder layers, hatchery and one-day old chicks.

Percentages of positive *S.aureus* isolation from different items of the hatchery, show that its working surfaces were heavily contaminated with *S. aureus* (75%), compared with the relatively low contaminated egg flats (20.8%), and with even lower egg shell percentage (16.6%) of *S. aureus* contamination (Table 2 and figure 4).

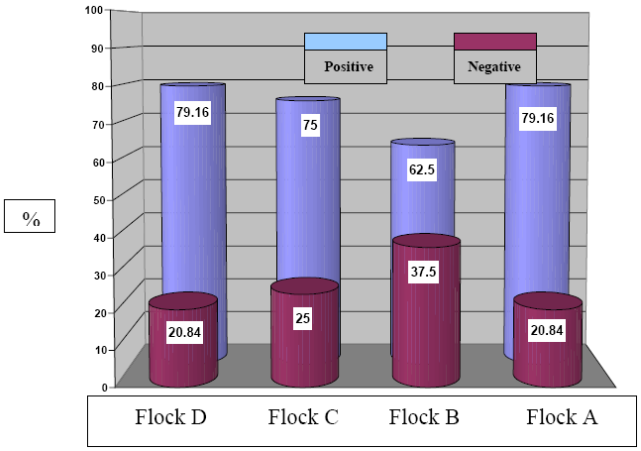


Figure 3: Percentages of positive *S. aureus* isolates from all samples of four breeder layer houses.

One day old chicks show relatively low percentage of *S. aureus* isolation (29.1%), when compared with other highest percentages of breeding flocks and hatchery.

Antimicrobial sensitivity

Figures 5 and 6 show the results of antimicrobial sensitivity (with their percentages) and resistant of isolated *S. aureus* from all items of breeder flocks, hatchery and one day old chicks. From these figures, it is evident that isolated *S. aureus* were 100% resistant to five antimicrobials, namely; enrofluxacin; methicillin; trimethoprim with sulfamethoxazol and vancomycin. In the opposite position, these isolates were 100% resistant to two antimicrobial,

ampicillin and amoxicillin. Other antimicrobials exhibited descending resistant from 100% of the five mentioned antimicrobials, and were as follows; gentamycin (90%); chloramphinicol (80%); penicillin (60%); erythromycin (55%); ciprofloxacin and colistin (45%); lincomycin and cephaloxin (30%); doxacllin (20%).

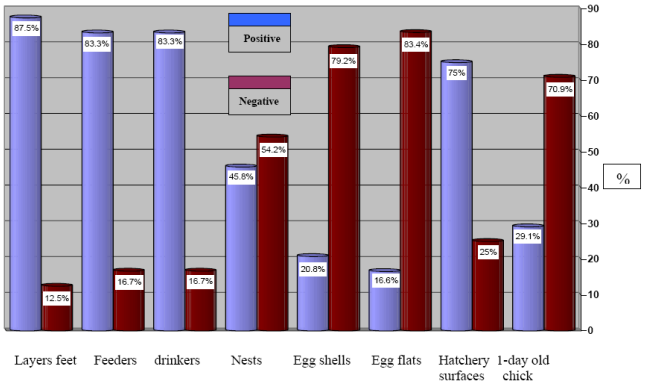


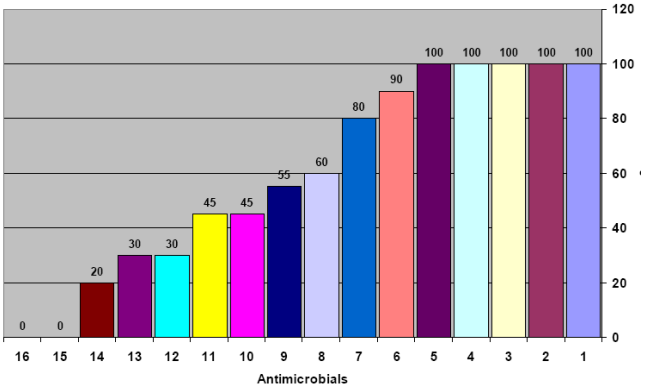
Figure 4: Percentages of positive and negative *S.aureus* isolates from all sample items.

Antimicrobial sensitivity

Figures 5 and 6 show the results of antimicrobial sensitivity (with their percentages) and resistant of isolated *S. aureus* from all items of breeder flocks, hatchery and one day old chicks. From these figures, it is evident that isolated *S. aureus* were 100% resistant to five antimicrobials, namely; enrofluxacin; methicillin; trimethoprim with sulfamethoxazol and vancomycin. In the opposite position, these isolates were 100% resistant to two antimicrobial, ampicillin and amoxicillin. Other antimicrobials exhibited descending resistant from 100% of the five mentioned antimicrobials, and were as follows; gentamycin (90%); chloramphinicol (80%); penicillin (60%); erythromycin (55%); ciprofloxacin and colistin (45%); lincomycin and cephaloxin (30%); doxacllin (20%).

Isolate NO.	Amp 10	Axc 25	Col 10	Cip 5	Dox 30	Cf 30	Ln 2	Ery 15	Pi 10 IU	Chl 30	Gen 10	Enr 5	Met 5	TS 25	Tet 30	van 30
	Antibiotics µg/disc															
1	R	R	R	R	R	R	R	I	I	I	I	S	S	S	S	S
2	R	R	R	R	R	R	I	I	I	I	I	S	S	S	S	S
3	R	R	R	R	I	I	I	I	I	I	S	S	S	S	S	S
4	R	R	R	R	I	I	I	I	I	I	S	S	S	S	S	S
5	R	R	R	R	I	I	I	I	I	S	S	S	S	S	S	S
6	R	R	R	R	I	I	I	I	I	S	S	S	S	S	S	S
7	R	R	R	R	I	I	I	I	I	S	S	S	S	S	S	S
8	R	R	R	R	I	I	I	I	I	S	S	S	S	S	S	S
9	R	R	R	R	I	I	I	I	S	S	S	S	S	S	S	S
10	R	R	I	I	I	I	I	S	S	S	S	S	S	S	S	S
11	R	R	I	I	I	I	I	S	S	S	S	S	S	S	S	S
12	R	R	S	S	I	I	I	S	S	S	S	S	S	S	S	S
13	R	R	S	S	I	I	I	S	S	S	S	S	S	S	S	S
14	R	R	S	S	I	I	I	S	S	S	S	S	S	S	S	S
15	R	R	S	S	I	S	S	S	S	S	S	S	S	S	S	S
16	R	R	S	S	I	S	S	S	S	S	S	S	S	S	S	S
17	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S
18	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S
19	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S
20	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S

Figure 5: Antimicrobial sensitivity, resistancy and intermediates of isolated *S. aureus* collected from all items of breeder flocks, hatchery and one day old chicks.



1= Enr; 2= Met; 3= TS; 4= Tet; 5= van; 6= Gen; 7= Chl; 8= Pi; 9= Ery; 10= Col; 11=Cip; 12=Cf; 13=Ln; 14=Dox; 15= Axc; 16= Amp

Figure 6: Antimicrobial sensitivity percentages of isolated *S. aureus* collected from all items of breeder flocks, hatchery and one day old chicks.

Table 2: Number and percentage of positive *S. aureus* samples in four breeder layer houses collected from different sites.

Source of swabs	Houses(flocks)				Total number of samples
	House A	House B	House C	House D	
Layers feet	5(83.3%)	4(66.6%)	5(83.3%)	6(100%)	21(87.5%)
Feeders	5(83.3%)	5(83.3 %)	5(83.3%)	5(83.3%)	20(83.3%)
Drinkers	6(100%)	4(66.6%)	5(83.3%)	5(83.3%)	20(83.3%)
Nests	3(50.0%)	2(33.3%)	3(50.0%)	3(50.0%)	11(45.8%)
Hatchery swabs	Number of samples		*S.aureus* detected		%
Egg flats	24		5		20.8
Egg shells	24		4		16.6
Hatchery Working surfaces	24		18		75.0
Chick swabs	Number of samples		*S.aureus* detected		%
1-day old chick	24		7		29.1

Discussion

The results gained in our study could be segregated to three categories; those obtained from breeder layer houses, that's to say, the flocks itself, and the other two categories were from hatchery and hatching chicks. About the first category, in which high percentage of *S. aureus* (62.5 to 79.16%), was recorded from four flocks and within each item of sampling in flock houses (45.8 to 87.5%). These figures still lower than those reported by (15) of 97.9%. Our results could be as a result of *S. aureus* colonization in 25 weeks aged layers in our study, since they are generally low in he first few weeks of chicks life, but then they tend to increase as chick grows older (15), and tend to be relatively high at seventh weeks of life after which time they were maintained at equally high levels (16) up to 25 weeks and more but after 50 weeks of age these organisms could be readily isolated from all hens (15). They were isolated from layers feet and the nests in which birds lay their eggs, organism may ultimately gained from feces in addition to their presence in the feeder and waterers swab samples. *S. aureus* populations are naturally found on body surfaces of live poultry without any previous history of staphylococcal disease (16). In addition, more than 80% of the airborne microorganisms found in poultry are Staphylococci and streptococci, which could contaminate birds, fomities and surfaces of their houses (17) as they were isolated from more than 50% of air samples during epidemiological study of environmental contamination of poultry farms (18). The origin of these organisms may be traced to their possible fecal or feed sources due to feed contamination of these organisms used to feeding breeder layers, or as a result of the people entering the poultry house and given feed to the fowls (19).

The second category of *S. aureus* isolation were swab samples from hatchery. Our findings stressed on the high prevalence of *S. aureus* isolation from working places, but not on the egg surfaces or their gathering flats a conclusion also reached by (15), when had referred to the high detection of these organisms in the debris from the hatcheries and on the working surfaces at the sexing and vaccination areas.

The third category of *S. aureus* isolation was from hatched one day old chicks, from which relatively low prevalence of these organisms isolation was detected. Their presence on chicks indicates that these organisms could colonize chicks from one day old, but in relatively low numbers, and then tend to be increased as the chicks grow older (15). *S. aureus* is an important opportunist that can cause superficial to life-threatening illnesses in poultry, this organism has been implicated in osteomyelitis, synovitis, and cellulites (2).

Whereas most infections can be treated with antibiotics, and because of the organism's propensity to acquire antimicrobial resistance, it is important to continually monitor antibiotic susceptibilities of clinical isolates. We surveyed 20 *S. areus* isolates for susceptibilities to a panel of 16 antimicrobial agents. We noticed that hundred percent of the isolates were resistant to at least two antimicrobials, ampicillin and amoxicillin, whereas about half (45%) of them were resistant to four antimicrobial agents, ampicillin, amoxicillin, colistin and ciprofloxacin. So, our findings surveyed more resistancy that those reported by (20), who reported that 16% of 77 *S.aureus* isolates were resistant to only two antimicrobials. On the opposite site, hundred percent of our isolates were susceptible to 5 antimicrobials, namely enrofloxacine, methicillin, trimethoprim and sulphamethoxazole, tetracycline and vancomycin. These finding were not entirely in agreement with (20), who reported less susceptibility to tetracycline (60%), lincomycin (81%), but agreed with their results of vancomycin.

We reported more resistant to ciprofloxacine (45%) than that reported by (21) of 30%, with less resistancy was reported by (21) of 7% versus 0% by us and 76 % susceptability to erythromycin versus 55% by us. The high rate of antimicrobial susceptibility may be due to the low using of these antimicrobials in layer breeders compared with broiler breeders or broilers. We suggest if these breeders were likely to acquire their staphylococci from human sources, a subsequent research, using phage typing, should be made on both strains specific to humans and layers. Although *S. aureus* is implicated in human food poisoning, but most poultry strains do not produce the enterotoxins that cause human food borne disease (11). Methicillin-resistant *S. aureus*, similar to strains causing disease in humans, has not been isolated in our study. However, transfer of antibiotic resistance between strains from animals and humans is thought to be infrequent (21).

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