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Detection of some virulence factors and antibiotic resistance of Staphylococcus aureus isolated from food source

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***Abstract*---**Because the *S. aureus* is not only associated with severe community and hospital acquired diseases, but it is also a leading cause of food-poisoning outbreaks, the focus of this research is to identify biotypes, virulence genes, and antimicrobial resistance patterns for *S. aureus* isolated from food . The current study was carried out from September to December 2021 , in which 20 *S. aureus* isolated from 150 food samples . A total of food samples were collected from a range of sources, including (milk, meat, cheese and celery). Only 20 (13.4%) of the 150 food samples screened positive for *S. aureus*, with 8 isolates from meat samples, 3 isolates from milk, 4 isolates from celery, and 5 isolates from Iraqi white cheese. Other *staphylococcus* species (*gallinarum*, *xylosus*, *sciuri*, and *lentus*) as well as other genera were obtained. All isolates were diagnosed using traditional biochemical assays and the Vitek method. Antimicrobial susceptibility was tested using the Kirby-Bauer method. All 20 food-borne *S. aureus* isolates were completely sensitive to the majority of antibiotics, which was demonstrated (100%). In food *S. aureus* isolates, only Cefotaxime (100%) was totally resistant, followed by Ceftazidime (60%) and Cefepime (40%). Food isolates had significantly more virulence genes, such as *mecA* encoding Methicillin resistance in (60%) of the isolates and *spa* encoding Staphylococcal protein A in (40%) of the isolates, whereas the *tst* and *luks* genes, which encode toxic shock syndrome toxin and panton-Valentine Leukocidin, were not found in any *S. aureus* isolates. Finally, the *icaA* gene was found in (40%) of food *S. aureus* isolates.

***Keywords*---**food-poisoning, virulence genes, mecA, spa, tst, luks, icaA.

Introduction

Food borne diseases (FBD) still cause a substantial public health, economic and social burden worldwide (WHO, 2015). It is associated with losses in productivity and high medical expenses every year (Garcia *et al* ., 2020). Foodborne pathogens are responsible for food intoxication and the *S.aureus* is a major bacterial pathogen often involved in food poisoning. This allows them to eliminate competing microorganisms, that are less able to endure withstand complex conditions (Kim *et al.*, 2018). The disease is caused by bacterial toxins found in food rather than by the direct effect of microbes on the patient (Murray *et al.*, 2020). Many food sources may serve as a substrate for many microorganisms which are transmitted during harvesting ,storage, food processing, and handling by multiple environmental sources such as water, food, insects, or even by the handlers (Ubiebi ,2017). While a majority of *S. aureus* strains are susceptible to front line antibiotics, horizontal gene transfer and chromosomal mutations have enabled the pathogen to acquire significant antibiotic resistance (Chambers and DeLeo , 2009). Presence of virulence factors appears to vary among *S. aureus* strains, some *S. aureus* produces toxins that may cause such diverse manifestations as septic shock , toxic shock syndrome and panton-Valentine Leukocidin (Adalbert *et al* .,2021) and By forming biofilms bacteria generate an environment that provides protection during growth within host by forming conglomeration of one or more types of microorganisms attached to a surface, in contrast to the planktonic state in which bacteria exist as individual organisms (Flemming *et al* ., 2016 ; Rumbaugh and Sauer ,2020).

Materials and Methods

Samples collection

A total of 150 food Samples collected from September to December of 2021 from different super market , house eat , butcher shops , popular markets as well as from farm in different region in Al-Najaf province. The samples were placed in separate sterile plastic bags and then immediately transported to the cool box with a lot of ice.

Samples Culture

All samples were inoculated on culture of selective medium, its called Blood and Mannitol salt agar, using the direct method of inoculation. They were then inoculated at 37°C for 18-24 hours (Cheesbrough,2010). The plates were examined for the presence of bacteria before being processed multiple times until pure isolates were obtained. A single pure isolated colony was transferred to Brain Heart Infusion broth for preservation.

Diagnosis of *S. aureus* isolates

Isolates were identified and diagnoses were made based on the characteristics of colony toward growing bacteria on media, as well as several important biochemical assays (MacFaddin, 2000). Biochemical assays were used to confirm the identity of suspected bacterial isolates that were Gram positive, coagulase

positive, oxidase negative, and non-motile. The final identification was carried out utilizing ID-GP cards and the automated VITEK-2 compact system.

Antibiotic Sensitivity Test

The sensitivity of all 20 *S. aureus* isolates to a group of antibiotics was determined using the Kirby- Bauer method, and the results were interpreted using the (CLSI, 2020).

S. aureus important gene identification

Polymerase Chain Reaction (PCR) was performed in monoplex patterns in order to amplify different fragments of genes under study for detecting *S. aureus* virulence factors genes. In Table (1) primer used in this study.

Table (1): Primer used in this study

Primer Type	(Primer Sequence (5'-3')		amplicon size (bp)	Reference
MecA	F	TAGAAATGACTGACGTCCG	154 bp	(Santos *et al.*,1999)
	R	TTGCGATCAATGTTACCGTAG		
Tst	F	AAG CCC TTT GTT GCT TGC G	445bp	(Becker *et al.*,1998)
	R	ATC GAA CTT TGG CCC ATACTT T		
Luks	F	CAGGAGGTAATGGTTCATTT	151 bp	(Al-Talib *et al.*,2009)
	R	ATGTCCAGACATTTTACCTAA		
Spa	F	ATCTGGTGGCGTAACACCTG	350bp	(Fatemeh Shakeri *et al.*, 2010)
	R	C GCTGCACCTAACGCTAATG		
IcaA	F	TCTCTTGCAGGAGCAATCAA	188bp	(Carla *et al* ., 2001)
	R	TCAGGCACTAACATCCAGCA		

Each 25 µL of PCR reaction mixture contained 2µL of forward gene primer, 2 µL of reverse gene primer, 4µL of free nuclease water, 5µL of DNA, and 12µL of master mix in a thin-walled PCR tube. The thermal cycler's settings were in Table (2).

Table (2): PCR program that apply in the thermo-cycler

PCR gene	Temperature (c) / Time					Cycle Number
	Initial denaturation	Cycling condition			Final extension	
		Denaturation	Annealing	Extension		
MecA	95/5 min	95/30 sec.	55/30 sec.	72/30 sec.	72/5 min.	35
Tst	95/5 min.	95/30 sec.	56/30 sec.	72/50 sec.	72/5 min.	35
Luks	95/5 min.	95/30 sec.	52/30 sec.	72/30 sec.	72/5 min.	35
Spa	95/5 min.	95/30 sec.	59/30 sec.	72/2 min.	72/5 min.	35
IcaA	94/5 min.	94/30 sec.	55.5 /30	72/30	72/1 min.	40

			sec.	sec.		

Results and Discussion

S. aureus were isolated from different food samples using specific media such as Mannitol Salt Agar, Dnase agar and CHROM™ agar.

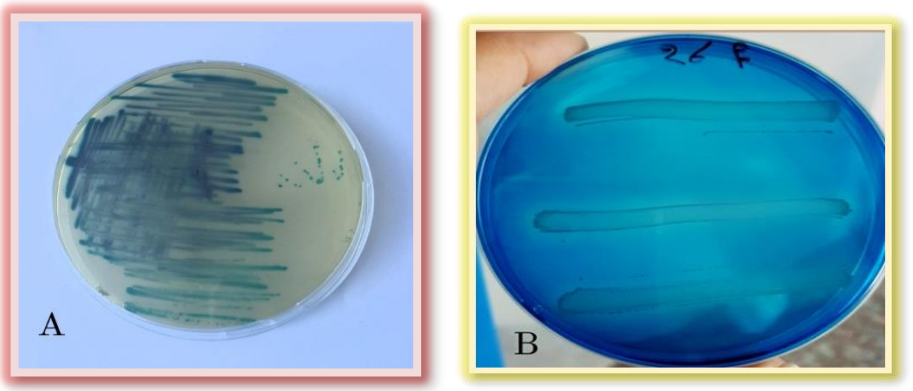

Figure (1) A: growth of *S. aureus* on Chrom ™ agar orientation medium B: growth of *S. aureus* on Dnase agar

Antimicrobial susceptibility test of food *S. aureus* isolate using disc diffusion method.

Antimicrobial susceptibility testing was performed on all of the detected *S. aureus* isolates, the results in a Table (3)

Table (3): Determination the Sensitivity of the food *S. aureus* to Antibiotics

No.	Class	Type of Antibiotics	Sensitive (%)	Resistance (%)
1	β-Lactams antibiotic	Vancomycin (VA)	20(100%)	0
2		Amoxicillin(AMC)	16(80%)	4(20%)
3	Cephems	Cefotaxime (CTX)	0	20(100%)
4		Ceftazidime (CAZ)	8(40%)	12(60%)
5	Aminoglycosides	Gentamicin (CN)	20(100%)	0
6		Amikacin (AK)	20(100%)	0
7	Fluoroquinolones	Levofloxacin (LEV)	20(%100)	0
8	Penems	Imipenem (IMP)	20(100%)	0
9	Macrolides	Azithromycin(AZM)	20(100%)	0
10		Erythromycin (E)	20(100%)	0
11	Carbapenems	Meropenem (MEM)	20(%100)	0
12	Sulfamethoxazole	Trimthoprim(TMP)	20(100%)	0
13	Cephalosporins	Cefepime (FEP)	12(60%)	8(40%)
14		Ceftriaxone (CTR)	20(100%)	0

Molecular Detection of Bacterial Isolates

Detection of *mecA* in food *S. aureus* isolates

A total of 20 *S. aureus* isolates were molecularly examined for the presence of *mecA* genes after being acquired from diverse food sources , Fig (2) illustrates the frequency of genes in the isolates under research as percentages: (60%) from *S. aureus* isolates.

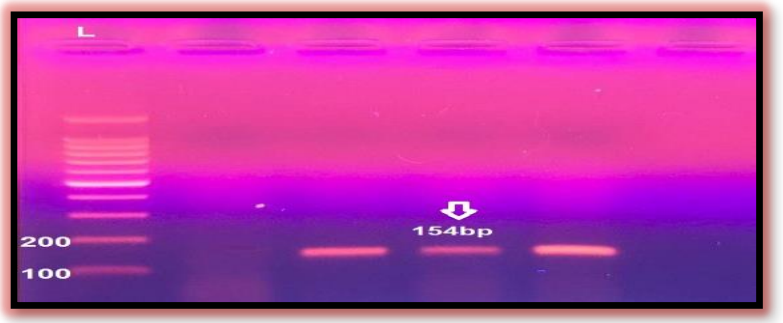

Figure (2): Electrophoresis diagram of Monoplex PCR generated products for extracted DNA of *S. aureus* isolates , utilizing Primer *mecA* gene with Product (154bp). For 1.5 hours, the electrophoresis was run at 70 volts. lane (L), DNA Molecular Size Marker (100 bp ladder) . Gene *mecA* produces positive results in 60% of the isolates.

The current study includes the molecular identification of genes responsible for resistance to major medications often employed in the nation against *S. aureus* isolates , such as *mecA*, which mediates Methicillin resistance in *S. aureus.* Methicillin resistance has occurred in *S. aureus* by mutation of a penicillin - binding protein , a chromosome - encoded protein . This type of resistance is transferred between *S. aureus* organisms by bacteriophages (Lakhundi *et al* ., 2018). The results of *S. aureus* food isolates show that 60% of them contained the *mecA* gene , this finding differs from that of (Can *et al* .,2017) that not detected *mecA* gene in any of the *S. aureus* strains in raw milk, cheese, minced meat.

Molecular detection of *tst* genes

polymerase chain reaction technique of the *S. aureus* isolates revealed that *tst* gene did not show positive result in any of the isolates, this result is identical with (Mello *et al* ., 2016) they not detected *tst* genes in any of the isolates . Other reports mentioned the appearance of this gene ,but in very low proportions such as (Baniardalan *et al* ., 2017) and (Chaalal *et al* ., 2018) they found the *tst* gene coding for toxic shock syndrome toxin was isolated rarely (1.3%) ,(3.2%).

Molecular detection of *luks* genes

The presence of the *luks* gene (151pb) was determined by polymerase chain reaction amplification among *S. aureus* isolates from diverse food sources .

The findings indicated that all food *S. aureus* isolates did not include the *luks* gene. the results of this study were identical to that of (Basanisi *et al* ., 2016) they showed that no *S. aureus* isolates carried *lukS-PV* genes , while the finding did not agree with that of (Basanisi *et al* ., 2017), who found that 50% of isolated strains carried PVL-encoding genes. gene coding for *luks* PVL (Panton-Valentine leukocidin) is a potent cytotoxin composed of two chromatographically different protein components, *LukS*-PV (slow) and *LukF*-PV (rapid). The active toxin induces neutrophil lysis by causing a hole in their membrane, a condition known as dermonecrosis (Jauneikaite *et al* ., 2020).

Molecular detection of *spa* genes

A total 20 of *S. aureus* isolates were molecularly tested for the presence of *spa* genes after being collected from various food sources . where was the gene frequency in the isolates under investigation as a percentage (60%). The *spa* is a surface protein of *S. aureus* that, in addition to being a virulence factor, is utilized to define the bacterium's unique identification . It is feasible to avoid epidemics, limit the number of illnesses, and lower the cost of nosocomial infections by molecular typing this protein.

Figure (3): Electrophoresis diagram of Monoplex PCR generated products for extracted DNA of *S. aureus* isolates utilizing Primer spa gene with Product (350bp). For 1.5 hours , the electrophoresis was run at 70 volts . lane (L), DNA Molecular Size Marker (100 bp ladder) Gene *spa* produces positive results in (60%) of the isolates.

Molecular detection of *icaA* genes

The molecular detection of *icaA* gene by using specific PCR primer for *S. aureus* isolates from different food source , Fig. (4) . The results of this experiment revealed positive amplification for (40%) of food *S. aureus* isolates. this result not identical with study of (Lampugnani *et al* ., 2020) who found all *S . aureus* strains were negative for *icaA* gene . *icaA* encoding slime layer production, it is the major part of biofilm formation. A microbial biofilm consists of a community of microbial cells that irreversibly attach to the substrate or each other. This community produces a surrounding matrix of extracellular polymeric materials (Parastan *et al* ., 2020).

Figure (4): Ethidium bromide – stained agarose gelelectrophoresis of PCR products from extracted total DNA of *S. aureus* using primer *icaA* gene with product 188bp . The electrophoresis was performed at 70 volt for 1.5 hr. lane (L), DNA molecular size marker (1500 bp ladder). Gene *icaA* produces positive results in (40%) of the isolates.

Conclusions

In food; *S. aureus* isolates, only Cefotaxime (100%) was totally resistant, followed by Ceftazidime (60%) and Cefepime (40%). Food isolates had significantly more virulence genes, such as *mecA* encoding Methicillin resistance in (60%) of the isolates and *spa* encoding Staphylococcal protein A in (40%) of the isolates, whereas the *tst* and *luks* genes, which encode toxic shock syndrome toxin and panton-Valentine Leukocidin, were not found in any *S. aureus* isolates.

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