



Prevalence of Multidrug-Resistant *Staphylococcus aureus* in Meals Served at Hospitals

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Abstract

Staphylococcus aureus (*S. aureus*) is one of the major causes of foodborne intoxication worldwide. Meals introduced to patients at hospitals are mandatory to be free from pathogens. Therefore, this study was conducted to investigate the prevalence of *S. aureus* in four basic meals hosted to hospitals in Menoufia government, Egypt including fried chicken, fried meat, grilled chicken, and grilled meat. The antimicrobial resistance of the recovered *S. aureus* isolates was screened. The public health significance of *S. aureus* was further discussed. The obtained results revealed that 31 out of 120 samples (25.6%) were found to be contaminated with *S. aureus*. The highest prevalence of *S. aureus* was found in grilled chicken (36.7%), followed by fried chicken (26.7%), grilled meat (23.3%), and fried meat (16.7%). The mean values (CFU/g) of *S. aureus* counts in grilled chicken, fried chicken, grilled meat and fried meat were $1.59 \times 10^4 \pm 0.26 \times 10^4$, $8.12 \times 10^3 \pm 2.04 \times 10^3$, $6.63 \times 10^3 \pm 1.51 \times 10^3$, and $2.97 \times 10^3 \pm 0.39 \times 10^3$ respectively. Most of the *S. aureus* isolates showed high resistance to Kanamycin (100%), Neomycin (93.5%), and Streptomycin (90.3%), respectively. The moderate resistance of *S. aureus* isolates was detected for erythromycin (58%), followed by gentamicin (32.3%), Oxacillin (19.4%), Enrofloxacin (6.5%), and Vancomycin (3.2%) respectively. Using polymerase chain reaction, the coding genes for the drug resistance against Methicillin, Vancomycin, Erythromycin, and Gentamicin were detected in several isolates. In conclusion, strict hygienic measure should be followed during preparation of the meals served at the hospitals to avoid their contamination with food poisoning organisms, particularly *S. aureus*.

Keywords: Meals, *Staphylococcus aureus*, Drug resistance.

Introduction

Meat are regarded as significant providers of essential amino acids, B complex vitamins, minerals, and protein. Conversely, it serves as a great growing medium for a wide range of harmful microorganisms. It has been underlined how crucial it is for hospitalized patients to eat healthfully and how eating tainted food could hinder their recuperation [1]. Outbreaks of foodborne infection at hospitals can be avoided, but they are made more likely by a number of variables, such as unsanitary kitchens, untrained food handlers, and staff carriers.

Given to patients who are ill, tainted food poses a risk to their health [2].

Food poisoning diseases are caused by the Gram-positive pathogenic bacterium *Staphylococcus aureus* (*S. aureus*) [3]. Global reports of epidemics of enterotoxins and *S. aureus* food poisoning (SFP) have been made [4, 5]. A total of 241,188 instances of SFP were reported in the United States between 2006 and 2008, according to the Centres for Disease Control and Prevention's assessment of foodborne illness [6]. These cases led to 1064 hospitalizations and 6 fatalities. According to Wu et al. [7], *S. aureus* is one of the most dangerous bacteria for humans. In

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China, microorganisms were responsible for 53.7% of instances of food poisoning in 2015. The sudden start of SFP, which causes vomiting, nausea, and cramping in the abdomen in patients [8]. Numerous additional illnesses, such as bacteremia, pneumonia, soft tissue infections, and toxic shock syndrome, are also caused by *S. aureus* [9].

Abuse of antibiotic used may caused *S. aureus* to become more resistant to drugs over time, and different regions are seeing distinct pandemic trends. Previous reports [10] have indicated that antibiotic-resistant *S. aureus* has been linked to outbreaks of foodborne disease, particularly methicillin-resistant *S. aureus* (MRSA) and multidrug-resistant *S. aureus* (MDR), which present a threat to public health security [11].

Multidrug resistant forms of bacteria are becoming more frequently as a result of the widespread use of antibiotics in recent decades, posing serious risks to public health. *S. aureus* is able to quickly develop resistance to almost all antibiotics due to its ability to adapt to its surroundings [12]. Food poisoning outbreaks have been linked to MDR *S. aureus*, which has been isolated from several food products [13, 14]. Furthermore, MRSA has received a lot of attention lately. It shown numerous antibiotic resistances, and the World Health Organization ranked it among the top 12 families of bacteria in 2017 for threat to human health [15]. Drug abuse without a prescription, excessive dosages, and pointless drug application have all contributed to the progressive rise in *S. aureus* drug resistance in recent decades and caused bacterial evolution [16, 17]. Multidrug-resistant *S. aureus* strains, particularly those that are MRSA, represent a significant health risk to humans, resulting in significant morbidity and mortality. This is particularly true in hospitals and among healthy individuals [18]. Livestock-associated MRSA is spread via foods obtained from animals [19, 20]. At abattoirs, meat processing facilities, carcasses, meat products, or ready-to-eat (RTE) foods may act as possible sources of MRSA and pose a risk to consumers [21, 22].

According to Holmes et al. [23], vancomycin has historically been used as a last-resort treatment for MRSA infections. Nevertheless, overuse of it results in diverse vancomycin-intermediate *S. aureus* strains, and vancomycin-resistant *S. aureus* [24].

One class of antibiotics called macrolides, which also contains erythromycin, is used to treat *S. aureus*-related infections. Erythromycin resistance is a result of overuse and uncontrolled usage among many infections. *S. aureus* resistance to erythromycin has been linked to a number of mechanisms. One of these mechanisms is the alteration of the ribosomal binding site, which lessens the capacity of erythromycin to bind to ribosomes. This change is

mediated by the production of ribosomal methylase, which is encoded by *erm* genes [25].

One class of antibiotics that is crucial in the management of staphylococcal infections is aminoglycosides. Aminoglycoside-modifying enzymes (AMEs), which are encoded by genetic elements, inactivate antibiotics and are the primary mechanism of resistance to aminoglycosides [26]. Thus, the most significant genes in this regard are those that encode aminoglycoside-6'-N-acetyltransferase/2"-O-phosphoryltransferase, aminoglycoside-4'-O-nucleotidyltransferase I, aminoglycoside-3'-O-phosphoryltransferase III, and streptomycin modifying enzyme, respectively. These genes are *aac* (6')-Ie + *aph* (2''), *ant* (4')-Ia, *aph* (3')-IIIa, and *ant* (6)-Ia. A bifunctional enzyme with AAC (6') and APH (2'') activity mediates staphylococci's resistance to gentamicin, kanamycin, and tobramycin. While the APH (3')-III enzyme inactivates neomycin, the ANT (4')-IA enzyme inactivates kanamycin, amikacin, neomycin, and tobramycin [27].

Thus, the current study set out to assess the *S. aureus* prevalence in the fried and grilled chicken and red meat at the hospital restaurants. The disk diffusion method was used to screen the obtained isolates' antibiograms. Furthermore, PCR was used to screen for the presence of vancomycin, gentamycin, methicillin, and erythromycin resistance genes in *S. aureus*.

Material and Methods

This study was done according to the guidelines of Benha University, and no living animals were used in the present study.

Collection of samples

A governmental hospital restaurant in Menoufia government, Egypt provided 120 randomized samples of grilled chicken, fried chicken, grilled meat, and fried meat (30 of each type). Samples (100 g/ each) were provided during serving (about 30 minutes after cooking). Every sample that was taken was separately stored in a sterile plastic bag, kept cold in an ice-box, and then brought straight into the laboratory at Food Hygiene and Control Department, Faculty of Veterinary Medicine, Benha University, where it was kept in perfect aseptic conditions. The collected samples underwent a bacteriological analysis as soon as it arrived to identify *S. aureus* and characterize its genes related to resistance to antibiotics.

Bacteriological examination:

Preparation of samples [28]

Ten-fold serial dilutions were made from the sample after 225 ml of 0.1 % sterile peptone water was precisely added to 25g of the sample and carefully blended for 1.5 minutes using a sterile blender.

Determination of total *S. aureus* count [29].

Using a sterile bent glass spreader, one millilitre from each of the created serial dilutions was spread over the Baird Parker agar plate. After being turned over, the control and inoculation plates were incubated for 48 hours at 37°C. The colonies were counted; they were lustrous black colonies. The black, shiny, round, smooth, convex, and narrowly white margined suspicious colonies of *S. aureus* were counted, and the number of colonies per milligram of *S. aureus* was determined.

Identification of *Staphylococcus aureus*:

The process involved morphological examination [30], followed by biochemical identification [31] and testing for the presence of hemolysis, coagulase, thermostable nuclease test "D-Nase activity," mannitol, growth at 10% NaCl, bile esculent test, catalase activity, oxidase, and fermentation of sugars [32].

Antibiotic susceptibility testing of *S. aureus*.

Using the Kirby Bauer disk diffusion test [33], the antibiotic susceptibility of all *S. aureus* isolates was assessed against 14 antibiotics on Mueller-Hinton agar plates (Oxoid, England). Trypticase Soy Broth (Oxoid, Basingstoke, UK) was used to cultivate *S. aureus*, and it was cultured for 18 hours at 37°C. After obtaining an optical density with sterile physiological saline, it was corrected to 0.5 McFarland standards and then plated on Müller Hinton Agar (Oxoid, Basingstoke, UK). To prevent inhibition zone overlap, the antibiotic discs were positioned far on the Müller Hinton Agar (Oxoid, Basingstoke, UK) surface. Incubation plates for 24 hours at 37°C. Following incubation, the inhibition zones were measured, and the Clinical Laboratory Standard Institute guidelines [34] were followed for the interpretation of the results. The following equation was used to compute the multiple antibiotic resistance index of the *S. aureus* isolate, in accordance with the formula provided by Singh et al. [35]:

$$\text{MAR index} = \frac{\text{Number of resistance profile antibiotics}}{\text{the number of used antibiotics}}$$

Polymerase Chain Reaction Detection of Methicillin, Erythromycin, Gentamicin and Vancomycin-Resistance Genes:

DNA Extraction by QIA amp kit [36]:

Utilizing the DNA extraction kit (Qiagen, GmbH, Germany) per the manufacturer's instructions, genomic DNA was extracted from 24-hour cultures of phenotypic MRSA" (*mecA*) and antibiotic resistance genes represented by Erythromycin (*ermA*), Gentamicin (*aac 6-aph 2*), and Vancomycin (*vanA*) of *S. aureus* isolates in BHI broth [36]. Thermo Fisher Scientific, Waltham, Massachusetts, USA, provided the NanoDrop™ 1000

spectrophotometer, which was used to measure the quantity and purity of DNA. As previously reported [37-39], the methicillin resistance gene (*mecA*), the erythromycin resistance gene (*ermA*), the gentamicin resistance gene (*aac 6-aph 2*), and the vancomycin resistance gene (*vanA*) were identified by PCR using primers (Pharmacia Biotech) described before.

Multiplex polymerase chain reaction:

In accordance with Perez-Roth et al. [40], a 25 µL total volume was used for the multiplex PCR, which included 1 µL of bacterial suspension extracted using the rapid DNA extraction method, 80 mM MgCl₂, PCR buffer, 3.5 mM Dntp mix (Fermentas), 10 picomoles µL⁻¹ of each primer, and 1 unit of Taq polymerase (BioSyntech Technologies). The following thermal cycling profile was used for the amplifications: a first denaturation step at 94°C for 5 minutes was followed by 10 cycles of amplification (denaturation at 94°C for 30 seconds, annealing at 64°C for 30 seconds, and extension at 72°C for 45 seconds); 25 cycles of amplification (denaturation at 94°C for 45 seconds, annealing at 50°C for 45 seconds, and extension at 72°C for 1 minute) and a final extension step at 72°C for 10 minutes. Following amplification, a 2% agarose gel containing 10 µL of the reaction mixture was electrophoresed to determine the sizes of the amplified products using a 100-bp molecular size standard ladder (MBI Fermentas). After staining the gel with ethidium bromide, the gel was photographed under a UV lamp.

Statistical analysis

The Analysis of Variance (ANOVA) test was used to statistically evaluate the obtained results [41].

Results

Out of the 120 examined samples, *S. aureus* was isolated from 31 samples (25.6%). *S. aureus* was isolated from grilled chicken at 11 out of 30 (36.7%), fried chicken at 8 out of 30 (26.7%), grilled meat at 7 out of 30 (23.3%), and fried meat at 5 out of 30 (16.7%), respectively.

It was revealed that the count of *S. aureus* in grilled chicken ranged from 4.0 x 10² to 3.7 x 10⁴ with a mean value of 1.59 x 10⁴ ± 0.26 x 10⁴ CFU/g, the range of *S. aureus* in fried chicken ranged from 2.0 x 10² to 1.9 x 10⁴ with a mean value of 8.12 x 10³ ± 2.04 x 10³ CFU/g, the range of *S. aureus* in grilled meat ranged from 1.0 x 10² to 1.1 x 10⁴ with a mean value of 6.63 x 10³ ± 1.51 x 10³ CFU/g, and the range of *S. aureus* in fried meat ranged from 1.0 x 10² to 8.0 x 10³ with a mean value of 2.97 x 10³ ± 0.39 x 10³ CFU/g (Table 2).

Table 3 shows the resistance of 31 isolates of *S. aureus* from grilled chick (11 isolates), fried chicken (8 isolates), grilled meat (7 isolates) and fried meat (5 isolates) against 14 antimicrobials using the disc

diffusion method. In our study, isolates of *S. aureus* demonstrated the peak resistance to Kanamycin, Neomycin and Streptomycin (100%, 93.5%, and 90.3% respectively), followed by Nalidixic acid, Penicillin, and Oxytetracycline (77.4%, 67.4% and 64.5%) respectively. The moderate resistance of *S. aureus* isolates was detected for erythromycin (58%), followed by sulfamethoxazole, Cephalothin, and Ciprofloxacin (48.4%, 41.9% and 41.9%), respectively. While the lower resistance of *S. aureus* isolates was found for Gentamicin, Oxacillin, Enrofloxacin and Vancomycin (32.3%, 19.4%, 6.5%, and 3.2%), respectively.

Table 4 shows detailed antimicrobial resistance profiles. MDR index among the 31 *S. aureus* isolates ranged from 0.071 to 1. As 21 isolates (67.7%) were resistant to three classes of antimicrobials. While 10 isolates (32.3%) were resistant to eight classes of antimicrobials, and one strain (3.2%) was resistant to more than eight classes of antimicrobials. The average MAR index value was 0.54.

Out of the 31 *S. aureus* isolates, 16 isolates (51.6 %) were screened by PCR for harboring MRSA-coding gene (*mecA*) and antibiotic resistance-coding genes, Erythromycin (*ermA*), Gentamicin (*aac 6-aph 2*) and Vancomycin (*vanA*) as presented in Fig. 1. The obtained results revealed that 9 out of 16 (56.25%) were +ve *ermA* gene, 7 out of 16 (43.75%) were +ve *aac (6)-aph* (Table 5), 4 out of 16 (25%) were +ve *mecA* gene, and 2 out of 16 (12.5%) were +ve *vanA* (Table 6).

Discussion

According to Wegndlandt *et al.* [42] and Sergelidis and Angelidis [43], *S. aureus* is thought to be the primary source of food poisoning in hospitals and one of the most frequent causes of nosocomial infections. Hospital meals are an essential component of medical care. Encouraging patients to eat properly and providing them with the nutrition they need to recover from sickness can be achieved with safe and comprehensive meals. *S. aureus* contamination of food can result from contaminated food produced by animals, from unsanitary manufacturing conditions, from food storage problems at retail, or from human sources.

The results of this study (Tables 2-6) showed that the prevalence of *S. aureus* in various hospital food samples, which included fried chicken, grilled chicken, and fried meat, was (25.6%). The present study's prevalence rate of *S. aureus* in hospital food samples was more than that of Portugal (11.10%) [44], and Iran (6.42%) [45]. However, research's prevalence rate of *S. aureus* in hospital food samples was lower than the 50% reported in Brazil [46] and the 50.8% reported in Egypt (Benha city) for ready-to-eat meat products sampled from restaurants and street vendors [47]. Additionally, findings are

consistent with the 33.26% reported prevalence in ready-to-eat meat products collected in all of South Africa's provinces [48]. The prevalence rates of *S. aureus* in hospital meals that our research examined were (36.7%, 26.7%, 23.3%, and 16.7%) for grilled chicken, fried chicken, grilled meat, and fried meat, respectively. These results are consistent with earlier reports [49, 50] that found *S. aureus* in samples of luncheon food, burgers, shawarma, and kofta that were examined.

Arab [51] looked at the bacteriological quality of cooked beef, they found $1.86 \times 10^3 \pm 0.64 \times 10^3$ CFU/g, which is almost identical to the counts of *S. aureus* found in the current experiment. The bacteriological quality of fried beef burgers was probably investigated by Ali [52], who discovered that the mean count of staphylococci was $1.85 \times 10^3 \pm 0.42 \times 10^3$ / (CFU/g). Mohamed *et al.* [53] also measured the mean counts of staphylococci at $2.10 \times 10^3 \pm 0.32 \times 10^3$ and $9.58 \times 10^3 \pm 2.08 \times 10^3$ CFU/g in fried beef and chicken flesh, respectively. The isolation of *S. aureus* from the examined samples, particularly heat-treated could be attributed to post-processing contamination and reflects poor personal hygiene during serving of the meals at the hospital [2].

Thirty-one *S. aureus* isolates' resistance profiles against several antibiotic classes partially matched findings from other investigations [54, 55]. Because of their ability to produce an exopolysaccharide barrier [56] and a wide variety of multidrug resistant genes on plasmids, which can be exchanged and spread between different species of Staphylococci [57], *S. aureus* strains are known to be frequently resistant to antibiotic therapy. Antimicrobial resistance monitoring is therefore crucial to determining the efficacy of novel antibiotic generations.

As a result of temperature fluctuations during storage, MRSA can multiply and spread to cooked goods. According to previous reports, the usage of antibiotics during livestock and poultry production may be connected to the greater MRSA contamination of the different RTE sandwiches under examination [58].

Conclusions

The study's findings revealed isolation of multidrug resistant *S. aureus* from meals served at hospitals. The recovered isolates harbored coding genes responsible for the observed MDR phenomenon. This could be because of improper handling practices and insufficient staff cleanliness. Hygiene precautions to prevent and minimize *S. aureus* food contamination are the cornerstone of the prevention of staphylococcal food-borne illness.

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Conflicts of interest

The authors declared no competing interests.

Funding statement

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TABLE 1. Primer sequences of *S. aureus* used for PCR system.

Target gene	Oligonucleotide sequence (5' → 3')	Product size (bp)	References
<i>mecA</i> (F)	5' AAAATCGATGGTAAAGGTTGGC'3	533	[38]
<i>mecA</i> (R)	5' AGTTCCTGGAGTACCGGATTTGC'3		
<i>ermA</i> (F)	5' TATCTTATCGTTGAGAAGGGATT '3	139	
<i>ermA</i> (R)	5' CTACACTTGGCTTAGGATGAAA '3		
<i>aac 6-aph 2</i> (F)	5' TTGGGAAGATGAAGTTTTTAGA '3	174	[39]
<i>aac 6-aph 2</i> (R)	5' CCTTTACTCCAATAATTTGGCT '3		
<i>vanA</i> (F)	5'CATGAATAGAATAAAAAGTTGCAATA'3	1030	
<i>vanA</i> (R)	5' CCCCTTTAACGCTAATACGATCAA '3		

TABLE 2. Statistical analytical results of *S. aureus* count/g in the examined meals served at governmental hospitals (n=30/ each).

Meals	+Ve samples		Min.	Max.	Mean ± S.E*
	No.	%			
Grilled chicken	11	36.7	4.0×10 ²	3.7×10 ⁴	1.59×10 ⁴ ± 0.26×10 ⁴ A
Fried chicken	8	26.7	2.0×10 ²	1.9×10 ⁴	8.12×10 ³ ± 2.04×10 ³ B
Grilled meat	7	23.3	1.0×10 ²	1.1×10 ⁴	6.63×10 ³ ± 1.51×10 ³ C
Fried meat	5	16.7	1.0×10 ²	8.0×10 ³	2.97×10 ³ ± 0.39×10 ³ D

*Mean values with different superscript letters in the same column are significantly

TABLE 3. Percentages of Antimicrobial susceptibility of *S. aureus* strains isolated from the examined samples of governmental hospital meals (n=31).

Antimicrobial agent	S		I		R	
	No.	%	No.	%	No.	%
Kanamycin (K)	-	-	-	-	31	100
Neomycin (N)	-	-	2	6.5	29	93.5
Streptomycin (S)	1	3.2	2	6.5	28	90.3
Nalidixic acid (NA)	3	9.7	4	12.9	24	77.4
Penicillin (P)	2	6.5	9	29.0	21	67.7
Oxytetracycline (T)	5	16.1	6	19.4	20	64.5
Erythromycin (E)	6	19.4	7	22.6	18	58
Sulphamethoxazol (SXT)	8	25.8	8	25.8	15	48.4
Cephalothin (CN)	14	45.2	4	12.9	13	41.9
Ciprofloxacin (CP)	15	48.4	3	9.7	13	41.9
Gentamicin (G)	17	54.8	4	12.9	10	32.3
Oxacillin (OX)	23	74.2	2	6.5	6	19.4
Enrofloxacin (EN)	24	77.4	5	16.1	2	6.5
Vancomycin (V)	27	87.1	3	9.7	1	3.2

TABLE 4. Antimicrobial resistance profile of *S. aureus* strains isolated from the examined samples of meat products (n=31).

No.	<i>S. aureus</i> serovar	Antimicrobial resistance profile	MAR index
1	<i>S. aureus</i>	K, N, S, NA, P, T, E, SXT, CN, CP, G, OX, EN, V	1
2	<i>S. aureus</i>	K, N, S, NA, P, T, E, SXT, CN, CP, G, OX, EN	0.928
3	<i>S. aureus</i>	K, N, S, NA, P, T, E, SXT, CN, CP, G, OX	0.857
4	<i>S. aureus</i>	K, N, S, NA, P, T, E, SXT, CN, CP, G, OX	0.857
5	<i>S. aureus</i>	K, N, S, NA, P, T, E, SXT, CN, CP, G, OX	0.857
6	<i>S. aureus</i>	K, N, S, NA, P, T, E, SXT, CN, CP, G, OX	0.857
7	<i>S. aureus</i>	K, N, S, NA, P, T, E, SXT, CN, CP, G	0.786
8	<i>S. aureus</i>	K, N, S, NA, P, T, E, SXT, CN, CP, G	0.786
9	<i>S. aureus</i>	K, N, S, NA, P, T, E, SXT, CN, CP, G	0.786
10	<i>S. aureus</i>	K, N, S, NA, P, T, E, SXT, CN, CP, G	0.786
11	<i>S. aureus</i>	K, N, S, NA, P, T, E, SXT, CN, CP	0.714
12	<i>S. aureus</i>	K, N, S, NA, P, T, E, SXT, CN, CP	0.714
13	<i>S. aureus</i>	K, N, S, NA, P, T, E, SXT, CN, CP	0.714
14	<i>S. aureus</i>	K, N, S, NA, P, T, E, SXT	0.571
15	<i>S. aureus</i>	K, N, S, NA, P, T, E, SXT	0.571
16	<i>S. aureus</i>	K, N, S, NA, P, T, E	0.500
17	<i>S. aureus</i>	K, N, S, NA, P, T, E	0.500
18	<i>S. aureus</i>	K, N, S, NA, P, T, E	0.500
19	<i>S. aureus</i>	K, N, S, NA, P, T	0.428
20	<i>S. aureus</i>	K, N, S, NA, P, T	0.428
21	<i>S. aureus</i>	K, N, S, NA, P	0.357
22	<i>S. aureus</i>	K, N, S, NA	0.286
23	<i>S. aureus</i>	K, N, S, NA	0.286
24	<i>S. aureus</i>	K, N, S, NA	0.286
25	<i>S. aureus</i>	K, N, S	0.214
26	<i>S. aureus</i>	K, N, S	0.214
27	<i>S. aureus</i>	K, N, S	0.214
28	<i>S. aureus</i>	K, N, S	0.214
29	<i>S. aureus</i>	K, N, S	0.214
30	<i>S. aureus</i>	K, N	0.143
31	<i>S. aureus</i>	K	0.071
Average	0.54		

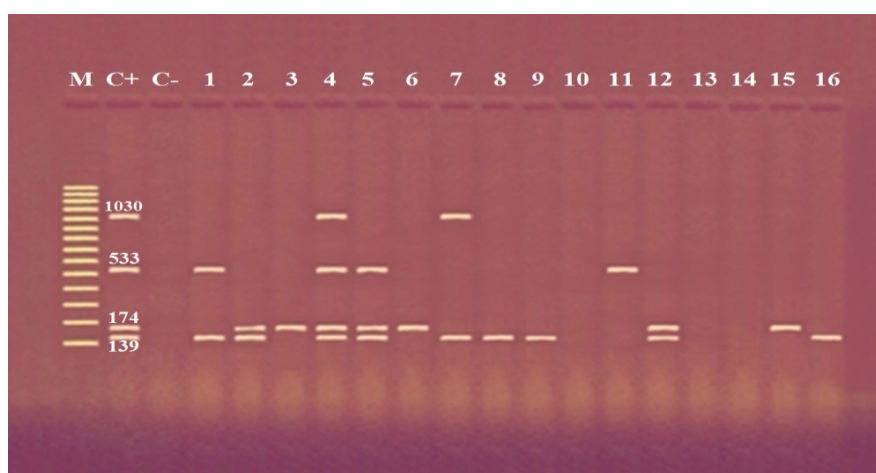
K: Kanamycin N: Neomycin S: Streptomycin NA: Nalidixic acid
P: Penicillin T: Oxytetracycline CN: Cephalotin E: Erythromycin
CP: Ciprofloxacin G: Gentamicin OX: Oxacillin EN: Enrofloxacin
V: Vancomycin SXT: Sulphamethoxazol

TABLE 5. Incidence of erythromycin and gentamicin Resistant *Staphylococcus aureus* by PCR using specific *ermA* gene and *aac (6)-aph (2)* gene.

Hospital meals	No of examined strains	+ve <i>ermA</i> gene		-ve <i>ermA</i> gene		+ve <i>aac (6)-aph (2)</i> gene		-ve <i>aac (6)-aph (2)</i> gene	
		No.	%	No.	%	No.	%	No.	%
Grilled chicken	4	3	75	1	25	3	75	1	25
Fried chicken	4	3	75	1	25	2	50	2	50
Grilled meat	4	2	50	2	50	1	25	3	75
Fried meat	4	1	25	3	75	1	25	3	75
Total	16	9	56.25	7	43.75	7	43.75	9	56.25

TABLE 6. Incidence of Methicillin and vancomycin Resistant *Staphylococcus aureus* (MRSA) by PCR using specific *mecA* gene and specific *vanA* gene.

Hospital meals	No of examined strains	+ve <i>mecA</i> gene		-ve <i>mecA</i> gene		+ve <i>vanA</i> gene		-ve <i>vanA</i> gene	
		No.	%	No.	%	No.	%	No.	%
Grilled chicken	4	2	75	1	25	1	25	3	75
Fried chicken	4	1	75	1	25	1	25	3	75
Grilled meat	4	1	50	0	0	0	0	4	100
Fried meat	4	0	25	0	0	0	0	4	100
Total	16	4	25	2	12.50	2	12.50	14	87.50

**Fig. 1.** Agarose gel electrophoresis of multiplex PCR of *ermA* (139 bp), *aac (6)-aph (2)* (174 bp), *mecA* (533 bp) and *vanA* (1030 bp) antibiotic resistance genes of *S. aureus*.

Lane M: 100 bp ladder as molecular size DNA marker.

Lane C+: Control positive for *ermA*, *aac (6)-aph (2)*, *mecA* and *vanA* genes.

Lane C-: Control negative.

Lanes 1, 2, 4, 5, 7, 8, 9, 12 & 16: Positive *S. aureus* strains for *ermA* gene.

Lanes 2, 3, 4, 5, 6, 12 & 15: Positive *S. aureus* strains for *aac (6)-aph (2)* gene.

Lanes 1, 4, 5 & 11: Positive *S. aureus* strains for *mecA* gene.

Lanes 4 & 7: Positive *S. aureus* strains for *vanA* gene.

Lanes 10, 13 & 14: Negative strains for *ermA*, *aac (6)-aph (2)*, *mecA* and *vanA* genes.

References

- Elkholy, R.A., Abou EL-Roos, N.A., Hussein, M.N. and Shaltout, F.A. Differential Microbiological Quality on Marketed Frozen Turkey Breast and Thigh Meat. *Egyptian Journal of Veterinary Sciences*, **56**(1), 1-10 (2025).
- El-Wehedy, S.E., Darwish, W.S., Tharwat, A.E. and Hafez, A.E.E. Hygienic status of meat served at hospitals and its improvement after HACCP implementation. *Japanese Journal of Veterinary Research*, **67**(1), 61-73 (2019).
- Zhou, K., Li, C., Chen, D., Pan, Y., Tao, Y., Qu, W., Liu, Z., Wang, X., Xie, S. A review on nanosystems as an effective approach against infections of *Staphylococcus aureus*. *International Journal of Nanomedicine*, **13**, 7333–7347 (2018).
- Saadati, A., Mashak, Z. and Yarmand, M.S. Prevalence and Molecular Characterization of Enterotoxin-and Antibiotic Resistance-Encoding Genes in the Methicillin-resistant *Staphylococcus aureus* Recovered from Poultry Meat. *Egyptian Journal of Veterinary Sciences*, **52**(2), 163-173 (2021).
- Darwish, W.S., El-Ghareeb, W.R., Alsayeqh, A.F. and Morshdy, A.E.M. Foodborne intoxications and toxicoinfections in the Middle East. In *Food Safety in the Middle East* (pp. 109-141). Academic Press, (2022).
- Scallan, E., Hoekstra, R.M., Angulo, F.J., Tauxe, R.V., Widdowson, M.-A., Roy, S.L., Jones, J.L. and Griffin, P.M. Foodborne illness acquired in the United States—Major pathogens. *Emerging Infectious Diseases*, **17**, 7–15 (2011).
- Wu, S., Huang, J., Wu, Q., Zhang, F., Zhang, J., Lei, T., Chen, M., Ding, Y., Xue, L. Prevalence and Characterization of *Staphylococcus aureus* Isolated From Retail Vegetables in China. *Frontiers in Microbiology*, **9**, 1263 (2018).
- Hennekinne, J.-A., De Buyser, M.-L. and Dragacci, S. *Staphylococcus aureus* and its food poisoning toxins: Characterization and outbreak investigation. *FEMS Microbiology, Reviews*, **36**, 815–836 (2012).
- Tong, S.Y.C., Davis, J.S., Eichenberger, E., Holland, T.L. and Fowler, V.G. Jr. *Staphylococcus aureus* infections: Epidemiology, pathophysiology, clinical manifestations, and management. *Clinical Microbiological Reviews*, **28**, 603–661 (2015).
- Alsayeqh, A.F., Baz, A.H.A. and Darwish, W.S. Antimicrobial-resistant foodborne pathogens in the Middle East: A systematic review. *Environmental Science and Pollution Research*, **28**(48), 68111-68133 (2021).
- Wang, W., Baloch, Z., Jiang, T., Zhang, C., Peng, Z., Li, F., Fanning, S., Ma, A. and Xu, J. Enterotoxigenicity and Antimicrobial Resistance of *Staphylococcus aureus* Isolated from Retail Food in China. *Frontiers in Microbiology*, **8**, 2256 (2017).
- Saleh, M., El Hady, A.M.M., R Mohamed, S., El-Shafei, A.A. and El-Shafei, M. Genetic Characterization of Methicillin Resistance *Staphylococcus aureus* Isolates of Poultry and Human Origin. *Egyptian Journal of Veterinary Sciences*, **52** (The 9th International Conference of Veterinary Research Division National Research Centre, Giza, Egypt 27th -29th September 2021), 61-68 (2021).
- Gharsa, H., Ben. S. K., Lozano, C., Gómezsan. E., Klibi N. and Ben. S. R. Prevalence, antibiotic resistance, virulence traits and genetic lineages of *Staphylococcus aureus* in healthy sheep in Tunisia. *Veterinary Microbiology*, **156**, 367–373 (2012).
- Papadopoulos, P., Papadopoulos, T., Angelidis, A. S., Boukouvala, E., Zdragas, A. and Papa A. Prevalence of *Staphylococcus aureus* and of methicillin-resistant *S. aureus* (MRSA) along the production chain of dairy products in north-western Greece. *Food Microbiology*, **69**, 43–50 (2018).
- Govindaraj, A. V. and Vanitha, A. 2018. WHO global priority pathogens list on antibiotic resistance: an urgent need for action to integrate one health data. *Perspectives in Public Health*, **138**, 87–88 (2018).
- Gharieb, R. M. A., Saad, M. F., Mohamed, A. S. and Tartor, Y. H. Characterization of two novel lytic bacteriophages for reducing biofilms of zoonotic multidrug-resistant *Staphylococcus aureus* and controlling their growth in milk. *LWT Food Science and Technology*, **124**, 109-145 (2020).
- Guo, Y., Song, G., Sun, M., Wang, J. and Wang, Y. Prevalence and therapies of antibiotic-resistance in *Staphylococcus aureus*. *Frontiers in Cellular and Infection Microbiology*, **10**, 107 (2020).
- Weber, J. T. Community-associated methicillin-resistant *Staphylococcus*. *Clinical Infectious Diseases*, **41** (4), S269-72 (2005).
- Darwish, W.S., Atia, A.S., Reda, L.M., Elhelaly, A.E., Thompson, L.A. and Saad Eldin, W.F. Chicken giblets and wastewater samples as possible sources of methicillin-resistant *Staphylococcus aureus*: Prevalence, enterotoxin production, and antibiotic susceptibility. *Journal of Food Safety*, **38**(4), e12478 (2018).
- Anjum, M.F., Marco-Jimenez, F., Duncan, D., Marin, C., Smith, R.P. and Evans, S.J. Livestock-Associated Methicillin-Resistant *Staphylococcus aureus* From Animals and Animal Products in the UK. *Frontiers in Microbiology*, **10**, 2136 (2019).
- Morshdy, A.E.M., Darwish, W.S., El-Dien, W.M.S. and Khalif, S.M. Prevalence of multidrug-resistant *Staphylococcus aureus* and *Salmonella enteritidis* in meat products retailed in Zagazig city, Egypt. *Slovenian Veterinary Research*, **55** (Suppl 20), 295–301(2018).
- Li, H., Andersen, P. S., Stegger, M., Sieber, R. N., Ingmer, H. and Staubrand, N. Antimicrobial resistance and virulence gene profiles of Methicillin-Resistant and -susceptible *Staphylococcus aureus* from food products in Denmark. *Frontiers in Microbiology*, **10**, 2681 (2019).
- Holmes, N. E., Tong, S. Y. C., Davis, J. S. and Hal, S. J. V. Treatment of Methicillin-Resistant *Staphylococcus aureus*: Vancomycin and Beyond. *Semin. Respir. Critical Care Medicine*, **36**(1), 17-30 (2015).

24. Amberpet, R., Sistla, S., Sugumar, M., Nagasundaram, N., Manoharan, M. and Parija, S. Detection of heterogeneous vancomycin-intermediate *Staphylococcus aureus* A preliminary report from south India. *Indian Journal of Medical Research*, **150**(2), 194-198 (2019).
25. Aktas, Z., Aridogan, A., Kayacan, C.B. and Aydin, D. Resistance to macrolide, lincosamide and streptogramin antibiotics in staphylococci isolated in Istanbul, Turkey. *Journal of Microbiology*, **45**, 286–290 (2007).
26. Hauschild, T., Sacha, P., Wieczorek, P., Zalewska, M., Kaczynska, K. and Tryniszewska, E. Aminoglycosides resistance in clinical isolates of *Staphylococcus aureus* from a University Hospital in Bialystok, Poland. *Folia Histochemica Cytobiologica*, **46** (2), 225–228 (2008).
27. Vakulenko, S.B. and Mobashery, S. Versatility of aminoglycosides and prospects for their future. *Clinical Microbiological Reviews*, **16**(3), 430–450 (2003).
28. International Standards Organization (ISO) (4833-1: 2013). Microbiology of food chain- Horizontal method for the enumeration of microorganisms. Part I; Colony count at 30°C by the pour plate technique. International Standards Organization, Geneva, Switzerland (2013).
29. Food and Drug Administration (FDA). *S. aureus*. Bacteriological analytical manual .8th Ed. Chapter12. Academic Press, Gaithersburg, UK (2001).
30. Cruickshank, R., Duguid, J., Marmion, B. and Swain, R.H. Medical Microbiology. 12th Ed., Edinburg, London and New York (1975).
31. MacFaddin, J.F. Biochemical tests for identification medical bacteria. Waryer Press Inc., Baltimore, Md. 21202 USA (2000).
32. Lachia, R., Genigeogis, C. and Hoeprich, P. Meta chromatic agar- diffusion methods for detecting Staphylococcal nuclease activity. *Applied Microbiology*, **21**, 585-587 (1971).
33. Bauer, A., Kirby, W., Sherris, J. C. and Turck, M. Antibiotic susceptibility testing by a standardized single disk method. *American Journal of Clinical Pathology*, **45**, 493 (1966).
34. CLSI. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing; approved guideline (M 100 S); 26edition, Wayne, PA: Clin. Lab. Stand. Inst (2016).
35. Singh, A., Yadav, S., Singh, S. and Bharti, P. Prevalence of Salmonella in chicken eggs collected from poultry farms and marketing channels and their antimicrobial resistance. *Food Research International*, **43**, 2027-2030 (2010).
36. Shah, D., Shringi, S., Besser, T. and Call, D. Molecular detection of foodborne pathogens, Boca Raton: CRC Press, In Liu, D. (Ed). Taylor & Francis group, Florida, USA, Pp. 369-389 (2009).
37. McClure, J. A., Conly, J. M., Lau, V., Elsayed, S., Louie, T., and Hutchins, W. Novel multiplex PCR assay for detection of the staphylococcal virulence marker Pantone-Valentine leukocidin genes and simultaneous discrimination of methicillin-susceptible from -resistant staphylococci. *Journal of Clinical Microbiology*, **44**, 1141–1144 (2006).
38. Bühlmann, M., K Bögli-Stuber, K., Droz, S. and Mühlemann, K. Rapid screening for carriage of methicillin-resistant *Staphylococcus aureus* by PCR and associated costs. *Journal of Clinical Microbiology*, **46** (7), 2151–2154 (2008).
39. Amghalia, E., AL-Haj, N., Shamsudin, M., Radu, S., Rosli, R., Neela, V. and Rahim, R. Multiplex PCR assays for the detection of clinically relevant antibiotic resistance genes in *Staphylococcus aureus* isolated from Malaysian hospitals. *Research Journal of Biological Sciences*, **4** (4), 444-448 (2009).
40. Perez-Roth, E., Claverie-Martin, F., Villar, J. and Mendez-Alvarez, S. Multiplex PCR for simultaneous identification of *Staphylococcus aureus* and detection of methicillin and mupirocin resistance. *Journal of Clinical Microbiology*, **39**, 4037-4041 (2001).
41. Feldman, D., Ganon, J., Haffman, R. and Simpson, J. *The Solution for Data Analysis and Presentation Graphics*. 2nd ed., Abacus Lancripts, Inc., Berkeley, USA (2003).
42. Wendlandt, S., Schwarz, S. and Silley, P. Methicillin-resistant *Staphylococcus aureus*: a food-borne pathogen? *Annual Review in Food Science and Technology*, **4**, 117–39 (2013).
43. Sergelidis, D. and Angelidis, A.S. Methicillin-resistant *Staphylococcus aureus*: a controversial food-borne pathogen. *Letters in Applied Microbiology*, **64**, 409–18 (2017).
44. Castro, A., Santos, C., Meireles, H. and Silva, J. Teixeira P. Food handlers as potential sources of dissemination of virulent strains of *Staphylococcus aureus* in the community. *Journal of Infection and Public Health*, **9**, 153–60 (2016).
45. Madahi, H., Rostami, F., Rahimi, E. and Dehkordi, F.S. Prevalence of enterotoxigenic *Staphylococcus aureus* isolated from chicken nugget in Iran. *Jundishapur Journal of Microbiology*, **7**, e10237 (2014).
46. Ferreira, J., Costa, W., Cerqueira, E., Carvalho, J., Oliveira, L. and Almeida, R. Food handler-associated methicillin-resistant *Staphylococcus aureus* in public hospitals in Salvador, Brazil. *Food Control*, **37**, 395–400 (2014).
47. Saad, M.S., Fatin, S.H., Fahim, A.S., Marionette, Z.N. and Marwa, Z.S. Prevalence of methicillin-resistant *Staphylococcus aureus* in some ready-to-eat meat products. *American Journal of Biomedical Science and Research*, **4**, 461–465 (2019).
48. Madoroba, E., Magwedere, K., Chaora, N.S., Matle, I., Muchadeyi, F. and Mathole, M.A. Microbial communities of meat and meat products: An exploratory analysis of the product quality and safety at selected enterprises in South Africa. *Microorganisms*, **9**(3), 507 (2021).
49. Abd Allah-Enas, M. A. Microbial and chemical evaluation of fast foods. *Thesis*, Master of Veterinary Medicine, Benha University, Egypt (2011).

50. Heweidy, A.Y. Prevalence of some foodborne micro-organisms in meat and meat products. Thesis, Master of Veterinary Medicine, Benha University, Egypt (2016).
51. Arab, W.S.S. Quality improvement of meat meals provided by a university student restaurant. Ph.D. Thesis (Meat Hygiene), Fac. Vet. Med., Benha University. Egypt (2010).
52. Ali, E.A.M. Microbial and chemical evaluation of fast foods. *M.V. Sc. Thesis*, (Meat Hygiene), Fac. of Vet. Med., Benha Univ. Egypt (2011).
53. Mohamed A. H., Reham A. A., Sherien M. E., Asmaa A.A.M. Bacterial Status of Food Meals served at Governmental Hospital. *Benha Veterinary Medical Journal*, **29**(1), 143-150 (2015).
54. Waters, A.E., Contente-Cuomo, T., Buchhagen, J., Liu, C.M., Watson, L., Pearce, K., Foster, J.T., Bowers, J., Driebe, E.M., Engelthaler, D.M. and Keim, P.S. Price LB. Multidrug-Resistant *Staphylococcus aureus* in US Meat and Poultry. *Clinical Infectious Diseases*, **52**, 1227–30 (2011).
55. Yurdakul, N.E., Erginkaya, Z., and Unal, E. Antibiotic resistance of enterococci, coagulase negative Staphylococci and *Staphylococcus aureus* isolated from chicken meat. *Czech Journal of Food Science*, **1**, 14–9 (2013).
56. Gundogan, N., Citak, S. and Turan, E. Slime production, DNase activity and antibiotic resistance of *Staphylococcus aureus* isolated from raw milk, pasteurized milk and ice cream samples. *Food Control*, **17**, 389–92 (2006).
57. Neihart, R.E., Fried, J.S. and Hodges, G.R. Coagulase-Positive Staphylococci. *South Medical Journal*, **81**, 491–500 (1988).
58. De Boer, E., Zwartkruis-Nahuis, J.T., Wit, B., Huijsdens, X.W., de Neeling, A.J., Bosch, T., van Oosterom, R.A., Vila, A. and Heuvelink, A.E. Prevalence of methicillin-resistant *Staphylococcus aureus* in meat. *International Journal of Food Microbiology*, **134**, 52–56 (2009).

تواجد المکور العنقودي الذهبی متعدد المقاومة للمضادات الحيوية في الوجبات المقدمة في احدي المستشفيات

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المستخلص

جديرا بالذكر أن المكورات العنقودية الذهبية أحد الأسباب الرئيسية للتسمم الغذائي في جميع أنحاء العالم. لذلك فمن الضروري ان تكون الوجبات المقدمة للمرضى في المستشفيات خالية من مسببات الأمراض. أجريت هذه الدراسة لبحث مدى انتشار جراثيم المكورات العنقودية الذهبية في عدد من الوجبات التي يتم تقديمها بأحد المستشفيات بمحافظة المنوفية، مصر، وشملت الوجبات الدجاج واللحوم المحمرة والدجاج واللحوم المشوية. بالإضافة إلى ذلك، تم فحص مقاومة المضادات الحيوية لعزلات المكورات العنقودية الذهبية. هذا وقد تمت مناقشة الأهمية الصحية لتلك الميكروبات. وكشفت النتائج المتحصل عليها أن عدد 31 من أصل 120 عينة (25.6%) وجدت ملوثة ببكتريا المكورات العنقودية الذهبية. تم الوقوف على أعلى معدل لانتشار البكتيريا العنقودية الذهبية في الدجاج المشوي (36.7%)، يليه الدجاج المحمر (26.7%)، واللحوم المشوية (23.3%)، واللحوم المحمرة (16.7%). كان متوسط العدد الكلي للبكتريا في الدجاج المشوي والدجاج المحمر واللحوم المشوية واللحوم المحمرة هو عدد $1.59 \times 10^4 \pm 0.26 \times 10^4$ ، $8.12 \times 10^3 \pm 2.04 \times 10^3$ ، $6.63 \times 10^3 \pm 1.51 \times 10^3$ ، و $2.97 \times 10^3 \pm 0.39 \times 10^3$ على التوالي. وأكدت النتائج أن معظم عزلات المكورات العنقودية الذهبية مقاومة عالية لمركبات الكاناميسين والنيوميسين والستربتوميسين بنسب 100%، 93.5%، 90.3% على التوالي. بينما تم الكشف عن المقاومة المعتدلة لعزلات المكورات العنقودية الذهبية للإريثروميسين (58%)، تليها الجنتاميسين، أوكساسيلين، الإنترافلوكساسين، والفانكوميسين (32.3%)، 19.4%، 6.5%، و 3.2% على التوالي. وتم استخدام تقنية تفاعل البلمرة المتسلسل للكشف عن جينات الضراوة لمقاومة الأدوية ضد الميثيسيلين، الفانكوميسين، الاريثروميسين، والجنتاميسين في عدة عزلات. وخلصت الدراسة الي ضرورة اتباع إجراءات صحية صارمة أثناء إعداد الوجبات المقدمة في المستشفيات من أجل تلافي تلوث الوجبات المقدمة للمرضى بميكروبات التسمم الغذائي وبصفة خاصة المکور العنقودي الذهبی.

الكلمات الدالة: المکور العنقودي الذهبی، وجبات المستشفيات، مقاومة للمضادات الحيوية.