Research Article

The Outstanding Effect of Casein and α-Lactalbumin on Multidrug Resistance *Staphylococcus aureus* Isolated from Ready to Eat Meat Products and Human Samples

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Abstract | This study was conducted to detect the presence of *S. aureus* in ready to eat meat products as grilled chickens, grilled beef kofta, and cooked beef meat sold in Assiut city restaurants, and among food handlers working in the same food premises. In addition to the sequencing of 23S rRNA gene and phylogenetic relation between *S. aureus* isolated from meat products and food handlers. Also, detect the antimicrobial profile and effect of two natural agents of cow milk proteins such as casein and α-lactalbumin on *S. aureus* isolates of chicken and beef meat and human. Bacteriological culture and PCR were used for *S. aureus* detection in 150 meat products (50 grilled chickens, 50 grilled beef kofta, and 50 cooked beef meat) as well as 92 food handlers. 23S rRNA gene sequencing was done. Disk diffusion method was used for antimicrobial resistance detection, while the impact of casein and α-lactalbumin was evaluated by well diffusion method. Results indicated that 53 (35.5%) and 35 (38.04%) of meat products and food handlers were positive for *S. aureus*, respectively. High degree of genetic homology was described between meat and human isolates of *S. aureus*. These isolates possess high antimicrobial resistance to clindamycine, ampicillin, doxycyclin, vancomycin and ceftriaxone. Casein and α-lactalbumin exhibited antibacterial effect on *S. aureus* isolates. Therefore, they can be used in food industry and human therapeutics but needs further studies for the best application methods.

Keywords | *S. aureus*, Meat products, Food handlers, 23S rRNA sequencing, Casein, α-lactalbumin

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INTRODUCTION

Staphylococcus aureus considered the most prevalent causative agent of food poisoning all over the world, also it infects diverse parts of the body such as skin, respiratory system, open wounds, soft tissue, and blood stream in case of elderly, young, and immunocompromised patients (Tiemersma et al., 2004; Tong et al., 2015). The most incriminated food in *S. aureus* infection includes raw meat and poultry and their products, milk and milk products, bakery products, and fast food meals (Mashouf et al., 2015). Food was contaminated by *S. aureus* when using ingredients originally contaminated, through handling and preparation by food handler as it colonizes the mucous membrane and skin of healthy individuals, and food storage below the sufficient temperature (Plata et al., 2009; Kadariya et al., 2014).

The molecular detection and sequencing of 23S rRNA gene was effective to detect in depth the gene specific for *S. aureus* (Salauddin et al., 2020).

The abundant use of antimicrobials with inadequate concentrations for patient treatment and growth promoter for animals and poultry induce the antimicrobial resistance for human and animal pathogens (Silbergeld et al., 2008). Exploring the antimicrobial profile of *S. aureus* is important for effective clinical treatment and decreases the health risks caused by this pathogen (Kot et al., 2020).

Milk composed of many nutritive components such as water, minerals, lipids, carbohydrates, vitamins, and proteins, and can be safely used in several fields of medicine and cosmetics (Ahmad et al., 2013). Milk proteins like casein, α-lactalbumin, β-lactalbumin, lysozyme, immunoglobulins, lactoferrin, and lactoperoxidase possess various antifungal, antibacterial, anticancer, antioxidant, and antiviral activity (Kazimierska and Kalinowska-Lis, 2021). Casein and α-lactalbumin have antimicrobial activity against *Bacillus subtilis*, *Listeria monocytogenes*, *Strept. pyogenes*, *S. epidermidis*, and *S. aureus* (Gobbetti et al., 2002).

This study investigated the microbial contamination of ready to eat meat products and food handler's hands with *S. aureus* as well as describe the role of food handlers in food contamination by study the phylogenetic relation between *S. aureus* from food and human sources. Also, the antimicrobial profile and the effect of two natural milk proteins such as casein and α-lactalbumin on *S. aureus* isolates were explored.

MATERIALS AND METHODS

Study design

Ready to eat meat products and food handlers samples were examined for the presence of *S. aureus* by bacteriological examination and PCR. Gene sequencing were done to study the relation between food and human isolates. All isolates were examined against 12 antimicrobials using disk diffusion method and exposed to casein and α-lactalbumin to study their antibacterial effect using well diffusion method.

Collection of samples

One hundred and fifty meat samples including grilled chickens, grilled beef kofta, and cooked beef meat (50 each) were purchased from various restaurants in different localities of Assiut city. Ninety-two hand swabs of food handlers who working in the same restaurants were collected and sent to the laboratory for examination.

Bacteriological examination of *S. aureus*

The preparation of meat samples was done according to (Okonko et al., 2013). Isolation and identification of *S. aureus* from meat products and food handlers samples were done according to (FDA, 2001; Hassanien and Abdel-Aziz, 2017).

Molecular identification of *S. aureus*

QIAamp DNA, Qiagen (cat. no. 51304) was used for DNA extraction from meat and human samples. PCR was implemented using Applied Biosystem Thermal Cycler 2720 for detection of 23S rRNA gene with primer sequence F: ACGGAGTTACAAAGGACGAC, and R: AGCTCAGCCTTAACGAGTAC with 1250 bp according to (Sunagar et al., 2010). The cycling condition was modified as following, 94°C for 5 min, then 35 cycles for 45 sec at 94°C, 45 sec at 55°C, 1 min at 72°C, and final extension for 12 min at 72°C. PCR product was examined by agarose gel electrophoresis (1%) with ethidium bromide and photographed using the light transilluminator (Biometra, Germany).

Gene sequencing and phylogenetic analysis

The purification of PCR fragments was done by QIA quick purification kit from Qiagen, Germany (cat. no. 28104). The purified product of 23S rRNA gene was sequenced by Applied Biosystems 3130 genetic analyzer in Animal Health Research Institute, Egypt. The BLAST® program was used to compare the identity of the sequence data with other *S. aureus* at GenBank entries. The phylogenetic tree was performed using neighbor-joining method in MEGA6 by Mega Align module of Laser gene DNA Star version 12.1, and 1000 bootstrap replications were used. The phylogenetic tree was conducted by 23S rRNA gene sequences of NCBI Genbank such as AP019751, CP042157, CP031265, CP040801, LR027876, CP054876, AP017891, LC036194, AP017377, AP017320, CP051482, CP051191, CP013621, HF937103, CP051484, CP051165, CP002110, CP059156, CP045435, CP029658, CP029667, CP051958, CP024649, CP060612, CP077908, CP077897, CP030403, and CP052975, Also, the sequences of meat products and food handlers isolates registered in the Genbank under the accession numbers OK597213 (AAW1) and OK597214 (AAW2), respectively.

Antimicrobial sensitivity test

The disk diffusion technique was conducted using antimicrobial disks from Oxoid to study the antimicrobial activity of all *S. aureus* isolates of food and human according to (CLSI, 2013).

Preparation of casein and α-lactalbumin

The preparation was done according to (Omara, 2019) with some modifications. The whole cow milk was centrifuged for 15 min at 5000 rpm to discard the fat layer and obtain skimmed milk. 250 ml of skimmed milk was warmed to 40 °C in water bath, and then add 1 ml acetic acid drop by drop with stirring to reach pH of 4.6. Keep the solution at 40 °C until no precipitations are observed, then filtered twice with cheesecloth and the residue is called casein.

The filtrate was heated for 5 min at 50 °C and filtered with Whitman fitter. Polyethylene glycol powder was added slowly with mixing until a final concentration of 30 % and kept at 4 °C for 24 hrs, then centrifuge for 20 min at 20000 rpm, and the pH of supernatant was adjusted to 7.8, and then recentrifuged to remove any precipitate. 25 ml of supernatant was diluted with 25 ml of Tris-HCL buffer (pH 7.5) until clear solution was obtained, and then the Tris-HCL was eluted with NaCL to produce α-lactalbumin (Richter et al., 1975).

Effect of casein and casein and α-lactalbumin on *S. aureus* isolates

All food and human isolates of *S. aureus* were cultured on Baird parker medium, then 1×10^6 CFU/ mL were plated on Müller Hinton agar with wells containing 80 μl of casein and α-lactalbumin in each well, and incubated for 24 hrs at 35 °C. The effect of casein and α-lactalbumin was detected by well diffusion method and the inhibition zone growth (Enayatifaed et al., 2021).

Statistical analysis

The antibacterial effect of casein and α-lactalbumin on *S. aureus* was described using SPSS 14 by mean and standard error. $P < 0.05$ indicated the significant difference.

RESULTS AND DISCUSSION

From 150 meat samples, 53 (35.5%) harbor *S. aureus*. Grilled chickens have the highest infection rate (46%), followed with grilled beef kofta (38%), and cooked beef meat (22%). Among 92 food handler swabs, 35 (38.04%) were positive for *S. aureus* (Table 1 and Figure 1). The sequence analysis of 23S rRNA gene and the phylogenetic tree revealed a high degree of similarity between the isolates from Genbank database and meat products (AAW1) and food handlers (AAW2) isolates (Figure 2).

S. aureus isolated from the majority of meat products and food handlers exhibited multidrug resistance against several antimicrobials of varied groups. The high resistance rates for meat products were observed with clindamycin (86.8%), ampicillin (84.9%), ceftriaxone (81.1%), doxycycline (73.9%), and vancomycin (69.8%), and highly sensitive to ciprofloxacin (84.9%), and ceftazidime (81.1%), while the food handlers isolates were resistant to clindamycin, ampicillin, ceftriaxone, doxycycline,and vancomycin in percentage of 91.4%, 82.9%, 80%, 71.4%, and 68.6%, respectively, and susceptible to ciprofloxacin (85.7%), and ceftazidime (82.9%) (Table 2).

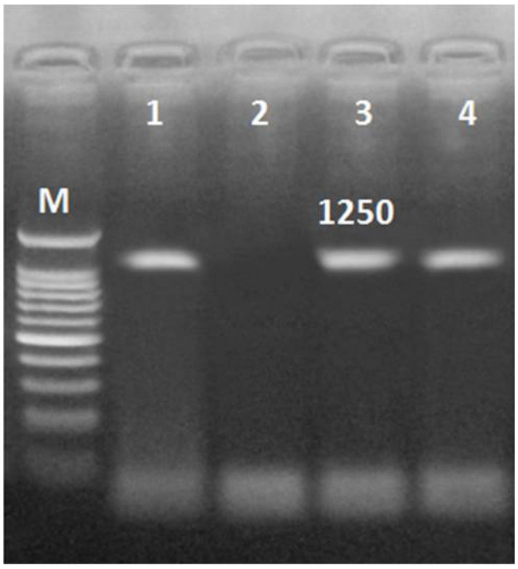

Figure 1: Result of PCR for 23S rRNA gene in meat products and food handlers' samples. M: marker, lane 1, 3, and 4: positive for *S. aureus*, lane 2: negative.

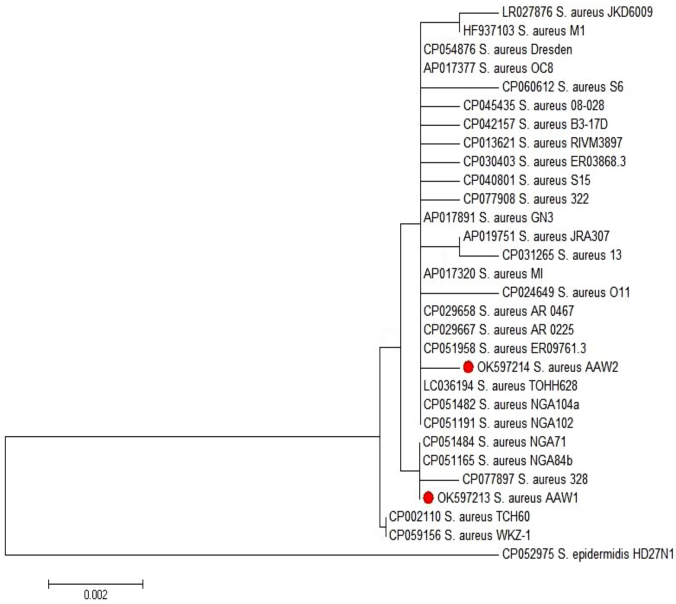

Figure 2: phylogenetic tree with neighbor joining for nucleotide sequence of 23S rRNA gene of *S. aureus*. AAW1: *S. aureus* isolates of meat products, and AAW2: *S. aureus* isolates of food handlers.

Casein represented a growth inhibitory effect on *S. aureus* isolates of meat products and food handlers at mean inhibition zones of 15.30±0.1778 and 12.09±0.2296, respectively. However, the α-lacatalbumin showed inhibitory effect at mean inhibition zones 15.02±0.1605 and 13.40 ± 0.1932 for *S. aureus* isolates of meat products and food handlers, respectively (Table 3).

S. aureus was investigated in some ready to eat meat products such as grilled chickens, grilled beef kofta, and cooked beef meat in percentage of 46%, 38%, and 22%, respectively (Table 1). The rate of *S. aureus* in grilled chickens was higher than other study conducted by (Hassan et al., 2009; Di´az-Lo´pez et al., 2011) with percentage of 40% and 5.7%, respectively. Lower results of *S. aureus* detection in grilled beef kofta were reported by (Shaltout et al., 2017) with detection rate of 32% and Haasan et al. (2020) with detection rate of 6.7%. Similarly, lower results were reported by Haghi et al. (2021) who detected *S. aureus* in 11.1% cooked beef meat samples. Presence of *S. aureus* in grilled chickens and kofta may be resulted from that cooking meat on grill does not provide sufficient heat to all meat mass which enables the growth of microorganisms inside meat especially when left several hours after cooking without refrigeration as some restaurants cooking large amount of food to be ready for serving (Réglier-Poupet et al., 2005). Although cooked beef meat was exposed to high temperature, *S. aureus* in cooked meat may be resulted from unhygienic practice of food handlers which represented in using contaminated equipment, inadequate cooking, and improper holding temperature (Ho et al., 2015).

Results in Table 1 indicated that food handlers' hands were contaminated with *S. aureus* in percentage of 38.04%. Nearly similar results were reported by (Bassyouni et al., 2012) who investigated *S. aureus* in 36.1% hand swabs of food handlers. While lower results were reported by (Castro et al., 2016; Beyene et al., 2019; Ahmed, 2020; Alves et al., 2021) in percentage of 11.1%, 11.3%, 25%, and 8.9%, respectively. These results revealed that food handlers play a great role in microbial contamination of food (Yap et al., 2019). Therefore, increased knowledge about hygienic practice, effective methods of food preparation and storage and improving the food handlers practice is the key factor in prevention of food contamination by pathogenic microorganisms (Conover and Gibson, 2016).

The sequence analysis of 23S rRNA gene in *S. aureus* isolates obtained from meat products (AAW1) and food handlers (AAW2) samples represented a great identity between the two strains. Also, the phylogenetic tree exhibited a high degree of similarity between AAW1, and AAW2, and those registered on the Genbank (Figure 2). This revealed that 23S rRNA gene have a role in epidemiological studies through clinical and molecular classification between several isolates of bacteria from varied sources (El-Maghraby et al., 2020). In addition, reflects the zoonotic importance of *S. aureus* that transmitted through food to humans as well as from food handlers to food (Da Silva et al., 2020).

Table 2 showed that *S. aureus* isolates of meat products and food handlers exhibited high resistance to clindamycin, ampicillin, ceftriaxone, doxycycline, and vancomycin, and sensitivity to ciprofloxacin, and ceftazidime. Similarly, Fri et al. (2020) and Elsawy et al. (2021) reported resistance to clindamycin, ampicillin, and vancomycin, while sensitive

Table 1: Incidence of *S. aureus* in ready to eat meat products and food handlers' samples.

Isolates	Meat products samples								Food handlers samples	
	Grilled chickens (n=50)		Grilled beef kofta (n=50)		Cooked beef meat (n=50)		Total (n=150)		Hand swabs (n=92)	
	No	%	No	%	No	%	No	%	No	%
S. aureus	23	46	19	38	11	22	53	35.5	35	38.04

Table 2: Antimicrobial profile of *S. aureus* isolated from meat products and food handlers' samples.

Antimicrobial	Meat products isolates (n= 53)						Food handlers' isolates (n= 35)					
	S		I		R		S		I		R	
	No	%	No	%	No	%	No	%	No	%	No	%
AMP	3	5.7	5	9.4	45	84.9	2	5.7	4	11.4	29	82.9
CAR	22	41.5	11	20.8	20	37.7	15	42.9	7	20	13	37.1
CRO	2	3.8	8	15.1	43	81.1	3	8.6	4	11.4	28	80
CTX	21	39.6	17	32.1	15	28.3	14	40	11	31.4	10	28.6
CAZ	43	81.1	7	13.2	3	5.7	29	82.9	3	8.6	3	8.6
VA	6	11.3	10	18.9	37	69.8	4	11.4	7	20	24	68.6
DO	6	11.3	8	15.1	39	73.9	4	11.4	6	17.1	25	71.4
AZM	17	32.1	12	22.6	24	45.3	12	34.4	6	17.1	17	48.6
DA	4	7.5	3	5.7	46	86.8	3	8.6	0	0	32	91.4
CIP	45	84.9	5	9.4	3	5.7	30	85.7	3	8.6	2	5.7
OFX	17	32.1	17	32.1	19	35.8	10	28.6	11	31.4	14	40
GAT	19	35.8	12	22.6	22	41.5	12	34.3	11	31.4	12	34.3

AMP: ampicillin 10 µg; CAR: carbenicillin 100 µg; CRO: ceftriaxone 30 µg; CTX: cefotaxime 30 µg; CAZ: ceftazidime 30 µg; VA: vancomycin 30 µg; DO: doxycycline 30 µg; AZM: azithromycin 15 µg; DA: clindamycin 10 µg; CIP: ciprofloxacin 5 µg; OFX: ofloxacin 5 µg; GAT: gatifloxacin 5 µg.

Table 3: The inhibitory effect of casein and α-lactalbumin on *S. aureus* isolates of meat and food handlers' samples.

S. aureus isolates	Casein			α-lactalbumin			P value
	Min	Max	Mean±SdE	Min	Max	Mean±SdE	
Meat products	14	19	15.30± 0.1778	13	18	15.02± 0.1605	0.05
Food handlers	9	14	12.09 ± 0.2296	12	15	13.40± 0.1932	

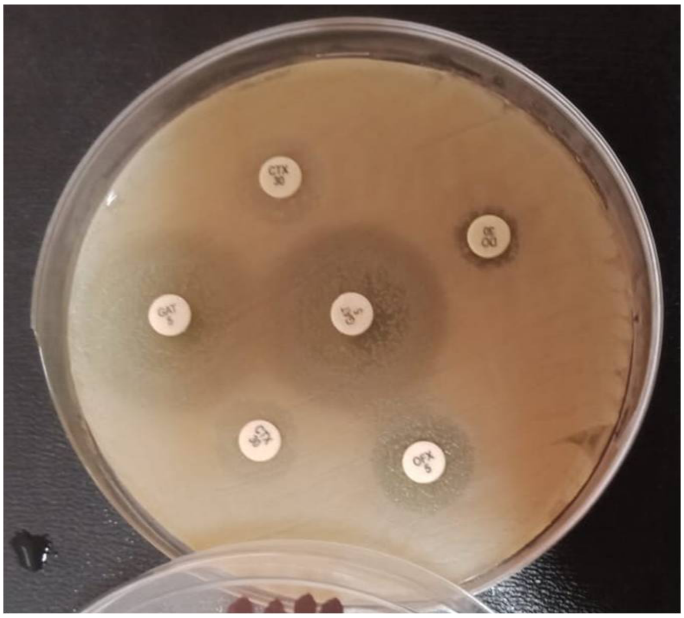

Figure 3: Antimicrobial resistance of *S. aureus* isolates.

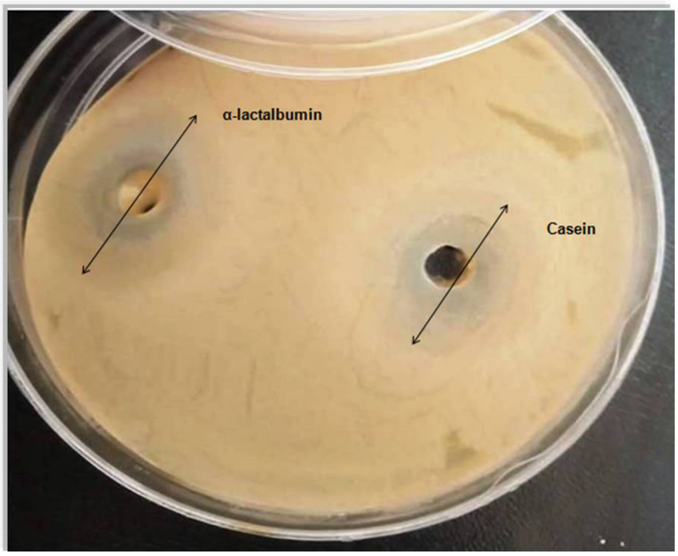

Figure 4: Effect of casein and α-lactalbumin on *S. aureus* isolates.

to ciprofloxacin. This resistance may be arising from indiscriminate usage of antimicrobials in parallel with absence of data about medications effects. The existence of bacterial resistance isolates in meat products and food handler's samples emphasizes the circulation of this resistance between food and human which restrain patients' recovery and expanded disease duration which may causing death to some patients (WHO, 2017).

The evolution of antimicrobial resistance microorganisms enhances to replace these antimicrobials with natural, safe, effective, and renewable agents such as casein and α-lactalbumin which extract from milk proteins (Belkhir et al., 2021).

In this study, casein and α-lactalbumin possess antibacterial impact against *S. aureus* isolates recovered from meat products and food handler's samples, and α-lactalbumin represented a higher effect than casein (Table 3). Similar results were reported by (Omara, 2019). In contrary, Pellegrini et al. (1999) cannot display antibacterial action of native α-lactalbumin. This difference may be related to the variations of milk source (sheep, cattle, goat, and camel) because a milk protein constituent varies between animal species (Ceballos et al., 2009). Our finding showed that α-lactalbumin represented a higher effect than casein; this may be explained why α-lactalbumin has more antibacterial peptide concentration than those in casein (Lopez-Exposito et al., 2006). Therefore, these results highlight the focus on using natural recycling agents in food industry and biomedicine for pathogens control.

CONCLUSIONS AND RECOMMENDATIONS

The great homology between AAW1 and AAW2 of *S. aureus* isolates recovered from meat products and food handlers, respectively, indicating the role of food handlers in transmission of *S. aureus* through food by following unhygienic practice during food handling and preparation. Therefore, educational and training programs about sanitary hygienic measure, selection of safe food ingredients, and methods of preparation and storage should be obligatory applied. Therapeutic programs for diseases should be established for monitoring the antibiotics effectiveness to prohibit the circulation of resistance strains of *S. aureus* among food and human. Application methods of alternative agents to antibiotics such as casein and α-lactalbumin for controlling *S. aureus* infection required additional studies.

NOVELTY STATEMENT

This study recorded a similar sequence between S. aureus isolated from ready to eat food and food handlers and regested in the Genebank as a first record. Also, detection the antibacterial activity of a natural wey protein component against the isolated multidrug resistance strains of S. aureus

AUTHOR'S CONTRIBUTION

Alshimaa A. Hassanien and Asmaa Osama Tolba: Conducted the idea and study design, collection of samples, and literature search. Alshimaa A. Hassanien , Asmaa Osama Tolba and Walaa M. Elsherif : Performed the laboratory work. Alshimaa A. Hassanien: Data analysis and manuscript writing. Asmaa A.A. Hussein: Supervised and editing and revised the manuscript. All authors revised and approved the final manuscript

Conflict of interest

The authors have declared no conflict of interest.

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