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Public Health Importance of Biofilm-Producing *Staphylococcus* Species in Poultry Farms, Nearby Wild Bird Residents, and Farm Workers in Egypt

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Abstract

Staphylococcus species is an important foodborne zoonotic pathogen causing diseases in livestock globally. This study focused on identifying biofilm-producing *Staphylococcus species* in six poultry farms and the nearby wild bird populations.120 samples, including poultry organ samples, (trachea and lung) and 80 environmental samples (litter, water, feed, and fans swabs), from 6 broiler farms in Egypt exhibiting respiratory manifestation. Also, workers nasal swabs along with 50 wild birds' faecal samples. Isolation and identification of *Staphylococcus* species were performed using conventional culture techniques and biochemical identification. PCR of the Staphylococcus 16S rRNA gene was performed. A colorimetric microtitration plate assay evaluated the isolates to produce biofilm. The *icaD, eno*, and *fnbA* genes were identified by PCR. A total of 45 isolates were obtained, all of which were identified as *Staphylococcus species* where 30% of organ samples and environmental sources: litter (25%), water (20%), feed (20%), and fan swabs (10%). Additionally, 44% of faecal samples from wild birds and 10% of farm workers nasal swabs Among these, 88.88% (39 out of 45) exhibited varying levels of biofilm production. Molecular analysis showed a high prevalence of biofilm-associated genes, with 35 isolates (77.7%) testing positive for *icaD*, 28 (62.2%) for *eno*, and 39 (86.6%) for *fnbA*. There were no significant differences in *Staphylococcus* isolation across animal sources or between resident and migratory wild birds ($p > 0.05$). Biofilm-producing *Staphylococcus* species are widely prevalent in poultry across Egypt, and the possibility of zoonotic transmission from wild birds to humans is an emerging area of research.

Keywords: Biofilm, Egypt, Poultry, Migratory Birds.

Introduction

The poultry industry is a major sector in global agriculture, significantly contributing to food safety and economic growth. Poultry and poultry products are the most widely consumed meat worldwide, particularly in developing countries [1]. However, the industry has a complicated chain that incorporates feed production, breeding, farming, processing, and marketing, with each segment contributing to the overall economic output [2].

In Egypt, poultry meat is an essential component of the national diet [3]. The industry employs millions of Egyptians by providing jobs in farming, processing, and distribution. Additionally, many small-scale farmers are engaged in poultry farming, contributing to income generation in rural communities [4].

Staphylococcus species are generally harmless bacteria that reside on the surface of human and animal skin as well as on their mucous membranes. However, they can become opportunistic pathogens, leading to various infectious diseases. These bacteria have a notable impact on the ecosystem, public health, and livestock production, causing conditions that range from mild to severe [5].

Staphylococcus includes more than 36 species and 21 subspecies, commonly present as normal residents on the skin, mucous membranes, and in the nostrils of healthy birds. **[6]**. *Staphylococcus aureus* is a bacteria found on the skin and in the nose of approximately 30% of humans, causing no harm but

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occasionally resulting in infections. In healthcare settings, infections can be serious or fatal, resulting in bloodstream infections (sepsis), pneumonia, endocarditis (heart valve infection), and osteomyelitis (bone infection). People with chronic illnesses, those who inject drugs, and those in intensive care or using medical devices are at a higher risk **[7].**

Staphylococcal infections can pose serious risks on poultry farms and can lead to various health problems. Furthermore, they carry the potential for zoonotic transmission to humans, raising significant public health concerns [8].

Farm workers, handlers, and others who have close contact with infected poultry can contract the infection through skin-to-skin contact, especially if they have cuts or abrasions resulting in skin infections such as boils, abscesses, or more severe conditions like cellulitis [9].

In Egypt, the prevalence of staphylococcal infection varies by region, with greater rates reported in ICU patients and post-surgical infections. The germs are prevalent in both hospital and community settings. Furthermore, it poses a public health danger in veterinary settings, with high frequency in domestic animals, bovine mastitis, and food products such as raw milk and fish. These findings emphasize the significance of ongoing surveillance, good hygiene, and infection control measures in preventing their spread throughout the human, animal, and food sectors [10]. Improperly handled or undercooked poultry meat and eggs can lead to food poisoning due to the production of toxins, resulting in rapid-onset foodborne illness characterized by symptoms such as nausea, vomiting, diarrhea, and abdominal pain [11].

Staphylococcus species can also persist on surfaces like equipment, cages, and feed troughs. Handling contaminated equipment can facilitate transmission, as touching these surfaces and subsequently touching the face, eyes, or open wounds can lead to infection [12,13].

Poor personal hygiene among farm workers heightens the risk of bacterial transmission. Additionally, overcrowded conditions and stress in birds can weaken their immune systems, making them more susceptible to infections and increasing their severity. This, in turn, raises the bacterial load and the risk of transmission to humans. Furthermore, mishandling poultry products during processing, transportation, or at retail can result in contamination posing a risk to consumers [14].

Over the past decade, interest has increased in the global prevalence of *Staphylococcus species* in wild animals, though information remains limited. Wild animals are seen as potential reservoirs or carriers for transmission. Their movement across habitats interactions with livestock and indirect contact with humans can facilitate bacterial spread, raising the risk of colonization and infection in both animals and humans [15,16,17].

The main virulence factor of *Staphylococcus species* is their ability to form biofilms on damaged tissues as it enhances bacterial survival by shielding them from the host's immune system and reducing antibiotic effectiveness [18,19].

Several genes contribute to the production of staphylococcal biofilms, though their regulatory mechanisms are not well understood [20,21] and these genes are associated with chronic infections, particularly those involving indwelling medical devices like catheters, prosthetic joints, and heart valves [22].

The *ica* (intercellular adhesion) locus is a key genetic determinant, with the *icaD* operon responsible for synthesizing poly-N-acetylglucosamine (PNAG), a vital component of the biofilm matrix [23]. The *fnbA* gene encodes Fibronectin-Binding Protein A, which facilitates initial adhesion to host tissues and medical devices, essential for biofilm formation [24].

Furthermore, the *eno* gene produces the essential glycolytic enzyme enolase, which catalyzes the transformation of 2-phosphoglycerate into phosphoenolpyruvate during glycolysis. This reaction is vital for the survival and growth of *Staphylococcus* bacteria, as it produces the energy necessary for various cellular activities [25]. Moreover, it can localize to the bacterial cell surface and bind to plasminogen, where Plasminogen-binding proteins play a role in bacterial adhesion to host tissues, which is a crucial step in both infection and biofilm formation.

In poultry farms, biofilm formation on equipment, surfaces, and within the birds can lead to chronic infections, decreased productivity, and higher mortality rates. Furthermore, migratory birds can act as carriers, introducing or spreading biofilm-forming *Staphylococcus species*, which complicates biosecurity measures. Therefore, this study aimed to detect biofilm-forming *Staphylococcus species* within poultry farms and the surrounding migratory birds in Egypt, as these strains may present a potential hazard to human and animal health through spread in the environment.

Material and methods

Samples collection and processing:

A total of 20 poultry organ samples (trachea and lung from freshly morbid chicken) and 80 environmental samples (litter, water, feed and fans swabs) were collected from 6 broiler farms in Egypt exhibiting respiratory manifestation during the period from May 2022 to April 2023. The samples

were transferred in boxes surrounding ice cubes and directly processed in the laboratory for bacterial isolation.

Between August 2022 and January 2023, a total of 50 faecal samples were taken from wild birds in the Giza and El-Fayoum governorates of Egypt. Of these, 30 were resident birds (7 hooded crows, 11 cattle egrets, and 12 laughing doves) and 20 were migratory birds (12 Northern shoveler ducks and 8 green-winged teal ducks). During the winter migration, the wild birds were caught using modified traps. Following trapping, faecal samples were collected, and the birds were liberated. The swabs were then placed in 2 ml of sterile saline solution (0.9% NaCl) and stored in an icebox until transport to the laboratory.

The 20 nasal swabs were obtained from apparently healthy farm workers working on broiler farms and in the vicinity where wild birds were trapped. Samples were collected using sterile swabs, subsequently They were then transferred to tubes containing trypticase soy broth and incubated at 37 °C for 24 hours.

Isolation and identification of Staphylococcus species:

Before plating on mannitol salt agar medium (Oxoid, Hampshire, UK), each inoculated sample was incubated aerobically at 37°C for 24 hours after being incubated overnight in 5 ml of Brain heart infusion broth (Oxoid- Hampshire- UK). suspected *Staphylococcus* species colonies were subculture to produce a pure culture and subsequently analysed for colony morphology, gram staining, standard biochemical tests, and coagulase tests [26, 27].

Molecular identification of Staphylococcus species:

Using the boiling approach, genomic DNA was extracted from isolates [28]. All *Staphylococcus* isolates were molecularly confirmed via PCR using *Staphylococcus* 16S rRNA primers to ascertain the *Staphylococcus* genus [29]. The reaction mixtures were prepared with a whole volume of 25 µl, comprising 3 µl of template DNA from each isolate, 12.5 µl of Emerald Amp MAX PCR master mix (Takara, Japan), 0.5 µl of each primer (10 pmol/l; Metabion-Germany), and added with PCR-grade water to reach 25 µl. The PCR amplicons underwent electrophoresis on agarose gel (1.5%) and were seen using ultraviolet light. The designated oligonucleotide primer set, and amplification settings are presented in Table 1.

Formation of the biofilm:

The biofilm quantification assay was performed in 96-well polystyrene microplates following the methodology described by Stepanović et al. [30]. A 200 µL aliquot of each staphylococcus isolate, grown in BHI broth at 37°C for 24 hours, was added three times to 96-well flat-bottom plastic microtiter plates with lids. The microplates were incubated at 37°C for 24 hours. After incubation, the bacterial suspensions in each well were discarded, and the wells were washed three times with 250 µL of sterile saline solution (0.9% NaCl). Each well was then treated with 200 µL of methanol for 15 minutes, followed by gradual elimination of the methanol, and the plates were left to dry at room temperature (30°C). Next, the wells were treated with 200 µL of crystal violet solution for 5 minutes, rinsed with running water, and allowed to dry at 30°C. Biofilm formation capacity was assessed by measuring absorbance using an enzyme-linked immunosorbent assay reader (BioRad, model 550, Srl, Italy) at a wavelength of 570 nm, and results were categorized according to Stepanović et al. [30].The negative control was BHI broth devoid of bacterial inoculum, and we determined the optical density (ODi) for every isolate (ODi) by averaging the results from three wells and comparing it to the optical density of the negative control (ODc). The results were put into four groups based on the difference between the mean optical density (OD) and the negative control (ODc): not adhering (−) if ODi < ODc; weakly adhering (+) if ODc < ODi ≤ 2 × ODc; moderately adhering (++) if 2 × ODc < ODi ≤ 4 × ODc; and strongly adhering (+++) if 4 × ODc < ODi.

The molecular detection for staphylococcus biofilm genes:

The *Staphylococcus* isolates were studied for the presence of the biofilm genes *icaD*, *eno*, and *fnbA*. The primers utilized were provided by Metabion, Germany, and the anticipated products are detailed in Table 1. Primers were employed in a 25-µl reaction comprising 12.5 µl of EmeraldAmp Max PCR Master Mix (Takara, Japan), 1 µl of each primer at a concentration of 20 pmol, 5.5 µl of water, and 5 µl of DNA template. The reaction was conducted in an Applied Biosystems 2720 thermal cycler. The PCR products were separated via electrophoresis on agarose gel (1.5%) (Applichem-Germany-GmbH) in 1x TBE buffer at ambient temperature using a gradient of 5V/cm. 20 microliters of the PCR products were deposited in each gel slot for examination. A GeneRuler 100 bp ladder (Fermentas-Thermo-Germany) and a GeneDirex 100-3000 bp DNA ladder H3 RTU (GeneDirex-Taiwan) were used to determine fragment sizes. The gel images were captured using a gel documentation system (Alpha Innotech, Biometra), and data analysis was conducted using specialized computer software.

Statistical analysis:

The chi-square test ($\chi 2$) was employed to ascertain the correlation among the *Staphylococcus* species occurrences in various animal and environmental samples. Statistics were considered significant when $p<0.05$.

Results

Occurrence of Staphylococcus species strains:

A total of 23 out of 120 samples (19.6%) tested positive for *Staphylococcus* species from 6 poultry farms in Egypt exhibiting respiratory manifestation and from apparently healthy farm workers residing in the poultry farms. using bacteriological identification. Poultry organs had the highest prevalence (30%), followed by litter (25%), feed and water (20% each), and fans and worker nasal swabs (10% each). The continuous finding of *Staphylococcus* species in ambient and human samples emphasizes potential cross-contamination and the farm environment's significance as a reservoir for bacterial persistence.

A total of 22 *Staphylococcus species* isolates (44%) were recovered from 50 faecal samples represented in 30 (50%) resident wild birds which probably reflect local contamination, revealing continuous exposure to the farm environment and 20 (40%) migratory wild birds which may act as carriers, initiating the infection into new environments.

All isolates were confirmed by identifying 16sDNA genes. There are no significant differences in isolating staphylo*coccus* from various animal sources. (p >0.05). furthermore, no distinction between resident wild birds and those that migrate (p>0.05).

Phenotypic and genotypic characterization of biofilm formation:

In the phenotypic test, regarded as the "gold standard" for biofilm detection, 88.88% (39 out of 45) of the isolates were recognized as biofilm producers. These isolates produced biofilms to varying extents and were classified as follows: about 17.7% (6/45) were weak biofilm producers, 33.3% (15/45) were moderate biofilm producers, and 35.5% (16/45) were strong producers (Table 5 - 6). There are no significant differences in the biofilm formation density of *Staphylococcus* from various animal sources (p >0.05). The presence of *icaD*, eno, and fnbA genes in the isolates was analyzed by PCR and revealed high prevalence where (35 (77.7%), 28 (62.2%), and 39 (86.6%) were positive for *icaD*, *eno,* and *fnbA* genes respectively) (Table 6).

According to our findings, *Staphylococcus* species were found to be prevalent in poultry farms, including in wild birds and farm workers, highlighting its potential role as a zoonotic pathogen and emphasizing the need for continuous surveillance and strict hygiene measures to prevent transmission between animals, humans, and the environment, which could pose a significant public health risk.

Discussion

Staphylococcal infections can cause severe health problems and economic losses in poultry farming. This study found that the overall isolation rate of *Staphylococcus species* from 20 poultry organ samples across six poultry farms was 30%, as shown in Table 2. Gonçalves-Tenório et al. stated similar observations [31].

In proportion to Pepe et al. [32] staphylococci are among the highest frequent bacteria found in the environment of poultry, capable of entering a bird's body through skin injuries or wounds, leading to localized or systemic infections. In severe cases, the infection can spread to internal organs, reducing growth, poor meat quality, and even mortality. Staphylococcal infections are especially concerning because of their potential for antibiotic resistance, which complicates treatment efforts in affected poultry [33]. Also, Poor hygiene and sanitation during poultry farming, slaughtering, and processing can facilitate the spread of Staphylococcal infections from the environment, handlers, or equipment to the birds [34,35].

Corresponding to Song et al. [36], Farmworkers and handlers can serve as carriers of *Staphylococcus* bacteria, inadvertently introducing them into the farm environment. Poor hygiene and improper handling of poultry increase the risk of bacterial spread. Additionally, contaminated equipment, feed, or water can serve as sources of *Staphylococcus* transmission. Regular interactions between humans and birds, such as during feeding, vaccination, or treatment, can further facilitate the spread of the bacteria, especially when biosecurity measures are not strictly enforced. and this is proven by the results we obtained in Table 2 where from 80 environmental samples (litter, water, feed, and fans swabs) and 20 nasal swaps from apparently healthy farm workers the results were 18.75% and 10% respectively.

The detection of *Staphylococcus species* in poultry farm environments such as feed, water, fans, walls, and litter signals serious contamination risks that can lead to poultry infections, this situation obscures the treatment and potentially endangers human health through the zoonotic transmission and the foodborne illnesses [36].

It is crucial to implement stringent biosecurity measures, maintain good hygiene practices, and conduct regular monitoring to mitigate economic losses and protect both poultry and public health. Effective environmental management, including thorough cleaning, disinfection, and litter control, is essential to reduce bacterial loads and prevent disease outbreaks [37]. Overall, the presence of Staphylococcal infection in poultry farms represents

a significant infection risk with important public health implications.

According to Table 4, A microbiological detection of the faecal examples of migratory birds showed the presence of *Staphylococcus species* with an incidence of 44%. This result was significantly higher than that reported by Dalton et al. [37] who detected 17.6% of *Saphylococcus* species in migratory birds in Saudi Arabia. This observation proves that migratory birds can carry greatly infective bacteria capable of infecting humans, either directly or indirectly through environmental transmission. Therefore, it is crucial to recognize the potential role of migratory birds in spreading these pathogens [38, 39].

Despite the significant role bacterial biofilms play in various diseases, they are often overlooked. The microtitration test showed that 88.88% of the isolates produced biofilms: 17.77% were weak biofilm producers, 33.33% were moderate producers, and 35.55% were strong biofilm producers. (Table 5). The current study results are in line with Pinto et al. [40] who discussed that the prevalence of biofilm-producing *Staphylococcus* species is notably high, particularly in healthcare environments. Nevertheless, the present study results contradict those results obtained by Mathur et al. [41] and Nasr et al. [42]. who reported 57.8% and 46% respectively displayed a biofilm-positive phenotype.

Biofilms allow bacteria to adhere to surfaces (for example bird feathers, beaks, or the environment) and resist environmental stressors. The PCR revealed high occurrence for those genes where 35 (77.7%), 28 (62.2%), and 39 (86.6%) were positive for *icaD, eno,* and *fnbA* genes, respectively. (Table 6). The ability of these strains to form biofilms after being isolated from chicken houses indicates that they can adapt and survive in the environment without a host [43]. Therefore, effective cleaning and disinfection practices are essential in poultry farms to prevent the growth and persistence of these pathogens in the farm environment.

Biofilm-producing *Staphylococcus* species in wild birds represent a significant concern for both wildlife and environmental health. Our results showed that both migratory and resident wild birds carry biofilm-producing *Staphylococcus* species where 9/10(90%) and 10/12 (83.33) were positive in migratory and resident wild birds' samples respectively (Table 5).

Biofilm formation in wild birds can result in chronic infections and act as reservoirs for pathogens, which can infect other animals or humans through contact [44]. The presence of biofilm-producing *Staphylococcus* in these birds is influenced by environmental factors, diet, and habitat. Birds in urban or agricultural areas are more likely to encounter human-related bacteria, increasing their risk of acquiring such strains [45].

Research on biofilm formation in poultry emphasizes the cause of bacterial communities in bird inhabitants and the role of wild birds' communities in the transmission of the diseases which can help in evolving approaches for monitoring wildlife health and mitigating the hazard of zoonoses [46].

Conclusion

In the present study, biofilm-producing *Staphylococcus* species were screened in poultry farms in Egypt and wild birds residing in the surrounding area. the persistence of biofilm-producing *Staphylococcus species* in our results highlights the need for enhanced prevention and treatment strategies and represents further evidence for the potential role of migratory wild birds in the global dissemination of biofilm genes that pose a serious challenge to the globe.

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Author contributions

All authors performed the collection of samples and the molecular detection of the target genes. Also, performed the analysis and interpretation of the data and the writing of the manuscript. All authors read and approved the final version of the manuscript.

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Consent for publication

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Competing interests

The authors declare no competing interests.

Ethical of approval

The research was conducted according to the guidelines of the Ethical Committee of the Faculty of Veterinary Medicine, Cairo University, Egypt, and approved by the Institutional Animal Care and Use Committee, ethics approval number; (vet CU13102024977).

TABLE 1. The sequence of oligonucleotide primers used for PCR amplification of the *Staphylococcus* 16S rRNA and biofilm genes.

	Primer sequence (5′to 3′)	Cycling Conditions	Bp	Reference
16S(rRNA)	**F:** AAC TCT GTT ATT AGG GAA GAACA **R:** CCA CCT TCCTCC GGT TTG TCA CC	94 ºC ,5 min; 30 cycles (94 ºC , 1 min; 50 ºC ,1 min; 72 ºC , 1 min) 72 ºC ,5 min	756	**[29]**
icaD	**F:** AAA CGTAAG AGA GGT GG **R:**GGC AAT ATG ATC AAGATA	(94˚C 5 min., 94˚C 30 sec., 49˚C 40 sec, 72˚C 40 sec, 72˚C 10 min).	381 bp	**[47]**
fnbA	**F:** CATAAATTGGGAGCAGCATCA **R:**ATCAGCAGCTGAATTCCCATT	(94˚C 5 min.,94˚C 30 sec.,58˚C 30 sec.,72˚C 30 sec.,72˚C 7 min.)	127 bp	**[48]**
Eno	**F:** ACGTGCAGCAGCTGACT **R:**CAACAGCATYCTTCAGTACCTTC	(94˚C 5 min.,94˚C 30 sec.,55˚C 30 sec.,72˚C 30 sec.,72˚C 7 min.)	205 bp	**[49]**

TABLE 2. the Occurrence of *Staphylococcus species* in the poultry farms and the workers:

Sample type	Sample size	Positive for *Staphylococcus species* (%)	X^2	*p*-value
Poultry organs	20	6 (30)	**4.158**	**0.384**
Litter	20	5 (25)		
Feed	20	4 (20)		
Water	20	4 (20)		
Fans swabs	20	2 (10)		
Worker nasal swaps	20	2 (10)		
Total	120	23 19.6)		

X^2: chi-square *The result is significant at $p < .05$.

TABLE 3. Types of migratory birds sampled.

Common name	Scientific name	Place	Condition	Sampling	Number of birds
Hooded Crow	*Corvus Cornix*	Giza,	Resident birds	Fecal sample	7
Laughing Dove	*Streptopelia Senegalensis*	Giza	Resident birds	Fecal sample	12
Cattle Egret	*Bubulcus Ibis*	Giza	Resident birds	Fecal sample	11
Northern Shoveler	*Anas Clypeata*	El-Fayoum	Migratory birds	Fecal sample	12
Green-Winged Teal	*Anas Carolinensis*	El-Fayoum	Migratory birds	Fecal sample	8
Total			**50**		

TABLE 4. The Occurrence of *Staphylococcus species* in wild birds' samples.

Wild Bird samples	Sample size	Positive for *Staphylococcus species* (%)	X^2	*p*-value
Migratory	20	10 (50)	**0.487**	**0.485**
Resident	30	12 (40)		
Total	50	22 (44)		

X^2: chi-square *The result is significant at $p < .05$.

TABLE 5. Phenotypic biofilm profile of 45 *Staphylococcus* isolates from different isolation sources.

Sample	Non-biofilm (%)	Weak (%)	Moderate (%)	Strong (%)	Total positive (%)
Poultry diseased organs	1/6(16.66)	2/6(33.3)	2/6(33.3)	1/6(16.66)	5(83.33)
Litter swabs	1/5(20)	1/5(20)	2/5(40)	1/5(20)	4(80)
Feed swabs	0/4(0)	1/ 4 (25)	2/4(50)	1/ 4 (25)	4(100)
Water swabs	1/ 4 (25)	1/ 4 (25)	2/4(50)	0/ 4(0)	3(75)
Fans swabs	0/2 (0)	0/2 (0)	0/2 (0)	2/2(100)	2 (100)
Worker nasal swaps	1/2 (0)	0/2 (0)	1/2 (50)	1/ 2(50)	2 /2 (100)
Migratory Wild birds	0/10(0)	1/10(10)	3/10(30)	5/10(50)	9/10(90)
Resident Wild birds	2/12(16.66)	2/12 (16.66)	3/12 (25%)	5/12 (41.66)	10/12 (83.33)
Total	6/45(13.33)	8/45(17.77)	15/45(33.33)	16/45(35.55)	39/45(88.88)
p-value	0.258	0.503	0.822	0.461	

The result is significant at $p < .05$.

TABLE 6. Phenotypic and genotypic biofilm profile of 45 *Staphylococcus* species:

Biofilm formation profile	Isolates number (%) Sum number (45)	The related genes		
		icaD	***fnbA***	***Eno***
Weak biofilm producer	8 (17.77)	7	5	8
Moderate biofilm producer	15 (33.33)	12	10	15
Strong biofilm producer	16 (35.55)	16	13	16
Total biofilm producer	39 (86.6)	35(77.7)	28(62.2)	39(86.6)

References

1. Attia, Y.A., Rahman, M.T., Hossain, M.J., Basiouni, S., Khafaga, A.F., Shehata, A.A. and Hafez, H.M. Poultry production and sustainability in developing countries under the COVID-19 crisis: Lessons learned. *Animals,* **12**(5),644. (2022). https://doi.org/10.3390/ani12050644

2. Javaid, M., Haleem, A., Singh, R.P. and Suman, R., Enhancing smart farming through the applications of Agriculture 4.0 technologies. *International Journal of Intelligent Networks,* **3**, 150-164(2022). .https://doi.org/10.1016/j.ijin.2022.09.004

3. Hassan Wassef, H., Food habits of the Egyptians: newly emerging trends. *EMHJ-Eastern Mediterranean Health Journal*, **10** (6), 898-915 (2004). https://doi.org/10.26719/2004.10.6.898

4. Dhillon, R. and Moncur, Q., Small-scale farming: A review of challenges and potential opportunities offered by technological advancements. *Sustainability*, **15**(21),15478(2023). https://doi.org/10.3390/su152115478

5. Rossi, C. C., Pereira, M. F., and Giambiagi-deMarval, M. Underrated Staphylococcus species and their role in antimicrobial resistance spreading. *Genetics and Molecular Biology,* **43,** e20190065(2020). https://doi.org/10.1590/1678-4685-GMB-2019-0065

6. Logue, C. M., Andreasen, C. B., Borst, L. B., Eriksson, H., Hampson, D. J., Sanchez, S., and Fulton, R. M. Other bacterial diseases. *Diseases of Poultry*, 995-1085. (2020). https://doi.org/10.1002/9781119371199.ch23

7. Szafraniec, G. M., Szeleszczuk, P., and Dolka, B. Review on skeletal disorders caused by Staphylococcus spp. in poultry. *Veterinary Quarterly*, **42**(1), 21-40. (2022). https://doi.org/10.1080/01652176.2022.2033880

8. Zaheer, Z., Rahman, S. U., Zaheer, I., Abbas, G., and Younas, T. Methicillin-resistant Staphylococcus aureus in poultry-an emerging concern related to future epidemic. *Matrix Sci. Medica*, **1**(1), 15-18. (2017). DOI: 10.26480/msm.01.2017.15.18

9. Varela, K., Brown, J. A., Lipton, B., Dunn, J. and Stanek, D., NASPHV Committee Consultants, ... and Yager, C. M. A review of zoonotic disease threats to pet owners: A compendium of measures to prevent zoonotic diseases associated with non-traditional pets such as rodents and other small mammals, reptiles, amphibians, backyard poultry, and other selected animals. *Vector-Borne and Zoonotic Diseases*, **22**(6), 303-360(2022). https://doi.org/10.1089/vbz.2022.0022

10. Abdeen, E. E., Mousa, W. S., Abdelsalam, S. Y., Heikal, H. S., Shawish, R. R., Nooruzzaman, M., and Abdeen, A. Prevalence and characterization of coagulase positive Staphylococci from food products and human specimens in Egypt. *Antibiotics,* **10**(1), 75. (2021). https://doi.org/10.3390/antibiotics10010075

11. Bintsis, T. Foodborne pathogens. *AIMS Microbiology*, **3**(3), 529. (2017). DOI: 10.3934/microbiol.2017.3.529

12. Ibrahim, A. N., Khalefa, H. S., and Mubarak, S. T. Residual contamination and biofilm formation by opportunistic pathogens Escherichia coli, Klebsiella pneumoniae, and Pseudomonas aeruginosa in poultry houses isolated from drinking water systems, fans, and floors. *Egyptian Journal of Veterinary Sciences*, **54**(6), 1041-1057(2023). DOI: 10.21608/ejvs.2023.211147.1502

13. Kalu, I. C., Kao, C. M., and Fritz, S. A. Management and prevention of Staphylococcus aureus infections in children. *Infectious disease clinics of North America,* **36**(1), 73-100. (2022). DOI: 10.1016/j.idc.2021.11.006

14. Bencardino, D., Amagliani, G., and Brandi, G. Carriage of Staphylococcus aureus among food handlers: An ongoing challenge in public health. *Food Control,* **130**, 108362. (2021). DOI:10.1016/J.FOODCONT.2021.108362.

15. Ahmed, Z. S., Elshafiee, E. A., Khalefa, H. S., Kadry, M., and Hamza, D. A. Evidence of colistin resistance genes (mcr-1 and mcr-2) in wild birds and its public health implication in Egypt. *Antimicrobial Resistance and Infection Control*, **8**, 1-8. (2019). DOI: 10.1186/s13756-019-0657-5.

16. Penna, B., Silva, M. B., Soares, A. E., Vasconcelos, A. T., Ramundo, M. S., Ferreira, F. A., and Figueiredo, A. M. Comparative genomics of MRSA strains from human and canine origins reveals similar virulence gene repertoire. *Scientific Reports*, **11**(1), 4724. (2021). DOI: 10.1038/s41598-021-83993-5.

17. Silveira, D. R., de Moraes, T. P., Kaefer, K., Bach, L. G., de Oliveira Barbosa, A., Moretti, V. D., and Timm, C. D. MRSA and enterobacteria of one health concern in wild animals undergoing rehabilitation. *Research, Society and Development,* **10**(1), e34810111809-e34810111809. (2021). DOI: https://doi.org/10.33448/rsd-v10i1.11809

18. Mack, D., Rohde, H., Harris, L. G., Davies, A. P., Horstkotte, M. A., and Knobloch, J. M. Biofilm formation in medical device-related infection. *The International Journal of Artificial Organs,* **29**(4), 343-359(2006). DOI: 10.1177/039139880602900404

19. Otto, M. Staphylococcus epidermidis—the'accidental'pathogen. *Nature Reviews Microbiology*, **7**(8), 555-567 (2009). DOI: 10.1038/nrmicro2182

20. Ferreira, A. A., Tette, P. A. S., Mendonça, R. C. S., Soares, A. D. S., andCarvalho, M. M. D. Detection of exopolysaccharide production and biofilm-related genes in Staphylococcus spp. isolated from a poultry processing plant. *Food Science and Technology*, **34**, 710-716 (2014). DOI:10.1590/1678-457X.6446

21. Laban, S. E., Arafa, A. A., Ibrahim, E. S., FE, E., and Khalefa, H. S. *International Journal of Veterinary Science,* **14**(1), 25-31. (2025). https://doi.org/10.47278/journal.ijvs/2024.197

22. Zhao, A., Sun, J., and Liu, Y. Understanding bacterial biofilms: From definition to treatment strategies. *Frontiers in Cellular and Infection Microbiology,* **13**, 1137947. (2023). DOI: 10.3389/fcimb.2023.1137947.

23. Cramton, S. E., Gerke, C., Schnell, N. F., Nichols, W. W., and Götz, F. The intercellular adhesion (ica) locus is present in Staphylococcus aureus and is required for biofilm formation. *Infection and Immunity*, **67**(10), 5427-5433. (1999). DOI: 10.1128/IAI.67.10.5427-5433.1999

24. Speziale, P., and Pietrocola, G. The multivalent role of fibronectin-binding proteins A and B (FnBPA and FnBPB) of Staphylococcus aureus in host infections. *Frontiers in Microbiology,* **11,** 2054. (2020). DOI: 10.3389/fmicb.2020.02054

25. Avilán, L., Gualdrón-López, M., Quiñones, W., González-González, L., Hannaert, V., Michels, P. A., and Concepción, J. L. Enolase: a key player in the metabolism and a probable virulence factor of trypanosomatid parasites—perspectives for its use as a therapeutic target. *Enzyme Research*, **2011** (1), 932549. (2011). DOI: 10.4061/2011/932549

26. Quinn, P. J., Markey, B. K., Leonard, F. C., Hartigan, P., Fanning, S., and Fitzpatrick, E. Veterinary microbiology and microbial disease. *John Wiley and Sons.* (2011). cabidigitallibrary.org/doi/full/10.5555/20123275549

27. Abdel-Moein, K. A., and Zaher, H. M. The nasal carriage of coagulase-negative staphylococci among animals and its public health implication. *Vector-Borne and Zoonotic Diseases,* **20**(12), 897-902. (2020). DOI: 10.1089/vbz.2020.2656

28. Oliveira, C. F. D., Paim, T. G. D. S., Reiter, K. C., Rieger, A., and D'azevedo, P. A. Evaluation of four different DNA extraction methods in coagulase-negative Staphylococci clinical isolates. *Revista do Instituto de Medicina Tropical de São Paulo,* **56**(1), 29-33. (2014). DOI:10.1590/S0036-46652014000100004

29. Zhang, K., Sparling, J., Chow, B. L., Elsayed, S., Hussain, Z., Church, D. L., and Conly, J. M. New quadriplex PCR assay for detection of methicillin and mupirocin resistance and simultaneous discrimination of Staphylococcus aureus from coagulase-negative staphylococci. *Journal of Clinical Microbiology,* **42**(11), 4947-4955(2004). DOI: 10.1128/JCM.42.11.4947-4955.2004

30. Stepanović, S., Vuković, D., Dakić, I., Savić, B., and Švabić-Vlahović, M. A modified microtiter-plate test for quantification of staphylococcal biofilm formation. *Journal of Microbiological Methods*, **40**(2), 175-179. (2000). DOI:10.1016/s0167-7012 (00)00122-6

31. Gonçalves-Tenório, A., Silva, B. N., Rodrigues, V., Cadavez, V., and Gonzales-Barron, U. Prevalence of pathogens in poultry meat: a meta-analysis of European published surveys. *Foods,* **7**(5), 69(2018). DOI: 10.3390/foods7050069

32. Pepe, O., Blaiotta, G., Bucci, F., Anastasio, M., Aponte, M., and Villani, F. Staphylococcus aureus and staphylococcal enterotoxin A in breaded chicken products: detection and behavior during the cooking process. *Applied and Environmental Microbiology,* **72**(11), 7057-7062(2006). DOI:10.1128/AEM.00198-06

33. Szafraniec, G. M., Szeleszczuk, P., and Dolka, B. Review on skeletal disorders caused by Staphylococcus spp. in poultry. *Veterinary Quarterly,* **42**(1), 21-40 (2022). DOI: 10.1080/01652176.2022.2033880

34. Haag, A. F., Fitzgerald, J. R., and Penadés, J. R. Staphylococcus aureus in Animals. *Microbiology Spectrum,* **7**(3), 10-1128. (2019). DOI: 10.1128/microbiolspec.GPP3-0060-2019

35. Abdelmalek, S., Kadry, M., Elshafiee, E. A., Hamed, W., Moussa, I. M., Al-Maary, K. S., and Elbehiry, A. Occurrence of Salmonella infection and antimicrobial susceptibility for local Salmonella isolates from different sources in a cross-sectional study. *Italian Journal of Food Safety*, **8**(4),8525 (2019). doi: 10.4081/ijfs.2019.8525.

36. Song, M., Tang, Q., Ding, Y., Tan, P., Zhang, Y., Wang, T., and Ma, X. Staphylococcus aureus and biofilms: transmission, threats, and promising. *Journal of Animal Science and Biotechnology,* **15**,44 *(*2024). DOI: 10.1186/s40104-024-01007-6

37. Dalton, K. R., Rock, C., Carroll, K. C., and Davis, M. F. One Health in hospitals: how understanding the dynamics of people, animals, and the hospital built-environment can be used to better inform interventions for antimicrobial- resistant gram-positive infections. *Antimicrobial Resistance and Infection Control*, **9**, 1-17. 461 (2020). DOI: 10.1186/s13756-020-00737-2

38. Khalefa, H. S., Ahmed, Z. S., Abdel-Kader, F., Ismail, E. M., and Elshafiee, E. A. Sequencing and phylogenetic analysis of the stn gene of Salmonella species isolated from different environmental sources at Lake Qarun protectorate: The role of migratory birds and public health importance. *Veterinary World,* **14**(10), 2764 (2021). DOI: 10.14202/vetworld.2021.2764-2772

39. Elshafiee, E. A., Khalefa, H. S., Al-Atfeehy, N. M., Amer, F., Hamza, D. A., and Ahmed, Z. S. Biofilms and efflux pump regulatory gene (mexR) in multidrug-resistant Pseudomonas aeruginosa isolated from migratory birds in Egypt. *Veterinary World*, **15**(10), 2425(2022). DOI: 10.14202/vetworld.2022.2425-2431

40. Pinto, H., Simões, M., and Borges, A. Prevalence and impact of biofilms on bloodstream and urinary tract infections: a systematic review and meta-analysis. *Antibiotics,* **10**(7), 825 (2021). DOI: 10.3390/antibiotics10070825

41. Mathur, T., Singhal, S., Khan, S., Upadhyay, D. J., Fatma, T., and Rattan, A. Detection of biofilm formation among the clinical isolates of staphylococci: an evaluation of three different screening methods. *Indian Journal of Medical Microbiology*, **24**(1), 25-29(2006). DOI: 10.4103/0255-0857.19890

42. Nasr, R. A., AbuShady, H. M., and Hussein, H. S. Biofilm formation and presence of icaAD gene in clinical isolates of staphylococci. *Egyptian Journal of Medical Human Genetics,* **13**(3), 269-274 (2012). DOI:10.1016/J.EJMHG.2012.04.007

43. Lamas, A., Miranda, J. M., Vázquez, B., Cepeda, A., and Franco, C. M. Biofilm formation, phenotypic production of cellulose and gene expression in Salmonella enterica decrease under anaerobic conditions. *International Journal of Food Microbiology,* **238,** 63-67 (2016). DOI: 10.1016/j.ijfoodmicro.2016.08.043

44. Mendhe, S., Badge, A., Ugemuge, S., and Chandi, D. Impact of Biofilms on Chronic Infections and Medical Challenges. *Cureus,* **15**(11), 48204 (2023). DOI: 10.7759/cureus.48204

45. Silva, V., Pereira, J. E., Maltez, L., Poeta, P., and Igrejas, G. Influence of environmental factors on biofilm formation of Staphylococci isolated from wastewater and surface water. *Pathogens,* **11**(10), 1069. (2022). DOI: 10.3390/pathogens11101069

46. Wang, X., Liu, M., Yu, C., Li, J., and Zhou, X. Biofilm formation: mechanistic insights and therapeutic targets. *Molecular Biomedicine*, **4**(1), 49(2023). https://doi.org/10.1186/s43556-023-00164-w

47. Ciftci, A., Findik, A., Onuk, E. E., and Savasan, S. Detection of methicillin resistance and slime factor production of Staphylococcus aureus in bovine mastitis. *Brazilian Journal of Microbiology*, **40**, 254-261. (2009). DOI: 10.1590/S1517-8382200900020000

48. Vancraeynest, D., Hermans, K., and Haesebrouck, F. Genotypic and phenotypic screening of high and low virulence Staphylococcus aureus isolates from rabbits for biofilm formation and MSCRAMMs. *Veterinary microbiology*, **103**(3-4), 241-247. (2004). https://doi.org/10.1016/j.vetmic.2004.09.002

49. Tristan, A., Ying, L., Bes, M., Etienne, J., Vandenesch, F., and Lina, G. Use of multiplex PCR to identify Staphylococcus aureus adhesins involved in human hematogenous infections. *Journal of clinical microbiology*, **41**(9), 4465 4467. (2003). DOI: 10.1128/JCM.41.9.4465-4467.2003

الأهمية الصحية العامة لأنواع المكورات العنقودية المنتجة للغشاء الحيوي في مزارع الدواجن، الطيور البرية المحيطة، وعمال المزارع في مصر.

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الملخص

تُعد أنواع المكورات العنقودية من مسببات الأمراض الحيوانية المنشأ التي تنتقل عن طريق الأغذية، والتي تعرف بتسببها في أمراض الماشية على مستوى العالم. هدفت هذه الدراسة على تحديد الأنواع المنتجة للغشاء الحيوي من المكورات العنقودية في ست مزارع دواجن وفي تجمعات الطيور البرية القريبة. تم جمع 120 عينة شملت عينات من أعضاء الدواجن (القصبة الهوائية والرئة من الدجاج المصاب حديثًا) و80 عينة بيئية (فرشة، مياه، أعلاف، ومسحات من المراوح) من ست مزارع دجاج تسمين في مصر تعاني من أعراض تنفسية. بالإضافة إلى ذلك، تم أخذ مسحات أنفية من العمال، و50 عينة براز من الطيور البرية. تم إجراء عزل أنواع المكورات العنقودية باستخدام تقنيات الزرع التقليدية والتعرف البيوكيميائي. كما تم الكشف عن جين 16S rRNA الخاص بالمكورات العنقودية باستخدام تقنية تفاعل البلمرة المتسلسل واستُخدمت طريقة اختبار الأطباق الدقيقة اللونية لتقييم قدرة العزلات على إنتاج الغشاء الحيوي. وتم تحديد جينات icaD وeno وfnbA بإستخدام تقنية تفاعل البلمرة المتسلسل. تم الحصول على 45 عزلة تم تحديدها جميعًا على أنها من أنواع المكورات العنقودية. وُجد أن 30% من عينات أعضاء الدواجن و25% من الفرشة و20% من المياه و20% من الأعلاف و10% من مسحات المراوح تحتوي على المكورات العنقودية. كما تم العثور على المكورات العنقودية في 44% من عينات براز الطيور البرية المقيمة والمهاجرة و10% من المسحات الأنفية لعمال المزارع. أظهرت 88.88% (39 من أصل 45) من العزلات مستويات متفاوتة من إنتاج الغشاء الحيوي. وأظهرت التحليلات الجزيئية انتشارًا واسعًا للجينات المرتبطة بالغشاء الحيوي، حيث كانت 35 عزلة (77.7%) إيجابية لجين icaD، و28 عزلة (62.2%) لجين eno، و39 عزلة (86.6%) لجين fnbA. لم تكن هناك فروق ذات دلالة إحصائية في عزل المكورات العنقودية بين المصادر الحيوانية أو بين الطيور البرية المقيمة والمهاجرة. تنتشر أنواع المكورات العنقودية المنتجة للغشاء الحيوي على نطاق واسع في الدواجن في مصر، كما أن احتمالية الانتقال الحيواني المنشأ من الطيور البرية إلى الانسان تمثل مجالًا ناشئًا للبحث.

الكلمات الدالة: الغشاء الحيوي، مصر، الدواجن، الطيور المهاجرة.