

## Using Integral System Stafilococchi Kit for Biochemical Identification and Susceptibility Testing of Coagulase Negative Staphylococcus Isolated from Broiler Chickens in Egypt

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**Abstract:** A total of 334 tracheal, cloacal and nasal swabs, heart, muscles and liver samples were investigated bacteriologically to detect the prevalence of coagulase negative Staphylococcus species among the apparently healthy broiler chickens. Out of 334 tested samples, 151 (45.2%) were Staphylococcus species positive. The most important conventional biochemical tests as well as INTEGRAL SYSTEM STAFILOCOCCI kit were used for identification of the Staphylococcus species isolates. Serotyping of the isolates were carried out using INTEGRAL SYSTEM STAFILOCOCCI kit and revealed the presence of *Staphylococcus aureus* (2.1%) and coagulase negative Staphylococci (43.1%) (*S. capitis*, *S. simulans*, *S. sciuri*, *S. haemolyticus*, *S. xylosus* and *S. saprophyticus*) among the positive samples. AntibioGram sensitivity testing for 20 isolates against 15 antibiotic agents was performed and it was found that (93.3%) of coagulase negative Staphylococcus (CoNS) isolates were sensitive to Fosfomycin. On the other hand, 86.7% of the isolates were sensitive to Rifampicin, 80% to Amoxicillin + Clavulanic acid, Ampicillin + Sulbactam, Ceftazidime, Cefuroxime and Levofloxacin (each) and Ciprofloxacin (66.7%). Moreover, 93.3% of isolates showed resistance to Claritromycin, Erythromycin and Roxitromycin while lower percentage of resistance was recorded against the remaining antibiotics. In regard to *Staphylococcus aureus* isolates, all strains were resistant to Azitromycin, Erythromycin and Roxitromycin and highly sensitive to Ampicillin + Sulbactam and Fosfomycin. Indeed, the present study reports the presence of multidrug resistance coagulase negative Staphylococci isolates among healthy broiler chicken farms in Egypt.

**Key words:** CoNS • AntibioGram sensitivity • Apparently healthy broiler chickens

### INTRODUCTION

Staphylococci are frequently found in poultry products for human consumption and can thus, spread to humans through the food chain [1, 2]. Staphylococci are considered to be of the most common causes of infections in birds [3]. Most infections are caused by coagulase positive Staphylococci, especially *Staphylococcus aureus*, but also coagulase negative Staphylococci seem to be associated with infections [4]. Disease conditions associated with pathogenic Staphylococci in older birds are most frequently seen in the heavier breeds (broilers, chickens) and include gangrenous dermatitis and sub-dermal abscesses with the wing tips and the

dorsal pelvic region as the sites most commonly affected [5, 6]. Coagulase-negative Staphylococci (CoNS) are most frequently isolated from blood cultures, accounting for one-third of nosocomial bacteraemia [7-9]. *Staphylococcus epidermidis*, *Staphylococcus haemolyticus* and *Staphylococcus saprophyticus* are increasingly often identified by researchers as the cause of nosocomial infections in humans [10, 11]. Whereas *Staphylococcus chromogenes*, *Staphylococcus simulans* and *Staphylococcus xylosus* are identified as the cause of infections in animals [12, 13]. Because of the widespread use of antibiotics, resistance profile of microorganisms is increasing among bacterial populations [14], therefore it will account for the existence of



Fig 1: Biochemical identification of *Staphylococcus* sp. using INTEGRAL SYSTEM STAFILOCOCCHI kit (A: *S. hemolyticus*, D: *S. aureus*, V: *S. simulans*).

antimicrobial resistance genes among *Staphylococci* of animal origin but these resistance genes might in some instances be transferred from *Staphylococci* of animal origin to *Staphylococci* which cause infections in humans and thereby need antimicrobial treatment [6]. Raw chicken meat is often consumed in many countries, so antibiotic resistant strains in chicken meat are regarded as a risk factor in the food chain [15]. The hazards associated with CoNS can be worsened by the notorious escalation of antibiotic resistance observed during the last decades in these bacteria [6].

The aim of the present study was to throw light on the prevalence and serotypes of coagulase negative *Staphylococcus* species isolated from samples collected from healthy broiler chickens using INTEGRAL SYSTEM STAFILOCOCCHI kit. In addition to determination of resistance profiles of isolated CoNS strains.

## MATERIALS AND METHODS

**Sample Collection:** A total of 334 tracheal, cloacal, nasal, muscles, liver and heart samples were collected from apparently healthy chickens in 25 broiler farms in: Giza, Menofia, Sharkia, Gharbia, Behera, Kafr el-Sheikh and Dakahlia governorates, Egypt during the period of work that extended from December 2012 till January 2014 with the aim of isolating *Staphylococcus* species.

**Isolation and Cultivation of *Staphylococci*:** The collected samples were cultured onto Nutrient agar, Blood agar medium, Mannitol Salt agar, Baird Parker agar then incubated for 24-48 hours at 37°C [14, 16]. The resulted colonies were examined for identifying morphological characteristic appearance of *Staphylococcus* species [17].

## Identification and Characterization of Coagulase Positive and Negative *Staphylococcus* Species:

The isolates were identified according to Quinn *et al.* [18] by using conventional techniques such as: catalase test, coagulase test, mannitol fermentation activity, pigment production onto nutrient agar, hemolytic activity on sheep blood agar, growth on Baird-Parker medium, as well as using INTEGRAL SYSTEM STAFILOCOCCHI kit (Liofilchem®).

**Serotyping of *Staphylococcus* Isolates:** A total of 20 isolates were selected and serotyped using INTEGRAL SYSTEM STAFILOCOCCHI kit based on biochemical tests as shown in (Figure 1) and (Table 2).

## Determination of Susceptibility of Coagulase Positive *S. Aureus* and Coagulase Negative *Staphylococcus* Species Isolates:

The susceptibility testing for 20 *Staphylococcus* species isolates were examined in vitro against 15 different antibacterial agents by using INTEGRAL SYSTEM STAFILOCOCCHI kit on the basis of the growth or inhibition of the micro-organisms in the median containing the antibiotic. The interpretation of the results was performed by assessing the change in colour of the various wells. The following antibiotics were used: Ampicillin/Sulbactam (32/16 µg/ml), Amoxycillin/clavulanic acid (8 µg/ml), Tobramycin (8 µg/ml), Ceftazidime (32 µg/m), Cefuroxime (32 µg/m), Levofloxacin (8 µg/ml), Azitromycin(8 µg/ml), Claritromycin (8 µg/ml), Roxitromycin(8 µg/ml), Erythromycin (8 µg/ml), Ciprofloxacin(4µg/ml), Gentamicin (8 µg/ml), Fosfomycin (200µg/ml), Rifampicin (4µg/ml) and Co-Trimazole (8 µg/ml).

## RESULTS

A total of 151 (45.2%) isolates were identified as *Staphylococcus* species. The isolation rate was 57.7% in tracheal samples while in cloacal and nasal samples, it was 47.1 and 28.9 % respectively (Table 1). As for birds' organs, the isolation rate was 60% in both heart and liver samples and 42.9% in muscles. On the other hand, *S. aureus* could be identified from tracheal, cloacal, nasal and muscles samples with an incidence of 2.6, 1.1, 1.9 and 7.1%. Serotyping of the isolates was carried out using INTEGRAL SYSTEM STAFILOCOCCI kit and revealed the presence of *Staphylococcus aureus* and coagulase negative *Staphylococci* (*S. capitis*, *S. simulans*, *S. sciuri*, *S. haemolyticus*, *S. xylosus* and *S. saprophyticus*) (Table 3). The highest resistance in most of isolated strains was detected against Erythromycin,

Clarithromycin and Roxitromycin whereas the best drugs of choice for the treatment of infections caused by bacteria mentioned in this study were: Fosfomycin, Rifampicin then Amoxicillin + Clavulanic acid, Ampicillin + Sulbactam, Ceftazidime, Cefuroxime and Levofloxacin, respectively (Table 3).

## DISCUSSION

*Staphylococci*, including *S. aureus* are known to cause a wide variety of diseases ranging in severity from slight skin infections to more severe diseases such as pneumonia and septicaemia [4]. Furthermore, *Staphylococcal* osteomyelitis has been recognized as one of the major problems in broiler chickens by Skeeles [19].

Table 1: Incidence of coagulase negative *Staphylococcus* and coagulase positive *S. aureus* regarding to the type of collected samples:

Samples		Coagulase -ve <i>Staph.</i>		Coagulase +ve <i>S. aureus</i>	
Type	Total no.	*No.	**Percentage	*No.	**Percentage
Tracheal	116	64	55.1	3	2.6
Cloacal	87	40	45.9	1	1.1
Nasal	107	29	27.1	2	1.9
Heart	5	3	60	0	0
Muscles	14	5	35.7	1	7.1
Liver	5	3	60	0	0
Total	334	144	43.1	7	2.1

\*No. Number of tested isolates

\*\*Percentage was calculated according to total number of examined samples

Table 2: The Biochemical characterization of *Staphylococcus* strains using the INTEGRAL SYSTEM STAFILOCOCCI kit:

No.	ADC	UR	MAL	TRE	MAN	XYL	SAC	MNN
1	+	-	+	+	+	-	+	-
2	+	+	-	+	+	-	+	+
3	+	+	+	+	+	-	+	+
4	+	-	-	-	+	-	+	+
5	+	+	-	+	+	-	+	+
6	+	-	-	-	+	-	+	+
7	+	+	-	-	+	+	+	+
8	+	+	+	+	+	-	+	+
9	-	+	-	+	+	-	+	+
10	+	-	-	-	+	-	+	+
11	-	-	+	+	+	-	+	+
12	-	-	+	+	+	-	+	-
13	-	+	+	+	+	-	+	-
14	+	-	+	+	+	-	+	+
15	-	+	+	+	+	+	+	+
16	+	+	+	+	+	-	+	+
17	-	+	+	+	+	+	+	+
18	-	-	-	+	+	-	+	+
19	+	-	-	-	+	-	+	+
20	+	-	-	-	-	-	+	-

+ : positive reaction - : negative reaction

ADC: arginine carboxylase UR: hydrolysis of urea MAL: fermentation of maltose

TRE: fermentation of trehalose MAN: fermentation of mannitol SAC: fermentation of sucrose

XYL: fermentation of xylose MNN: fermentation of mannose

Table 3: The results of resistance and sensitivity of the isolated strains among *Staphylococci*:

No.	Serotype	CN	TOB	AUG	AMS	CAZ	CXM	CIP	LEV	AZM	CLR	ERY	ROX	FOS	RD	SXT	C
1	<i>S. haemolyticus</i>	R	R	S	I	I	S	R	I	R	R	R	R	S	R	R	R
2	<i>S. simulans</i>	S	S	S	I	S	S	I	S	R	R	R	R	S	S	I	R
3	<i>S. aureus</i>	R	R	R	I	R	I	R	R	R	R	R	R	I	R	I	R
4	<i>S. capitis</i>	S	S	I	S	I	S	S	S	S	S	S	S	S	S	S	R
5	<i>S. simulans</i>	I	I	S	S	S	S	R	R	R	R	R	R	S	S	R	R
6	<i>S. capitis</i>	R	R	R	R	I	R	R	R	R	R	R	R	R	R	R	R
7	Unidentified <i>S. species</i>	R	R	S	S	S	S	S	S	R	R	R	R	S	S	R	R
8	<i>S. aureus</i>	S	S	S	S	S	S	S	S	R	R	R	R	S	S	R	R
9	<i>S. simulans</i>	S	S	S	S	S	S	S	S	R	R	R	R	S	S	S	R
10	<i>S. capitis</i>	R	R	S	S	R	I	I	S	R	R	R	R	S	S	R	R
11	<i>S. sciuri</i>	R	R	S	S	S	S	I	I	R	R	R	R	S	S	R	R
12	<i>S. sciuri</i>	S	S	S	S	S	S	S	S	R	R	R	R	S	S	S	R
13	<i>S. saprophyticus</i>	R	R	R	S	R	R	R	R	R	R	R	R	S	S	R	R
14	<i>S. haemolyticus</i>	R	R	S	S	S	S	I	S	R	R	R	R	S	S	R	R
15	<i>S. xylosus</i>	S	S	S	S	S	S	S	S	R	R	R	R	S	S	S	R
16	<i>S. aureus</i>	R	R	R	S	R	R	I	I	R	I	R	R	S	I	R	R
17	<i>S. xylosus</i>	S	S	I	S	I	S	S	S	R	R	R	R	S	S	R	R
18	<i>S. sciuri</i>	R	R	R	R	R	R	R	S	R	R	R	R	S	I	R	R
19	<i>S. capitis</i>	R	R	I	R	I	S	S	I	I	R	R	R	I	I	R	R
20	Unidentified <i>S. species</i>	R	R	R	R	R	I	R	R	R	R	R	R	R	I	R	R

S: Sensitive R: Resistant I: Intermediate resistant

CN: Gentamycin- 8 µg/ml

TOB: Tobramycin- 8 µg/ml

AUG: Amoxicillin + Clavulanic acid -8 µg/ml

AMS: Ampicillin + Sulbactam - 32/16 µg/ml

CAZ: Cefazidime - 32 µg/ml

CXM: Cefuroxime - 32 µg/ml

CIP: Ciprofloxacin - 4 µg/ml

LEV: Levofloxacin - 8 µg/ml

AZM: Azitromycin - 8 µg/ml

CLR: Claritromycin - 8 µg/ml

ERY: Erythromycin - 8 µg/ml

ROX: Roxitromycin - 8 µg/ml

FOS: Fosfomycin - 200µg/ml

RD: Rifampicin - 4µg/ml

SXT: Co- Trimazole - 8µg/ml

C: Growth control for the susceptibility

The purpose of the present study was to investigate the prevalence and serotypes of coagulase negative *Staphylococcus* species isolated from samples collected from apparently healthy broiler chickens using INTEGRAL SYSTEM STAFILOCOCCI kit. In addition to determination of resistance profiles of isolated CoNS strains.

A total of 334 tracheal (n=116), cloacal (n=87), nasal (n=107), muscles (n=14), liver (n=5) and heart (n=5) samples were collected from apparently healthy chickens in broiler farms located in different governorates of Egypt.

The *Staphylococci* isolates were screened by colonial morphology, diffusible pigment production, ability to induce hemolysis and Gram staining. Results present in (Table 1) concluded that 151 (45.2%) of *Staphylococcus* species isolates were isolated from apparently healthy chickens and this is in line with the results obtained by Yurdakul *et al.* [15] who isolated 26 (48%) of *Staphylococcus* species isolates and 4 (4%) of *Enterococci* isolates from 50 chicken meat samples.

Coagulase negative *Staphylococci* were isolated on Mannitol Salt Agar at 37°C for 24 hours as described by El-Jakee *et al.* [14] and typical *Staphylococci* colonies

were obtained. As presented in (Table 2), all *Staphylococcus* isolates were mannitol fermenter positive except one isolate which was negative. The ability for the reduction of tellurite varied in different *Staphylococcus* strains [20]. On the other hand, all isolates were catalase positive but on blood agar medium, most species of coagulase negative *Staphylococcus* species were non-haemolytic. The obtained results indicated that coagulase negative *Staphylococcus* represent 95.4% (144/151) from the total determined *Staphylococcus* isolates. These results are in agreement with those stated by Awan and Matsumoto [4] who found that among 79 *Staphylococcus* isolates, 77 (97.5%) were coagulase-negative. On the other hand, they were inconsistent with the results of Youssef. and Hamed [21] who isolated CoNS from apparently healthy chickens in a farm in Ismailia governorate with incidence rate of 3.4%.

Results in (Table 1) reported that *Staphylococcus* species were isolated from 47.1% of the collected cloacal samples and these results disagreed with the results of SrideviDhanarani, *et al.* [22]. They isolated 120 bacteria from the poultry litter samples and the predominant organisms were *Staphylococcus* (29.1%).

The results of our study concluded that coagulase negative Staphylococcus could be isolated from nares of chicken with incidence rate of 27.1% and this is matched with the results of Kawano *et al.* [23] who isolated CoNS from nares of 72 (25.7%) of the 280 chickens tested in single farm in Japan but a higher incidence was detected by Trkylmaz [24].

In a very recent study, 58 strains of coagulase negative Staphylococci (CoNS) isolated from ready-to-eat food of animal origin bought in the retail market were tested by Wioleta Chajęcka *et al.* [25]. The following species were identified in this study: *S. xylosus* (n ¼ 29/50%), *S. epidermidis* (n ¼ 16/27.6%), *Staphylococcus lentus* (n ¼ 7/12.1%), *S. saprophyticus* (n ¼ 4/6.9%), *Staphylococcus hyicus* (n ¼ 1/1.7%) and *S. simulans* (n ¼ 1/1.7%) while, Glay *et al.* [26], isolated and identified 74 coagulase negative Staphylococci as: 16 *S. simulans* (22%), 11 *S. hyicus* (15%), 8 *S. saprophyticus* (11%), 6 *S. epidermidis* (8%), 4 *S. schleiferi subsp. schleiferi* (5%), 4 *S. arlettae* (5%), 4 *S. lentus* (5%), 4 *S. gallinarum* (5%), 3 *S. chromogenes* (4%), 3 *S. warneri* (4%), 3 *S. haemolyticus* (4%), 2 *S. caprae* (3%), 2 *S. auricularis* (3%), 2 *S. xylosus* (3%) and 2 *S. cohnii* (3%).

Serotyping of 20 isolates revealed the presence of 4 *S. capitis* (20%), 3 *S. aureus* (15%), 3 *S. simulans* (15%), 2 *S. xylosus* (10%), 2 *S. haemolyticus* (10%), 3 *S. sciuri* (15%) and 1 *S. saprophyticus* (5%) in apparently healthy chicken samples.

A total of 7 (4.6%) *S. aureus* isolates were isolated as presented in (Table 1) from nasal, tracheal and cloacal swabs and muscles with an incidence of 6.4, 4.4, 2.4 and 16.6% from the total recovered Staphylococcus isolates respectively. These results are matched with those obtained by Yurdakuet *et al.* [15] and in contrary with the results of Hanning *et al.* [27] who isolated *S. aureus* with higher rate.

Antibiotics are used unconsciously to protect chickens against infections and it is observed that the level of antibiotic resistance has been rising more and more. As illustrated in (Table 3), all strains of *S. aureus* were resistant to Azitromycin, Erythromycin and Roxitromycin. These results disagree with the results of Goswam *et al.* [28] and Baba *et al.* [29] who concluded that 13.33 % and 3.1 % of *S. aureus* were resistant to Erythromycin and with the results of da Silva *et al.* [30] and Gundogan *et al.* [31] who reported low resistance of *S. aureus* strains against Erythromycin.

Results obtained in (Table 3) showed that 66.7% (2/3) of *S. aureus* isolates were resistant to: Gentamycin, Tobramycin, Amoxicillin + Clavulanic acid, Ceftazidime,

Clarithromycin and Co-Trimazole and sensitive to Ampicillin + Sulbactam and Fosfomycin and these results is linked to the results obtained by El-Jakee *et al.* [32] who stated that *S. aureus* showed resistance to Amoxycillin, amoxicillin clavulanic acid and Gentamicin (66.7% each). Most researches were directed to antibiotic resistance of Staphylococci isolated from food focusing on the *S. aureus* species, whereas less attention is paid to the group of coagulase-negative Staphylococci (CoNS) [31].

Results of this study showed that 87% of coagulase negative Staphylococci strains were resistant to Erythromycin, Roxitromycin and Clarithromycin but 33.3% of isolates were resistant to Ciprofloxacin. These findings are in accordance with the findings of Unal and Cinar [13] and Gao *et al.* [33] who stated that coagulase negative Staphylococci strains were resistant to Erythromycin (68.1%), Tetracycline (77.2%), Vancomycin (59%), Teicoplanin (9%), Chloramphenicol (27.2%) and Ciprofloxacin (27.2%) and disagree with the findings of Wioleta Chajęcka-Wierzchowska *et al.* [25] who reported low prevalence (13.8%) of CoNS resistance to Erythromycin.

## CONCLUSIONS

- Coagulase negative *Staphylococci* (CoNS) were considered as non-pathogenic microorganism for many years but the results of recent researches suggest that CoNS can be potentially pathogenic.
- Our results proved that coagulase negative *Staphylococci* strains were present in a considerable rate in apparently healthy broiler chickens.
- INTEGRAL SYSTEM STAFILOCOCCI kit was used in this study for identification and susceptibility testing of Staphylococcus species. It was more accurate, easier, time saver and relatively cheaper than conventional techniques.

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