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Prevalence of enterotoxigenic *Staphylococcus aureus* in meat and chicken meals served at governmental hospital.

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ABSTRACT

Staphylococcus aureus (*S. aureus*) is a major contributor to zoonotic diseases worldwide, primarily caused by food contamination with enterotoxins. This study aimed to investigate the occurrence and ability of *S. aureus* bacteria to produce enterotoxins in meat meals served at governmental hospitals in Menoufia, Egypt. A total of 120 random samples of meat meals, consisting of grilled chicken, fried chicken, grilled meat, and fried meat (30 each) were obtained from a restaurant in a governmental hospital. The collected samples were examined for detection and identification of staph.aurues strains. In all, 25.6% of the analyzed samples were found to be contaminated with *S. aureus*. The latter was most commonly recovered from grilled chicken (36.7%), followed by fried chicken (26.7%), grilled beef (23.3%), and fried meat (16.7%). The average *S. aureus* count (CFU/g) in grilled chicken, fried chicken, grilled meat, and fried meat were $15.9\pm2.6\times10^3$, $8.12\pm2.04\times10^3$, $6.63\pm1.51\times10^3$, and $2.97\pm0.39\times10^3$, respectively. The prevalence of *S. aureus* strains capable of producing enterotoxins (SE) was 29.0%. Among the examined enterotoxogenic staph. Aureus isolated strains, SEA was the most detected enterotoxin, accounting for 13.3% of the isolates. This was followed by SEC (10.0%), SED (3.3%), and a combination of SEA and SED (3.3%). *Staphylococcus species*, such as *Staphylococcus xylosus*, *Staphylococcus epidermidis*, *Staphylococcus intermedius*, and *Staphylococcus saprophyticus*, were also isolated at variable rates. The investigation revealed isolation of enterotoxigenic *S. aureus* strains from meat meals served in governmental hospitals, emphasizing the potential risks they pose to public health. Therefore, strict hygienic measures should be adopted during the preparation of meat meals served at hospitals.

1. INTRODUCTION

Poultry and red meat are regarded as significant sources of protein, indispensable amino acids, B complex vitamins, and minerals. Conversely, it serves as an optimal surface for the proliferation of certain harmful microorganisms. The significance of ensuring the safety of food for patients in hospitals and the potential harm that tainted food could cause to their rehabilitation has been highlighted (Kandela, 1999). Foodborne diseases in hospitals can be prevented, but are exacerbated by various variables, such as staff carriers, unsanitary conditions in the kitchens, and inadequate training of food workers. The risk of contaminated food in hospitals lies in the fact that it is served to those who are in a state of poor health (Custovic and Ibrahimagic, 2005).

Staphylococcus aureus (S. aureus) is a Gram-positive pathogenic bacterium can cause various zoonotic diseases and food poisoning (Zhou et al., 2018). The global occurrence of foodborne illnesses resulting from *S. aureus* and its enterotoxins has been documented in many studies (Hennekinne et al., 2012; Wang et al., 2019). According to a study conducted by the Centre for Disease Control and Prevention, there were 241,188 occurrences of foodborne illness known as Staphylococcal Food Poisoning (SFP) in the United States between 2006 and 2008. W u et al. (2018). This resulted in 1064 people being admitted to the hospital and 6 deaths (Scallan et al., 2011). *Staphylococcus aureus* is a highly significant pathogenic bacterium for humans (Wu et al., 2018). In China, microorganisms were responsible for 53.7% of food poisoning cases in 22015. Staphylococcal food poisoning (SFP)is characterized by a sudden and swift occurrence, causing patients to experience symptoms such as nausea, vomiting, and stomach cramps (Hennekinne et al., 2012). *S. aureus* is responsible for several disorders such as SFP, toxic shock syndrome, bacteremia, pneumonia, and soft tissue infections (Foster, 2004; Tong et al., 2015). The pathogenicity of *S. aureus* is enhanced through the presence of many virulence factors, including as staphylococcal enterotoxins (SEs), toxic shock syndrome toxin-1, hemolysins, and fibronectin-binding proteins (Puah et al., 2016).

Staphylococcal enterotoxins (SEs) are the primary agents responsible for SFP and are produced by staphylococci that test positive for coagulase. SEs refer to gastrointestinal exotoxins (Argudín et al., 2010). The SEs remain viable in the digestive tract following ingestion by humans due to its ability to withstand high temperatures, proteolytic enzymes, and other environmental factors (Shanehbandi et al., 2014;

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Benkerroum 2018). The identification of SEs is a reliable approach to confirm outbreaks because of their consistent characteristics and the ability of strains to produce enterotoxins. More than 20 enterotoxins of *S. aureus* have been discovered W u et al. (2018). Es are categorized into classical genes and non-classical genes (referred to as new SEs) based on serological classification. The classical genes, namely SEA, SEB, SEC, SED, and SEE, are the most prevalent enterotoxins found in over 90% of cases of SFP outbreaks. On the other hand, non-classical genes refer to newly discovered enterotoxins that have been isolated in only 5% of cases (Mashouf et al., 2015; Benkerroum 2018; Johler et al., 2018). Consuming food that is contaminated with enterotoxigenic *S. aureus* can result in foodborne outbreaks, as a result of the stable characteristics of SEs and the minimal amount needed to cause symptoms. The enterotoxins produced by *S. aureus* are well-recognized as the primary cause of SFP (Fisher et al., 2018). The objective of this study was to assess the occurrence and toxin-producing ability of *S. aureus* in meat meals served at hospitals, namely those containing grilled and fried beef and chicken.

2. MATERIAL AND METHODS

Ethical approval:
This study was conducted according to the guidelines of Scientific Research Ethics Committee, Faculty of Veterinary Medicine, Benha University, Egypt. (Ethical Approval Number: BUFVTM 17-10-23).

2.1. Collection of samples:
A total of 120 random samples of meat meals, consisting of grilled chicken, fried chicken, grilled meat, and fried meat (30 each), sample size was 250 gm of cooked meat meals were obtained from a restaurant in a governmental hospital in Menoufia, Egypt. The gathered samples were individually stored in sterile plastic bags and preserved in an ice box. They were then transferred immediately to the animal research institute Shebein El Kom Laboratory under strict aseptic conditions with P-value (.01), collected and examined from January to December 2023. The collected samples underwent bacteriological analysis to identify the presence of Staphylococcus spp. and investigate the enterotoxigenicity of the isolated strains.

2.2. Bacteriological examination:
2.2.1. Preparation of samples (ISO 4833 -1, 2013).
Precisely 225 ml of 0.1% sterile peptone water was added to 25 g of the sample and blended well for 1.5 minutes using a sterile blender. Subsequently, ten-fold serial dilutions were made.

2.2.2. Determination of Staphylococcus aureus count (FDA, 2001).
Using a sterile bent glass spreader, one ml from each of the created serial dilutions was evenly distributed on a Baired Parker agar plate. The plates were turned upside down and placed in an incubator set at a temperature of 37 °C for duration of 48 hours. The colonies, which were glossy and black in color, were counted. The colonies presumed to be *S. aureus* are observed as black, glossy, circular, smooth, and convex with a thin white border.
They are surrounded by a transparent area that extends into the opaque medium.
These colonies were counted, and the number of *S. aureus* per gram was estimated.

2.2.3. Identification of Staphylococcus spp.:
The recovered isolates were initially analyzed through morphological examination (Cruickshank et al., 1975), followed by biochemical identification (McFadden, 2000). Subsequently, it underwent various tests including catalase and oxidase activities, mannitol fermentation, growth in 10% NaCl, bile esculent test, detection of hemolysis, coagulase test, thermostable nuclease test for "D-Nase activity," detection of Arginine decarboxylase (ADH) (Lachia et al., 1971), fermentation of sugars, and finally serological identification.

2.3. Detection and typing of enterotoxin (Shingaki et al., 1981):
Staphylococcus aureus strains were grown in tryptone soy broth supplemented with 5% sodium chloride in an orbital shaker (Lab-Line Instruments, Melrose Park, Calif.) operating at 200 rpm. The cultures were then incubated at 37 °C for 24 hours. Following the growth phase, the culture underwent centrifugation at a force of 900 times the acceleration due to gravity for duration of 20 minutes. The resulting liquid, located above the sediment, was then examined at a temperature of 4 °C to determine the existence of SEs. The enterotoxins were identified using the commercially available SET-RPLA test, following the directions provided by the manufacturer. To summarize, latex reagents that have been sensitized with antisera are combined with diluted supernatant and left to incubate overnight to detect SEA, SEB, SEC, and SED.

2.5. Statistical analysis:
The collected results were evaluated statistically using the Analysis of Variance (ANOVA) test with P-value (.01), as described by Feldman et al. (2003).

3. RESULTS

The obtained results revealed that out of the 120 examined meat meal samples *S. aureus* was isolated at 25.6%. *S. aureus* was isolated from grilled chicken at 36.7%, fried chicken at 26.7%, grilled meat at 23.3%, and fried meat at 16.7% (Fig. 1).

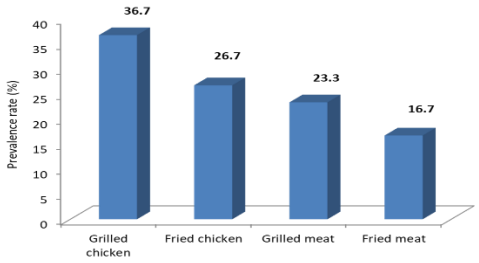

Fig. 1 Prevalence (%) of *S. aureus* in the examined sample

The count of *S. aureus* in grilled chicken ranged from 4.0 $\times 10^2$ to 3.7×10^4 with a mean value of $15.9\pm2.6\times10^3$ CFU/g. *S. aureus* in fried chicken ranged from 2.0×10^2 to 1.9×10^4 with a mean value of $8.12\pm2.04\times10^3$ CFU/g. The range of *S. aureus* in grilled meat was 1.0×10^2 to 1.1×10^4 with a mean value of $6.63\pm1.51\times10^3$ CFU/g. The range of *S. aureus* in fried meat was 1.0×10^2 to 8.0×10^3 with a mean value of $2.97\pm0.39\times10^3$ CFU/g (Fig. 2).

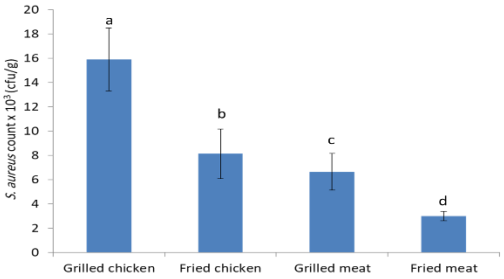

Fig. 2 *Staphylococcus aureus* count/g in the examined meals served at governmental hospitals. Columns with different letters are significantly different at p< 0.05.

The results in Table (1) indicated that 36.7%, 26.7%, 20%, and 10% of the examined grilled chicken, fried chicken, grilled meat, and fried meat had unacceptable *S. aureus* counts (> 10^2) according to EOS (2004).

Table (2) showed the ability of the recovered *S. aureus* isolates to produce enterotoxins. 9 out of 31 (29.0%) isolates produced SE. Two isolates produced SEA (6.7%), one isolate produced SEC (3.3%), one isolate produced SEA+SEC (3.3%) from grilled chicken. One isolate produced SEA (3.3%) and one isolate produced SED (3.3%) from fried chicken. One isolate produced SEA (3.3%), and one isolate produced SEC (3.3%) from grilled meat.

One isolate produced SEC (3.3%) from fried meat. With significant differences (P- value more than .01).

Staphylococcus species, such as *Staphylococcus xylosus*, *Staphylococcus epidermidis*, *Staphylococcus intermedius*, and *Staphylococcus saprophyticus*, were also isolated at variable rates from the examined samples (Table 3).

Table 1 Acceptability of the examined meat samples at governmental hospital based on their *Staphylococcus aureus* count /g (n=30).

Meat meals	*S. aureus* count /g (EOS, 2004)	Accepted samples		Unaccepted samples	
		No.	%	No.	%
Grilled chicken	> 10^2	19	63.3	11	36.7
Fried chicken	> 10^2	22	73.3	8	26.7
Grilled meat	> 10^2	24	80.0	6	20.0
Fried meat	> 10^2	27	90.0	3	10.0

Table 2 Occurrence of enterotoxin secreted by *S. aureus* strains isolated from meat meals served at the governmental hospital.

Enterotoxin	Grilled chicken		Fried chicken		Grilled meat		Fried meat	
	No.	%	No.	%	No.	%	No.	%
A	2	6.7	1	3.3	1	3.3	-	-
C	1	3.3	-	-	1	3.3	1	3.3
D	-	-	1	3.3	-	-	-	-
A+C	1	3.3	-	-	-	-	-	-
Total	4	13.3	2	6.7	2	6.7	1	3.3

Table 3 Incidence of *Staphylococcus species* isolated from the examined samples of governmental hospital meat meals (n=30).

Species	========== Hospital meat meals ==========									
	Grilled chicken		Fried chicken		Grilled meal		Fried meat		Total (n=120)	
	No.	%	No.	%	No.	%	No.	%	No.	%
Staphylococcus aureus	11	36.7	8	26.7	7	23.3	5	16.7	31	25.8
Staphylococcus xylosus	4	13.3	2	6.7	1	3.3	1	3.3	8	6.7
Staphylococcus epidermidis	6	20.0	10	33.3	5	16.7	2	6.7	23	19.2
Staphylococcus intermedius	1	3.3	2	6.7	2	6.7	1	3.3	6	5.0
Staphylococcus saprophyticus	2	6.7	1	3.3	1	3.3	0	0.0	4	3.3

4. **DISCUSSION**

Staphylococcus aureus is widely recognized as a major contributor to hospital-acquired infections, as well as being responsible for the majority of food poisoning cases in healthcare facilities (Wendlandt et al., 2013; Sergelidis and Angelidis, 2017). Hospital meals are an essential component of patient care. Providing patients with safe and nutritionally balanced meals helps promote healthy eating habits and provides the necessary nutrients for their recovery from sickness. Contamination of food with *S. aureus* can happen due to contaminated food-producing animals or inadequate hygiene throughout manufacturing, retail, and storage procedures. In addition, people can carry these bacteria.

The research findings indicated that the prevalence of *S. aureus* in various types of hospital meat meal samples, such as grilled chicken, fried chicken, grilled beef, and fried meat, was 25.6%. In the current research, the prevalence rate of *S. aureus* in hospital meat meal samples was found to be greater than that reported in Iran (6.42%) (Madahi et al., 2014) and Portugal (11.10%) (Castro et al., 2016). It was found that *S. aureus* was less common in hospital food samples than in ready-to-eat meat products from restaurants and street vendors in Benha City, Egypt (50.8%; Saad et al., 2019; Madoroba et al., 2021). In Brazil, the rate was 50% (Ferreira et al., 2014); in South Africa, it was 33.26%; and in Australia, it was 50.8% as mentioned by (Saad et al., 2019). The prevalence rates of *S. aureus* in hospital meals from our research were as follows: 36.7% for grilled chicken, 26.7% for fried chicken, 23.3% for grilled meat, and 16.7% for fried meat. These findings were in agreement with those recorded by Abd Allah-Enas (2011) and Heweidy (2016). They also found *S. aureus* in kofta, burger, shawarma, and luncheon samples at rates of 35%, 25%, 25%, and 8.6%, respectively.

According to the presented data here, the average counts of *S. aureus* in the examined samples agreed with previous reports. For instance, Arab (2010) produced nearly the same findings when investigating the bacteriological quality of cooked meat. The average count of staphylococci was determined to be 1.86±0.64×103 / g. Ali (2011) conducted a study on the bacteriological quality of fried beef burgers and indicated that the average count of staphylococci was 1.85±0.42x103 (cfu/g). Mohamed et al. (2015) found that the average count of staphylococci in fried chicken meat was 2.10±0.32×103 cfu/g, whereas in fried beef meat it was 9.58±2.08×103 cfu/g. The data presented showed that 36.7%, 26.7%, 20%, and 10% of the analyzed grilled chicken, fried chicken, grilled beef, and fried meat samples had *S. aureus* counts over 102, which is considered unacceptable (EOS, 2004). Likely, Salem et al. (2019) found that 68.8% of the retailed minced meat had unacceptable *S. aureus* counts exceeding the Egyptian standards. Besides, Saad et al. (2019) confirmed that unacceptable *S. aureus* counts were in ready-to-eat meat products in Egypt.

In this investigation, the prevalence of enterotoxin production by *S. aureus* was found to be 29.0%, which is comparable to the rates reported in Malaysia (30.8%) and USA (25.8%) (Puah et al., 2016; Ge et al., 2017). In this investigation, the SEA gene was the most commonly found among the tested isolates of enterotoxogenic staph. Aureus strains, accounting for 13.3% of the total. It was followed by the SEC gene at 10.0%, the SED gene at 3.3%, and a combination of the SEA and SED genes at 3.3%. These findings are consistent with reports from China and other countries (Wang et al., 2014; Puah et al., 2016; Ge et al., 2017). According to a study conducted by Zhang et al. (2015), the SEA and SED genes were the most commonly identified SEs genes in *S. aureus* isolates from retail products in China. Argudín et al. (2010) and Gholamzad et al. (2015) found that the SEA gene was frequently detected

in contaminated foods and is associated with staphylococcus-related food poisoning cases worldwide. Additionally, the SEB gene has been identified as a potential source of more severe poisoning compared to other enterotoxins.

5. CONCLUSIONS

The results of this study clearly demonstrated that meat meals from governmental hospitals have a high prevalence of *S. aureus* which secrete certain enterotoxins, leading to food poisoning. Specifically, it was found that the samples of grilled and fried chicken had a higher percentage of contamination with *S. aureus* compared to samples of grilled and fried meat. This can be attributed to poor hygienic handling and inadequate personnel hygiene. The prevention of staphylococcal food-borne illness relies on implementing hygiene protocols to prevent and minimize the contamination of food by S. aureus. Our findings emphasize the significance of monitoring enterotoxigenicity. The study showed that meals served at government hospitals might act as potential sources of enterotoxigenic *S. aureus* strains, which highlights the health risks they may pose.

CONFLICT OF INTEREST

The authors announce that they have no conflict of interest

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