



Antibiogram Assay and Pathogenesis of *Staphylococcus aureus* in Baby Chicks

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Abstract | Intensive usage of antibiotics in the poultry sector has become a major area of concern with the consequent emergence of antibiotic-resistant bacteria (ARB) and accordingly their access into the food chain, thus threatening the human health. In the current study, A total of 250 specimens from different local hatcheries of Luxor province (southern Egypt) have been examined for the incidence of *Staphylococcus aureus* using standard microbiological methods and biochemical tests. The overall prevalence was 27.60% ($n = 69/250$). Only 30 isolates have been screened for in vitro drug sensitivity bioassay against 18 antimicrobial discs. Our findings revealed that all isolates showed high resistance against β -lactams, including oxacillin (83.33%, $n = 25/30$), ampicillin (80.00%, $n = 24/30$), penicillin (73.33%, $n = 22/30$), and amoxicillin (60.00%, $n = 18/30$). On the other hand, the highest susceptibility was shown for vancomycin (93.33%, $n = 28/30$), trimethoprim sulphamethoxazole (80.00%, $n = 24/30$), and chloramphenicol (70.00%, $n = 21/30$). Results from a pathogenicity study conducted on 300 one-day-old chicks (SPF) manifested the highest mortality rate of 34.00% in one infected group, and most deaths were in the first three days of all infected groups. Based on the current study, constant monitoring of antibiotic susceptibility phenotypes is deemed prudent and necessary for evaluating the utility of certain antibiotics for treating *S. aureus* infections. Additionally, any alteration of resistance over the years should be identified, and all centers in charge must determine their own resistance profiles, as reducing the rate of antibiotic resistance will contribute to reducing the cost of treatment. Such measures can lead to improvement of the poultry industry, which represents a vital source of economy in Egypt.

Keywords | Baby chicks, Pathogenicity index, Prevalence, Sensitivity bioassay, *S. aureus*

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INTRODUCTION

Staphylococcus spp. are substantial commensal pathogens of medical and veterinary prominence, and they have the potential to cause life-threatening disorders in several susceptible hosts (Gosbell and Van Hal, 2013). It is easily spread between animals and under certain conditions to

humans through dermal infection or direct contact with animal excretions such as saliva, or through aerosols released during coughing and sneezing. Among several *Staphylococcus* spp., *S. aureus* is the most common and frequent pathogen causing food poisoning and food-related infections (Costa *et al.*, 2012). Furthermore, *S. aureus* can compromise the poultry industry by causing

severe economic losses due to reduced farm productivity and increased culls rate during processing (Jordan and Pattison, 1996).

Animal sources of *Staphylococcus* strains can potentially be harmful to humans, as most strains show resistance to antibiotics and cause zoonoses (Stapleton and Taylor, 2002).

In general, 25% of people are carriers for *S. aureus* according to studies conducted by the Centers for Disease Control and Prevention in 2011; infections vary from superficial skin infections to fatal disease (CDC, 2011). The severity of illness depends on several factors, among them the emergence of antibiotic-resistant strains which contribute to the evolution of methicillin-resistant *S. aureus* (MRSA). Poultry products are a potential source of transmitting antibiotic-resistant *Staphylococcus* strains to humans, manifesting as food-borne infections (Abulreesh and Organji, 2011).

Poor hygienic and sanitary conditions, along with a shortage of data about the health status of local hatcheries in the Luxor Province, motivated us to explore the existence of microbial load, particularly *S. aureus* pathogen in the first week following hatching.

In the present study, we investigate *S. aureus* significance inside local hatcheries of the Luxor province and explore the antibiotic resistance evolution within poultry farms by conducting a phenotypic characterization of isolated strains from baby chicks. Furthermore, we screened for their susceptibility toward commonly used antibiotics followed by a pathogenicity study for the same isolated strains.

MATERIALS AND METHODS

STUDY AREA

The study was conducted at local hatcheries of the Luxor province, southern Egypt, in which five sub-districts were included: Esna, Arment, El-Monshah, El-Tode, and El-Hebil, as shown in Table 1.

SAMPLING

All chicks were subjected to necropsy before sampling in order to record any gross lesions on their viscera, especially for the yolk sac. Post-mortem examination was done according to Chauhan and Roy (2007). After necropsy, yolk sac samples were collected aseptically using sterile swabs, and transferred to Tryptic-soy broth in sterile test tubes until they were submitted for bacteriological examination in the reference lab for control on poultry production, Luxor, Egypt. All samples were collected under aseptic

conditions and safety precautions according to Kitai et al. (2005); Lee (2003); Middleton et al. (2005); Rodgers et al. (2003); Rosky and Hamdy (1972); Swayne et al. (1998). Organs including liver and yolk material were collected from live diseased or freshly dead one day old baby chicks.

Table 1: Number and sources of samples collected from different local hatcheries of Luxor Province.

Sample sources	Diseased baby chicks		Dead in shell Embryo
	Liver	Yolk sac	
Esna	17	13	20
Arment	15	15	20
EL-Monshah	11	19	20
EL-Tode	9	21	20
EL-Hebail	15	15	20
Total	67	83	100

The study protocol was approved by the committee of animal welfare and ethics, Laboratory Animal Control Guidelines of the Animal Health Research Institute, Cairo, Egypt.

STAPHYLOCOCCUS AUREUS ISOLATION AND IDENTIFICATION

Pre-enriched nonselective medium (buffered peptone water) was inoculated with the collected samples at ambient temperature and then incubated at 37°C for 24 h under aerobic conditions. A loopful of the inoculated medium was transferred onto blood agar and then incubated for 24 h at 37°C. Another loopful of enriched broth was sub-cultured on Mannitol Salt Agar (MSA) and incubated at 37°C for 24 h, then confirmed on Baird Parker's Agar (BPA) containing egg yolk tellurite (EYT) and incubated at 37°C for 48 h in ambient air. Presumptive colonies were collected and maintained for gram stains and characterization by oxidase test, coagulase test, and slide catalase test according to Quinn et al. (2002). Suspected colonies were identified on the basis of staining reaction, cellular morphology, and hemolytic pattern on blood agar according to Swayne et al. (1998).

ANTIMICROBIAL SUSCEPTIBILITY TEST

Only 30 *S. aureus* isolates were screened for in vitro antimicrobial susceptibility against a panel of 18 antimicrobials discs as shown in Table 3.

Profiling was conducted by the Kirby-Bauer disk diffusion method on Mueller-Hinton Agar (Bauer et al., 1966; CLSI, 2014). Plates were inverted and incubated at 35°C for 24 h. Diameters of inhibition zones were measured, and results were interpreted as sensitive (S), intermediate (I), and resistant (R) according to CLSI (2014).

Table 2: Overall and individual prevalence of *S. aureus* isolated from different tissues in each subdistrict.

Source	Coagulase +ve%			Coagulase -ve%		
	Diseased baby chicks		Dead-in-shell	Diseased baby chicks		Dead-in-shell
	Liver	Yolk sac	Embryo	Liver	Yolk sac	Embryo
Esna	11.76	76.92	25.00	88.24	23.08	15.00
Arment	6.67	46.67	30.00	93.33	53.33	20.00
EL-Momshah	9.09	15.79	40.00	90.90	84.21	15.00
EL-Tode	22.22	23.81	20.00	77.77	76.19	40.00
EL-Hebail	6.67	26.67	45.00	93.33	73.33	40.00
Total	11.94	34.94	32.00	89.55	65.06	26.00
	27.60			17.60		

Table 3: Phenotypic profile of antibiogram assay of *S. aureus* against 18 antimicrobials according to CLSI, 2014.

Antimicrobial discs	Classification	<i>S. aureus</i> %		
		Resistant	Sensitive	Intermediate
Oxacillin	β-lactam	83.33	13.00	3.67
Ampicillin	β-lactam	80.00	20.00	0.00
Penicillin G	β-lactam	73.33	26.67	.000
Norfloxacin	Fluoroquinolone	.0070	23.33	6.67
Clindamycin	Lincosamide	.0070	.0030	.000
Streptomycin	Aminoglycoside	63.33	.0033	6.67
Amoxicillin	β-lactam	.0060	16.67	23.33
Tetracycline	Tetracycline	56.67	.0040	3.33
Neomycin	Aminoglycoside	56.67	.0037	6.33
Oxytetracycline	Tetracycline	.0055	.0045	.000
Erythromycin	Macrolide	.0050	.0020	.0030
Spectinomycin	Aminoglycoside	.0050	33.33	6.67
Amikacin	Aminoglycoside	.0050	.0030	.0020
Gentamicin	Aminoglycoside	43.33	.0030	16.67
Enrofloxacin	Fluoroquinolone	.0030	.0050	.0020
Chloramphenicol	Chloramphenicol	26.67	.0070	3.33
Trimethoprim-Sulpha	Folate pathway antag-onist	16.67	.0080	3.33
Vancomycin	Glycopeptide	6.67	93.33	.000

PATHOGENICITY STUDY

A total of 300 one-day-old baby chicks (SPF) were used for the pathogenicity study of *S. aureus* isolates. Chicks were divided into 6 equal groups (A-F), each one consisting of 50 birds, randomly chosen.

Groups (A to E) were infected once orally by 10⁶ dilutions of the same isolated strains from different hatchery locations (Esna, Arment, El -Monshah, El-Tode, and El-Hebail), respectively. Group F was injected with nutrient broth and served as a negative control. All chick groups were kept separate and received a starter ration without any medication.

The temperature was maintained between 21°C and 25°C, and relative humidity was (30-40%) with a 12-h photoperiod. Birds were observed daily for 10 days, during which clinical signs and mortality were recorded. After 10 days, all the remaining live birds were euthanized for post-mortem scoring.

RESULTS AND DISCUSSION

PREVALENCE OF COAGULASE +VE *S. AUREUS* FROM BABY CHICKS AND DEAD IN-SHELL EMBRYOS

Staphylococcosis has a crucial influence on veterinary and medical sectors. In poultry, it is correlated with several

clinical disorders such as tenosynovitis, omphalitis, femoral head necrosis, infected hock and stifle joints, and bumblefoot (Suleiman et al., 2013). *S. aureus* has been recognized as the second most important bacterium accountable for yolk sac infections (Chauhan and Roy, 2007; Rehman et al., 1996).

Our findings revealed that overall prevalence of *S. aureus* was 27.60% as coagulase positive ($n = 69/250$), while coagulase-negative samples comprised 17.60% ($n = 44/250$) as shown in Table 2. These results are in the line with those obtained by Shareef and Mansour (2009). Our prevalence was higher than Rizk and Bekhiet (2001), who identified *S. aureus* as 12.60% from chickens with facial edema, and Mohammed (2006), who reported 8.00% incidence from chicken musculoskeletal abscesses. On the contrary, our results were lower than Zhu et al. (1999) who identified 49.00% of *S. aureus* isolates in live birds; (Amare et al., 2013) who isolated 41.67% from baby chicks, and Khalil and Enas (2012), who recorded a 20.00% prevalence in litter samples from Behera (Egypt).

Yolk sac represents (20-25%) of the live weight of a chick at birth, and it is usually resorbed during the first week (Khan et al., 2004). However, several factors contribute to the delay of yolk resorption resulting in its retention. Bacterial contamination is considered as the most frequently factor involved in this health problem. Our findings showed that yolk sacs from diseased baby chicks recorded a higher incidence for *S. aureus* 34.94% ($n = 29/83$) than liver 11.94% (8/67), as shown in Table 2. This is attributed to the fact that *Staphylococcus aureus* is one of the significant factors for yolk sac infections in baby chicks.

ANTIMICROBIAL SUSCEPTIBILITY ASSAY

Poor husbandry practices with minimum levels of biosafety and biosecurity practices necessitate the overdependence and improper utilization of antibiotics as a prophylaxis or as growth promoters in poultry farms, and hence, these antibiotics have found wide clinical and veterinary applications with indiscriminate use in Egypt. The antimicrobial sensitivity profile of the current study exhibited that resistance of *S. aureus* to antibiotics varied considerably; the highest resistance was recorded to β -lactams including oxacillin (83.33%), ampicillin (80.00%), penicillin (73.33%), and amoxicillin (60.00%), as shown in Table 3.

Previous investigations demonstrated variant patterns of *S. aureus* resistance against penicillin, oxacillin, erythromycin, gentamicin, neomycin, and tetracycline (Nemati et al., 2008). Furthermore, Shareef and Mansour (2009) reported that *S. aureus* isolates tolerated two antimicrobials (ampicillin and amoxicillin), which agreed with our study.

On the contrary, graded sensitivity was observed for other antimicrobials such as gentamycin, chloramphenicol, penicillin, and erythromycin, while 100% sensitivity was demonstrated to enrofloxacin. Our investigations were also in agreement with those of Losito et al. (2005), who reported that all *S. aureus* isolates were susceptible to vancomycin.

Antimicrobial agents sold in Egypt and other developing countries are combinations of several active components of these antibiotics at subtherapeutic/ substandard doses; consequently, a single drug may have mixed ingredients of those at a required dose. Limited policies for the regulation of drug acquisition, additionally with the use of several drug combinations, may contribute to the antimicrobial resistance observed in this study and other studies. As such, our findings may be congruous or may be lower or higher than others, due to the difference in conditions associated with the environment where this study has been conducted. Moreover, an organism's propensity to acquire antimicrobial resistance necessitates conducting a frequent susceptibility monitoring of the clinical isolates against commonly used antibiotics (Werckenthin et al., 2001; White et al., 2003).

PATHOGENICITY STUDY

The pathogenicity index was studied for 300 one-day-old chicks experimentally infected with the same 30 isolated strains from different hatcheries as mentioned above. All infected groups manifested general signs of depression: closed eyes, drooping wings, poor growth, weakness, huddling together, and watery diarrhea. Moreover, the umbilicus was inflamed with bluish-black discoloration and a pungent odor. The abdomen felt soft, mushy, flabby, and enlarged. In contrast, the control group remained active throughout the experimental period. Additionally, all infected birds were off-feed and water as reported before by Moustafa and Hussein (1999). On the other hand, significant post-mortem lesions exhibited air sacculitis and pericarditis with fibrinous exudates. In addition, congestion of breast and thigh muscles with unabsorbed yolk sac and petechial hemorrhages on the coronary fat were found. Congestion of liver and kidney with septicemia was observed in some birds.

Infected yolk sac weight/body-weight ratio was higher than the control group (data not shown). These observations were expounded as the weight of the unabsorbed yolk was higher, while body weight was lower in all infected groups. Our findings were similar with those observed by Anjam (1997), Khan et al. (2002).

The mortality rate post-infection is shown in Table 4; variant patterns have been observed among infected

groups due to assorted virulence between isolated strains, according to different localities. The data revealed that group (A) infected with *S. aureus* (from Esna) demonstrated the highest mortality (34.00%), and most deaths were in the first 3 days after hatching. This may explain the severity of these isolates, which showed the highest resistant among others in the antibiogram assay. This interpretation seems reasonable, as the Esna territory is recognized by massive poultry production without establishment of proper hygienic measures, combined with intensive abuse of antimicrobial agents without regulations.

Table 4: Mortality pattern of experimentally infected baby chicks.

<i>S. aureus</i> sources	Mortality %	Mortality / day			
		3 rd	5 th	7 th	9 th
Esna	34.00	11	4	1	1
Arment	26.00	7	3	2	1
El-Monshah	12.00	3	1	1	1
El-Tode	14.00	5	2	0	0
El-Hebail	10.00	4	1	0	0
Control	0.00	0	0	0	0

Re-isolation of *S. aureus* from experimentally infected birds demonstrated that yolk sacs recorded the highest rate amongst all infected groups, followed by liver and heart as shown in Table 5, which is in agreement with Devriese (1975) and Narine (1973).

Table 5: Prevalence of re-isolated *S. aureus* from dead chickens.

<i>S. aureus</i> isolates	Reisolated <i>S. aureus</i>		
	Yolk sac %	Liver %	Heart %
Group-A	41.18	41.18	5.88
Group-B	38.46	15.38	0.00
Group-C	50.00	50.00	16.67
Group-D	57.14	57.14	0.00
Group-E	40.00	20.00	40.00
Group-F	0.00	0.00	0.00

Since yolk is very rich in water and lipid, it promotes the rapid growth of pathogens. Additionally, the body temperature of the chick, higher than that of egg incubation, is an ideal factor for bacterial growth including *Staphylococcus* spp. That can lead to infection of the yolk sac (Khan et al., 2004), which represents the main cause of chick mortality during the first week post-hatching (Rai et al., 2005; Yassin et al., 2009). Consequent mortality ranging from 1.7% to 8.6% would cause high economic losses (Awobajo et al., 2007).

In the current survey, individual incidence of *S. aureus* was

the same in yolk sacs and livers from hatcheries of Esna, El-Monshah and El-Tode: 41.18%, 50.00% and 57.00%, respectively. On the contrary, prevalence of *S. aureus* was higher in yolk sacs than liver for hatcheries of Arment and El-Hebail. We could not compare the prevalence post-infection in hearts because we did not collect samples from heart tissues in the original survey.

CONCLUSIONS AND RECOMMENDATIONS

Based on the previous investigations, the following study fills a significant gap about *S. aureus* infections within local hatcheries of the Luxor province.

Despite the susceptibility of isolated strains to many antibiotics which may be effective for chickens in the first week of age, the emergence of multidrug-resistant bacteria presents a real danger to animal and human health. Further investigations are demanded to better control risk factors. Periodic surveillance programs are also needed to reduce the frequent use of antibiotics in poultry farms.

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NOVELTY STATEMENT

Our study provided the 1st survey to investigate the incidence of *S. aureus* among chicken embryos of Luxor province. Furthermore, our isolates exhibited resistance toward β lactam and other antibiotics which may be acquired from their hens, and consequently, this is alarm that resistance gene can be transmitted through progeny.

AUTHOR'S CONTRIBUTION

All authors contributed equally to the manuscript.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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