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Prevalence of *Staphylococcus aureus* in The food Chain, Humans, and The environment in Mansoura

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Abstract

Staphylococcus aureus (*S. aureus*) is responsible for most foodborne outbreaks. This study aimed to evaluate the prevalence of *S. aureus* in the food chain, humans, and environment in Mansoura to evaluate one health aspect. A total of 210 samples from raw milk, meat (minced meat, meat by products, and processed meat products), and poultry meat were randomly selected (seventy samples from each); in addition, twenty samples were taken from their surrounding environment and the hands of their workers. These samples were collected from small markets, and student residences located in Mansoura, Egypt, during the period of September 2020 and March 2021. For *S. aureus* isolation, samples were subjected to standard culture techniques. Identification of *S. aureus* colonies was performed by molecular confirmation using PCR for the biochemically suspected *S. aureus* isolates by utilizing a molecular marker that targeted the species-specific thermonuclease-*nuc* gene. Out of 230 samples, 50 isolates were determined to be *S. aureus*. The overall prevalence of *S. aureus* isolates in our study was 21.7% (50/230). *S. aureus* was present in 35.7% (25/70) of the raw milk sampled. *S. aureus* isolates were found in 13.3% (4/30), 10% (2/20), and 15% (3/20) of the meat, meat by-products, and processed meat products, respectively. Fourteen (20%) of the seventy samples of chicken meat contained isolates of *S. aureus*. Ten percent (1/10) of the worker's hand swabs and samples from nearby surfaces contained *S. aureus* isolates. To improve one health aspect, tracking food and monitoring farm animals must be built.

Keywords: *S. aureus*, Poultry, Meat, Environment, Workers.

Introduction

Staphylococcus aureus, a Gram-positive bacterium, is a facultative pathogenic organism that infects a variety of animal species and is responsible for causing diseases in both animals and humans [1]. In the dairy economy, *S. aureus* is responsible for major, huge economic losses as it is the main causative agent for mastitis in ruminants, which leads to severe public health hazards [2]. Human infection by *S. aureus* is mostly related to nosocomial and community-acquired infections [3]. *S. aureus* is classified as a facultative anaerobe due to its ability to thrive in environments with or without oxygen. *S. aureus* possesses a wide range of virulence factors that cause disease [4] and food poisoning due to enterotoxins ingestion [3].

One Health, the theory of coordinated cooperation and integration between humans, animals, and the environment, has recently become a growing emphasis [5]. The aim of One Health is to concentrate on an interdisciplinary strategy to treat zoonotic illnesses at the human-animal-environmental interface [6]. Seventy-five percent of newly discovered infectious illnesses (EIDs) have animal origins [7, 8, 9, 10, 11]. The World Health Organization (WHO) mentioned that 43% of the total global illness burden is attributed to infectious diseases, most of which are zoonotic [12].

The main reservoir for *S. aureus*' infection is infected mammary glands; as well, dairy product contamination can occur anywhere in the processing or production chain, but most notably during milking processing [1]. The ingestion of this contaminated milk is considered a serious health hazard to humans [2]. *S. aureus* can spread from processing and packaging workers and the environment [13], as well as from different food sources [14]. In Egypt, most of the milk produced is based on small-scale farms. Those small-scale milk producers are mostly the main suppliers to the processing plants or direct local consumption. Any improper hygiene during milk in

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this small-scale milking will lead to larger contamination and bigger problems that will lead to serious problems for consumers. This highlights how important it is to monitor milk and other animal products for the presence of virulent *S. aureus*.

S. aureus contamination of meat throughout the food chain is a challenging problem. The contamination could potentially come from both animals and humans. It has previously been demonstrated that food handlers carrying *S. aureus* in their noses or hands are the primary cause of infection in humans. To limit the chances of meat contamination and food illness, poor hygiene at that level should be avoided. This may be problematic due to the large number of small slaughter and meat processing operations in Africa [15].

An additional source of infection is food producing animals have on their intestinal tract, nose, and skin. The main factors that determine the degree of contamination are the duration of animal transportation, the techniques utilized to move the animals from one location to another, the holding conditions, the geographic location, and temperature changes [16]. Furthermore, it is vital to maintain food handling and slaughter procedures to decrease the danger of pathogen contamination. Inadequate food handling procedures and improper storage conditions promote the growth of *S. aureus*, resulting in the development of enterotoxins in food [17].

Few studies have focused on investigating *S. aureus* contamination throughout the food chain [18]. In Egypt, *S. aureus* was isolated from different sources, including food [19, 20, 21, 22] and animal and human sources [23, 24, 21]. Raw milk is susceptible to contamination from pathogenic microorganisms due to its traditional methods of collection, processing, and transportation. As well, in villages and traditional areas, most people buy meat slaughtered outside abattoirs. workers sometimes underestimate the hygiene on farms or abattoirs. All these factors are considered hazards that may increase the chances of contamination of food used for human consumption. Therefore, this study focused on detecting the prevalence of *S. aureus* in different aspects, food (raw milk, raw meat, its byproducts, processed meat products, and raw poultry meat), environment (surfaces surrounding samples), and workers' hands in Mansoura, Egypt, to provide a science-based conceptual underpinning for accurate management of the spread of *S. aureus* from the source to the fork and to assist the involved parties in implementing safety risk management measures.

Methodology

Sampling size

For *S. aureus* isolation, a total of 70 samples of raw milk, 70 samples of meat and its byproducts (liver, spleen, and lung), and processed meat products (sausage, smoked meat, and beef turkey) were obtained from small holders and retail establishments. In addition, 70 samples of poultry meat came from small markets that serve prepared food and student residences. About 50 gm were collected from all the samples in a sterile manner. Additionally, twenty swab samples in Tryptone Soya Broth (TSB; Oxoid, UK) were taken from surfaces surrounding the collected samples in small businesses that provide prepared food, private housing for students, retail stores, and their employees' hands.

All the samples were collected from Mansoura, Egypt, during the period between September 2020 and March 2021 and were sent for microbiological analysis to the department of Bacteriology, Mycology, and Immunology, the Faculty of Veterinary Medicine, Mansoura University.

Sample preparation and S. aureus isolation

A total of 10 g of meat, meat byproducts, processed meat, and poultry samples were suspended in 90 ml of Tryptone Soya Broth (TSB; Oxoid, UK) in a sterile plastic bag. Samples were forcefully shaken and grinded for a duration of 2 minutes. Subsequently, a volume of 0.1 ml from each sample was streaked onto Baird Parker agar, which contains 5% egg yolk and 1% potassium tellurite (Oxoid, UK), and blood agar with 7% sheep blood (Oxoid, UK). All cultured plates were incubated under aerobic conditions at a temperature of 37 °C for 24 to 48 hrs. Milk samples, environmental swabs (from surfaces), and workers' hand swabs were directly streaked onto Baird Parker agar and blood agar plates as described above. Typical black colonies with a clear halo zone on Baird Parker agar and β-hemolysis on blood agar were picked and sub-cultured onto Tryptone Soya Agar (TSA; Oxoid, UK) [25, 26].

All the suspected colonies were assessed based on their colony morphology and Gram-staining characteristics. The identification of suspected colonies was conducted through the application of traditional biochemical tests, including catalase, oxidase, urease, coagulase activity, and nitrate reduction tests [25, 26]. Preservation of all suspected *S. aureus* samples was performed in 30% glycerol at -20°C for molecular examination.

Molecular examination:

DNA extraction

Each presumptive *S. aureus* strain was subjected to DNA extraction by suspending three to five colonies in 200 µl of sterile nuclease-free water, followed by boiling for ten minutes, and centrifuging for one minute at 10,000 rpm. Afterwards, the supernatant was transferred to a sterile Eppendorf tube and utilized as a DNA template for additional

molecular analysis. The DNA samples that were produced were kept at -20 °C.

Molecular characterization of S. aureus isolates

To validate the *S. aureus* species, DNA templates of the suspected *S. aureus* DNA samples were submitted to molecular PCR targeting the thermonuclease (*nuc*) gene. A total of 12.5 µL of 2x PCR master mix (WizPureTM, Gyeonggi-do, Korea), 1 µL of each primer (20 pmol; Metabion, Germany), 5 µL of template DNA, and nuclease-free water completed the reaction mixture (25 µL). The PCR thermocycler conditions were as follows: primary denaturation at 94˚C for 5 min, followed by 35 cycles of denaturation at 60˚C for 1 min, annealing at 72˚C for 1 min, and extension at 72˚C for 1 min; the final extension step was done at 72˚C for 10 min [27].

According to Oliveira et al. [27], the primer sequence is summarized in Table 1. Agarose gel electrophoresis was used to visualize approximately 5 µl of each PCR result on a 1% agarose gel. Gels were photographed using a UV transilluminator, viewed using Gel Doc (Cleaver Scientific Ltd. UV Gel Documentation System, USA), and stained with ethidium bromide. Positive and negative controls were used [28, 29].

Results

Identification of S. aureus isolates

All the bacteria that appear on Baird Parker agar showing typical black colonies with a clear halo zone and on blood agar with a complete clear zone of β-hemolysis, were suspected to be *S. aureus*. All suspected isolates showed positive results in biochemical and molecular examination targeting the *nuc* gene were confirmed as *S. aureus* isolates.

Prevalence of S. aureus isolates

A total of fifty isolates among 230 samples were confirmed to be *S. aureus*. In our investigation, the overall prevalence of *S. aureus* isolates was 21.7% (50/230). In raw milk samples, 35.7% (25/70) of samples were *S. aureus*. Meat, meat byproducts, and processed meat products showed *S. aureus* isolates in 13.3% (4/30), 10% (2/20), and 15% (3/20), respectively. Out of 70 poultry meat samples, 14 (20%) harbored *S. aureus* isolates. Swabs from the worker's hand and samples surrounding surfaces showed *S. aureus* isolates in 10% (1/10) for each.

Discussion

S. aureus is a major pathogen in the worldwide public health crisis, standing third among foodborne pathogens [30, 31]. *S. aureus* is the most prevalent foodborne bacteria that is frequently connected to cases of foodborne infections and illnesses. *S. aureus* is a significant contributor to illnesses in both humans and animals [1, 4, 32]. The tendency of *S. aureus* to have a wide range of virulence factors that contribute to bacterial invasion determines its ability to cause disease [4, 32]. Milk, its products, raw meat, meat byproducts, eggs, and fish are frequent sources of *S. aureus* [34]. *S. aureus*-contaminated foods have the potential to spread the disease to consumers. This transmission can occur through a variety of sources, including food-contact surfaces, food handlers, food-producing animals, processing tools, and air [35].

Pathogenic *S. aureus* colonizing dairy cattle and contaminating raw milk as a direct consequence is still a key problem for the dairy industry and the public's health [33]. In our study, *S. aureus* isolates were investigated in 35.7% (25/70) of raw milk samples, and the contamination rate was highly noticeable, which matched the results of Traversa et al. [36] and Ahmed et al. [37]. In China, the *S. aureus* contamination rate was identified at 27.7% and 28.9% in raw milk samples by Liu et al. [36] and Zhao et al. [39], respectively. On the contrary, in an Iranian study, *S. aureus* was observed at a lower frequency of 12.4% in raw milk [40].

Contamination of the final product will arise from substandard food processing and animal husbandry techniques. *S. aureus* contamination in milk is usually linked to bovine mastitis or human carriers, which can contaminate finished foodstuffs [21]. The discrepancies in the *S. aureus* prevalence rate between the mentioned studies may be attributed to the accuracy of the detection techniques, type of breeding system or animal, milking method, or hygienic manners [30, 21].

Consequently, raw milk harvesting, processing, distribution, and marketing should be regulated. Simultaneously, suitable professional training for the workers at each stage is required to limit raw milk pollution produced by unfavorable situations and to avoid further danger to consumer health.

Meat and meat by-products were found to be the main distinguishing reservoirs for *S. aureus* [41]. *S. aureus* contamination of raw meat can happen at any point of processing, from farm, abattoir to table [42]. Typically, an appropriate heat application during food preparation can kill all vegetative *S. aureus* strains but cannot destroy SEs [43]. In our study, the total prevalence of *S. aureus* in meat samples investigated was 12.9% (9/70). Meat, meat byproducts, and processed meat products showed *S. aureus* isolates in 5.7% (4/70), 2.9% (2/70), and 4.3% (3/70), respectively. Out of 70 poultry meat samples, 14 (20%) harboured *S. aureus* isolates.

Previous inquiries revealed a comparable incidence of *S. aureus* strains in raw red meat, like our observation: 15% in Egypt [44] and 20.5% in China [43]. According to earlier research, a slightly higher incidence (26.31% of raw red meat samples) in Iran tested positive for *S. aureus* [46], 27.8% in the United States [45], 29.4% in Algeria [33], 32.8% in Japan [46], 34.3% in Ethiopia [49], and 35.4% in Korea [50]. Morocco, Ghana, Colombia, Georgia,

and Poland had significantly higher rates of *S. aureus* contamination, with percentages of 40.38%, 45%, 46%, 63%, and 68%, respectively [51, 52, 53, 54, 55]. Several variables impact the variation in prevalence data on *S. aureus* in raw meat gathered from different countries: sampling protocols, seasons and locations, identification methods, packing processes, handling, and retail points [44].

Reports have indicated a comparable prevalence of *S. aureus* to that of this study, with percentages of 25%, 24.2%, and 17.8% in Bhargava et al. [56], Wang et al. [57], Hanson et al. [58], respectively. Our study's results regarding the prevalence of *S. aureus* in retail chicken meat were less significant than other reports in India (46.61%), in Turkey (55%), in China (67.9%), and in Bangladesh (71%) [59, 60, 41, 61]. The aforementioned researchers have linked the high frequency of *S. aureus* to inadequate personal hygiene, contaminated processing water, and inadequate or non-existent tool and work surface cleaning in retail establishments.

The discrepancy in prevalence could be attributed to the number of samples examined, the sampling process, and the sanitary conditions of retail outlets in different nations. The aggregation of several poultry species in these markets raises the likelihood of cross-contamination, a high diversity of diseases, and the establishment of new strains [62, 63]. Poor hygiene and overcrowding increase pathogen transmission and spread through direct and indirect contact.

Swabs from the worker's hand and samples surrounding surfaces showed *S. aureus* isolates in 1.4% (1/70) for each. Food workers infected with *S. aureus* can spread the bacteria from their hands to food at any step of preparation, posing a significant hazard of cross-contamination [64]. As a result, food workers' poor personal hygiene would have major consequences for food safety, encouraging the spread of *S. aureus* strains [65, 66]. *S. aureus* is able to stick to various surfaces, which is a crucial virulence feature that facilitates its colonization and raises the possibility of recurrent cross-contamination [67]. A comprehensive evaluation of all possible cross-contamination occurrences is necessary to support risk management [68]. The link between workers, contact surfaces, and food permits the transmission of *S. aureus* through food and humans [66, 69].

Hand washing with soap and water, or an ethanol- or alcohol-based hand sanitizer (60%) is the most critical practice for preventing the transmission of various pathogens [70]. Furthermore, the proper usage of disposable gloves has been considered an efficient technique to limit infection transmission from bare hands to food [71]. To summarize, regular hand washing frequent glove changes can be factors helpful in reducing the risk of contamination [71, 72]. The manufacturing environment's hygiene and training handlers for good manufacturing practices are crucial in lowering the risk of cross-contamination.

Conclusion

To sum up, *S. aureus* spreads through animal sources, food, the environment, and humans. A structured, integrated One Health surveillance system might offer a practical means of lowering the incidence of *S. aureus* in Egypt. There are still obstacles to the implementation of integrated national human-animal disease surveillance. Epidemiologists, public health officials, and animal health authorities should collaborate to address zoonotic disease outbreaks such as *S. aureus* as a result of a greater understanding of One Health principles.

Conflict of Interest

The authors have no conflicts of interest to declare.

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Ethical approval

The study was carried out in accordance with the guidelines of the animal research ethical committee of Mansoura University's faculty of veterinary medicine (Code number: M/74).

Author contributions

Conceptualization, A.S, A.A; methodology, M.H.; validation, A.S, A.A; investigation, A.S., A.A., M.H. writing-original draft preparation, M.H. writing-review and editing, A.S.; supervision, A.S., A.A.

TABLE 1. Primers and PCR cycle used in this study:

Genes	Primer Sequence (5′–3′)	Amplicon (bp)	Reference
Nuc	Forward: GCGATTGATGGTGATACGGTT Reverse: AGCCAAGCCTTGACGAACTAAAG	270	[25]

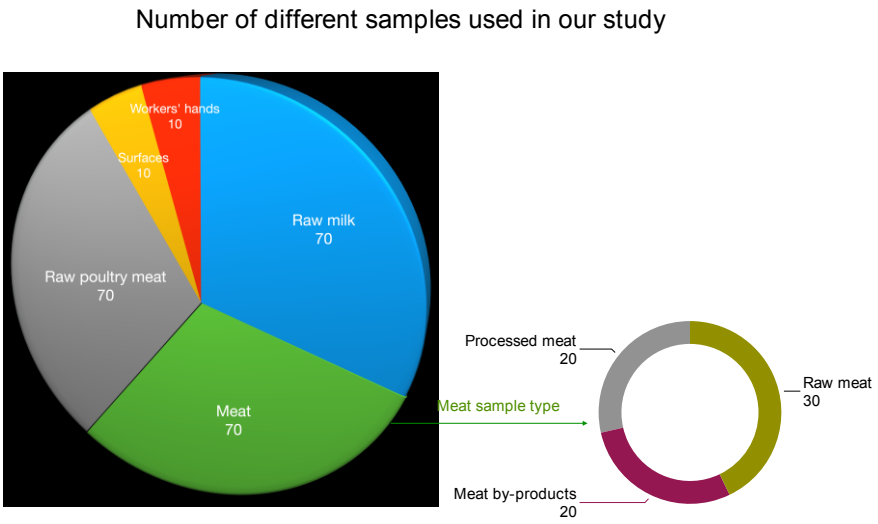

Fig. 1. Number of different samples used in this study

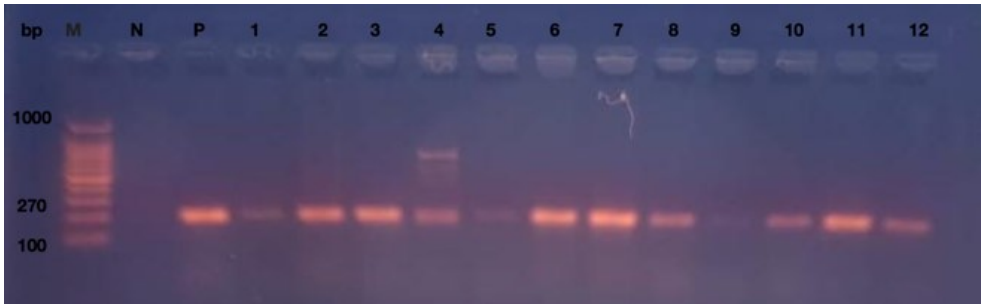

Fig. 2. PCR amplification of *nuc* gene at 270 bp. Lane M: 100 bp DNA ladder; lane N is the negative control; lane P is the positive control; lanes 1, 2, 3, 6, 7, 8, 10, 11, and 12 are the positive samples and the rest are negative samples

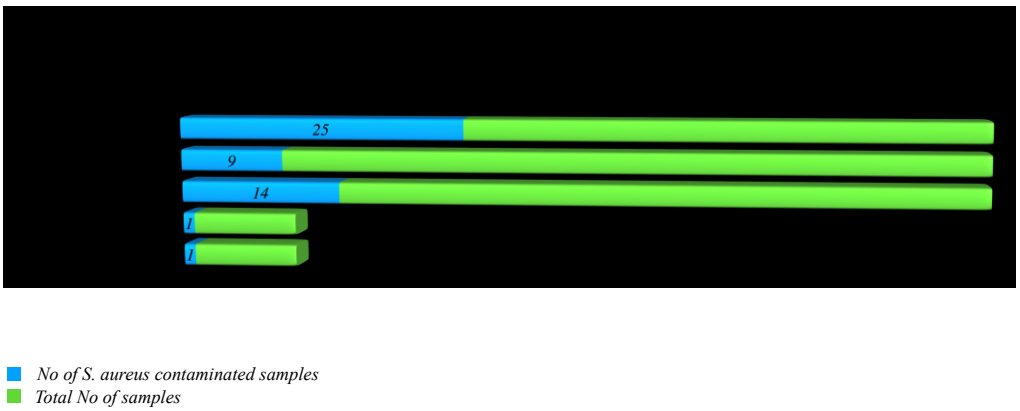

Fig. 3 Number of *S. aureus* contaminated samples examined in our study

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مدى انتشار المكورات العنقودية الذهبية في السلسلة الغذائية والبشر والبيئة في المنصورة

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الملخص

تعد المكورات العنقودية الذهبية (*S. aureus*) مسؤولة عن معظم حالات تفشي الأمراض المنقولة بالغذاء. هدفت هذه الدراسة إلى تقييم مدى انتشار بكتيريا المكورات العنقودية الذهبية في السلسلة الغذائية والإنسان والبيئة في مدينة المنصورة لتقييم one health. تم اختيار 210 عينات من الحليب الخام، واللحوم (اللحم المفروم، واللحوم المصنعة، ومنتجات اللحوم المصنعة)، ولحوم الدواجن (سبعون عينة من كل منها)؛ بالإضافة إلى ذلك تم أخذ عشرين عينة من البيئة المحيطة بهم وأيدي العاملين لديهم. تم جمع هذه العينات من الأسواق الصغيرة ومساكن الطلاب الواقعة في المنصورة، مصر، خلال الفترة من سبتمبر 2020 إلى مارس 2021. بالنسبة لعزل المكورات العنقودية الذهبية، تم إخضاع العينات لتقنيات الاستنبات القياسية. تم تحديد هوية مستعمرات المكورات العنقودية الذهبية عن طريق التأكيد الجزيئي باستخدام تفاعل البوليميراز المتسلسل (PCR) لعزلات المكورات العنقودية الذهبية المشتبه بها كيميائيًا باستخدام علامة جزيئية تستهدف جين نوكلياز-نوك الحراري الخاص بالأنواع. من أصل 230 عينة، تم تحديد 50 عزلة على أنها S. aureus. كان معدل الانتشار الإجمالي لعزلات المكورات العنقودية الذهبية في دراستنا 21.7٪ (230/50). كانت *S. aureus* موجودة في 35.7% (70/25) من عينات الحليب الخام. تم العثور على عزلات المكورات العنقودية الذهبية في 13.3% (30/4)، 10% (20/2)، و15% (20/3) من اللحوم ومنتجات اللحوم الثانوية ومنتجات اللحوم المصنعة، على التوالي. أربعة عشر (20٪) من سبعين عينة من لحم الدجاج تحتوي على عزلات بكتريا المكورات العنقودية الذهبية. عشرة بالمائة (10/1) من مسحات يد العامل وعينات من الأسطح المجاورة تحتوي على معزولات بكتريا المكورة العنقودية البرتقالية. لتحسين جانب صحي واحد، يجب بناء تتبع الغذاء ومراقبة حيوانات المزرعة.

الكلمات الدالة: S. Aureus الدواجن، اللحوم، البيئة، العمال.