Detection of Indicator Genes in Methicillin-Resistant Staphylococcus Aureus (MRSA) Isolated From Meat Samples

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Staphylococcus Aureus, MRSA, Meat, ,Detection Genes, Antimicrobial Resistance

ABSTRACT
Aims: The presence of Staphylococcus aureus, particularly methicillin-resistant S. aureus (MRSA), on retail meat products is becoming more and more of a concern. The aim of the present study is to examine the recovered strains of methicillin-resistant Staphylococcus aureus (MRSA) from different retail beef in Najaf/ Iraq. Methodology and results: The phenotypically by conventional identification. Genotypical examination was made also by polymerase chain reaction (PCR) to detect the genes some diagnostic genes for the enterotoxins sea, seb, sed. More than 200 strains of Staphylococcus aureus, from different butchers meat shops. MRSA strains were characterized by streaking on different selective media and biochemically identified as Staphylococcus aureus MRSA. The results showed that the appearance of sea genes in 51% of the isolates. Seb gene was in 18.90% of the isolates while 5.40 % of the isolates displayed sed gene. Also, Resistance towards six different antimicrobial agents was assessed and revealed that the tested strains of Staphylococcus aureus showed different level of resistance for Streptomycin 100%, Cotrimoxazole 100% , Cephalothin 45.90%and Tetracycline 32.3%. Conclusion, significance and impact of study: the majority of molecular isolate types have been connected to human infections globally, demonstrating that these S. aureus strains in Iraq have a potential for serious pathogenicity. The presence of the genes (sea, sed, and seb) in the MERSA isolates from meat in this study is alarming and raising public health concerns.

1. Introduction

The responsibility for ensuring the safety of food has recently fallen to numerous pathogens. It is well known that *Staphylococcus aureus* is one of the main foodborne pathogens in fresh and ready-to-eat products and is to blame for a number of infections around the world (Aycicek *et al*., 2005). *S.* aureus can grow at temperatures ranging from 15 to 45 degrees Celsius and at NaCl concentrations up to 15%. At room temperature, this bacterium grows promptly and produces toxins that can be harmful to humans. *S. aureus* was naturally present in many places around the world, but food was its primary point of infection (Jackson et al., 2013). Around 241,000 foodborne illnesses in the USA occur annually (Jones and Yackley, 2018). This bacteria was the third most common pathogen in China in 2013 after *Vibrio parahaemolyticus* (27.8%) and *Salmonella* (23.1%), where outbreaks of foodborne bacteria were responsible for 12.5% of cases (Wei-We *et al*., 2018). Methicillin-resistant *S. aureus* (MRSA) is a significant threat to human health, with multi-drug resistance being a top concern. Retail meat items from animals that are used to produce food, such as swine, poultry, and cattle, are now among the food sources of *S. aureus*, in addition to the staphylococcal food poisoning it previously caused (Jackson *et al*., 2013) Additionally, MRSA has been discovered in both animals used for meat production and in retail meat. When MRSA-positive animals are slaughtered, the environment around them and the animal carcasses themselves may serve as points of contamination, which is how retail meat is thought to become contaminated (Wei-We *et al*., 2018). Staphylococcal food poisoning (SFP) is usually associated with foods rich in protein, such as meat and dairy products, which are complex in terms of microbial content, salt, pH, and available nutrients. In most cases, food can reach a temperature that prompt the growth of *Staphylococcus aureus* (Bean *et al*., 199). Meat and meat products were among the food items surveyed, and it is well known that they serve as a significant *S. aureus* reservoir and are linked to several outbreaks. *S. aureus* from meat research can be used to implement system monitoring (Wu *et al*., 2018). The researcher (Giannatale *et al*., 2011) examined 350 different samples of food products of animal origin and found that the percentage of contamination in fresh meat products reached 19.3%, as the number of colonies formed in these meats ranged between (5-720 cfu/g), while the number of Colonies formed in canned meat ranged between (30 - 2900 cfu / g), and the percentage of contamination in fresh cheese was 13.3%,

as the number of colonies formed reached (750 - 2800 cfu / g). In another study conducted by the researcher (Oh *et al*., 2007) in Ready-to-eat foods contaminated with *Staphylococcus aureus,* where he collected 3332 samples, 47% of the isolated strains were capable of forming one or more types of staphylococcal enterotoxins SEA, AEB, SEC, and 90% of these strains were able to form at least SEA enterotoxins. The prevalence and levels of *S. aureus* from Iraq are therefore being investigated in this study's retail meat and meat products. However, the quantitative and qualitative information about the toxins produced by this bacterium in retail meat from various regions of Iraq was limited. Antibiotic susceptibility tests were used to determine the virulence background of the *S. aureus* isolates following isolation and identification, demonstrating that these *S. aureus* strains in Iraq have a potential for serious pathogenicity. Therefore, this study was carried out to give a clear understanding about the plan for The Public Health.

2. Methodology

Samples Collection, isolation and identification of *Staphylococcus aureus*

200 meat samples were collected for the period 10/ 1/2022 to 2/ 05/ 2022, from different local markets in Najaf /Iraq. Meat samples included (70 samples of fresh Red meat(R), 70 samples of frozen meat (F), 30 samples of Canned mead (C) and 30 samples of Sausages (S) Table 1. All collected samples were processed and identification of *Staphylococcus aureus* was achieved using differential culture media (Mannitol salt agar, Blood agar and *MeReSa* ChromoSelect Agar (Himedia/China) . The latter medium used for detection of mecithillin resistance staphylococcus. The isolates grew on this medium were selected for genes of enterotoxins detection. Vitek compact system was also applied to confirmed the diagnosis. Isolates of *Staphylococcus aureus* grew well on mannitol salt agar forming yellow colonies

Table 1: Distribution of samples under study

Samples	Abbreviation	No.of collected samples
Fresh meat	R	70
Frozen meat	F	70
Canned meat	C	30
Sausage	S	30
Total		200

Antibiotic Susceptibility Test:

The antimicrobial susceptibility examination of the collected bacterial isolates were accomplished by the disk diffusion method using Muller Hinton agar (Oxoid) based Clinical and LaboratoryStandards Institute (CLSI, 2017) and the antimicrobial agents used are shown in table 2. The results were explicate as susceptible, intermediate, or resistant, according to the standards outlined in (CLSI, 2017).

Table **2**: Antibiotics used for susceptibility testing of *Staphylococcus aureus* isolates and inhibition measurements according to Clinical and Laboratory Standards Institute (CLSI, 2017)

Antibiotic	Inhibition zones			concentration	symbol
	S	I	R		
Cotrimoxazole	≥16	11 - 15	≤10	25 µg/disk	COT
Cephalothin	≥18	15 – 17	≤14	30 µg/disk	CEP
Streptomycin	≥15	12−14	≤11	10 µg/disk	S
Tetracycline	≥15	12−14	≤11	30 µg/disk	TE

Ciprofloxacin	≥21	16−20	≥15	15 µg/disk	CIP
Levofloxacin	≥17	14 – 16	≤13	5 µg/disk	LE

PCR detection of the genes sea, seb and sed

Three genes (sea, seb and sed) in *Staphylococcus aureus* were selected for detection of enterotoxin production. Using the online tool at http://www.ncbi.nlm.nih.gov, the gene sequences were annotated in accordance with the GenBank sequence database of the National Center for Biotechnology Information (NCBI). The most conserved sequence of the two genes was identified through the Multiple Sequence Alignment (SRS) of these sequences. The conserved gene sequences were used by Primer Quest through Integrated DNA Technology (IDT) to select specific primers for each gene. The primers used in this study were produced ad supplied by Promega, USA. The sequence of primers is listed in (Table1). In brief, one colony was chosen from the isolated samples and grown on LB broth medium overnight at 37°C. Various amounts (from 50 to 100 l) were plated on selective LB agar plates. The plates were incubated for 18 hours at 37 °C. Then, with sterile toothpicks, 2 to 4 colonies were selected at random from each plate, and then they were boiled in 50 ml of water for five minutes at 95 °C. Then samples were centrifuged at 12,000 rpm for 60 second, direct colony PCR of the supernatant was implemented with Dream Taq™ Green PCR Master Mix (Promega/USA). PCR cycling setting were as follows: initial denaturation at 94 $C^{o}$for 2 minutes for 35 cycles, Annealing for 57 C^{o} 2 minutes and 72°C for 40 seconds and a final extension was at 72°C for 7 minutes. An aliquot of each reaction was electrophoresed on a 1 % agarose gel (Bio Basic/ Canada) marked with ethidium bromide and paralleled with 100 bp plus DNA Molecular Weight Marker (Bioline, UK).

Table 3: Specific amplification primer sets for the tested genes.

Gene	Primer type	Oligonucleotide sequence (5'_3')	Size of amplified fragment (bp)	Reference
sea	F R	GGTTATCAATGTGCGGGTGG CGGCACTTTTTTCTCTTCGG5	102	**This study**
seb	F R	GTATGGTGGTGTAACTGAGC CCAAATAGTGACGAGTTAGG	164	**This study**
sed	F R	CCAATAATAGGAGAAAATAAAAG ATTGGTATTTTTTTTCGTTC	278	**This study**

3. Results and discussion

More than 200 random samples of fresh, frozen and processed meat were collected in the local markets of Najaf province /Iraq. Samples were distributed in the form of four groups, which included (70 samples of fresh meat, (70) samples of frozen meat, (30) samples of meat Imported canned and (30) samples of processed meat (sausages) from different origins that were collected from several local markets in different regions of Al-Najaf province.

Isolation and identification of *S. aureus*

The results showed that 56% (112) of the samples did not show any growth in Mannitol Salt Agar, while 44% (88 samples) of the samples showed growth on the same medium, 19% (33 isolates) were growing on the medium without fermenting the Mannitol sugar. 25% (50 isolates) showed growth on the medium and fermented sugar Mannitol as shown in Figure 1, from the sugar fermented isolates were selected 37 isolates were positive on MRSA and these were selected for further evaluation. .

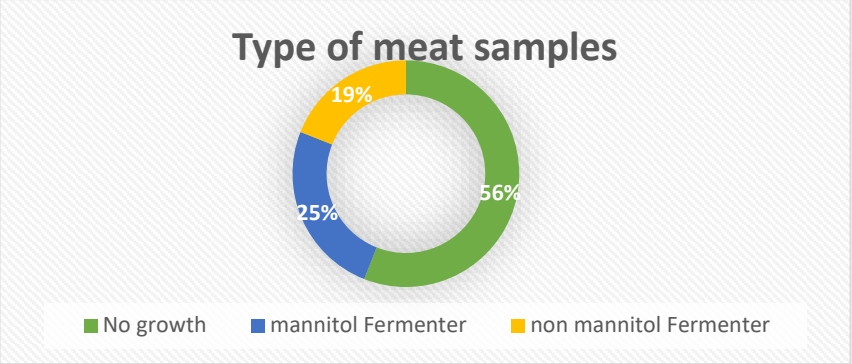


Figure 1: Percentage of isolates growing on Mannitol salt agar

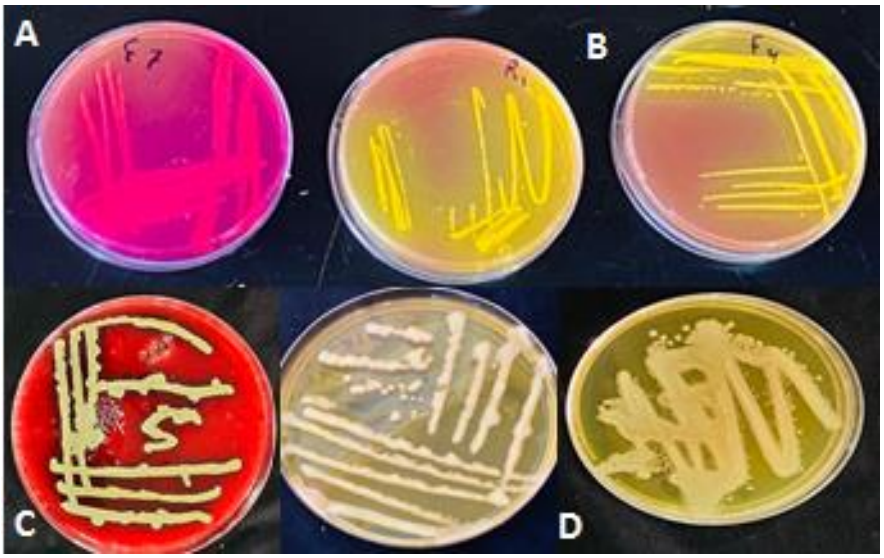


Figure 2 : Growth of *S. aureus* bacteria in different culture media.(A) and (B) sample growing on Mannitol salt agar.(C) sample growing on Blood agar.(D) confirmation of *S. aureus* on Chromogenic MRSA Selective Agar.

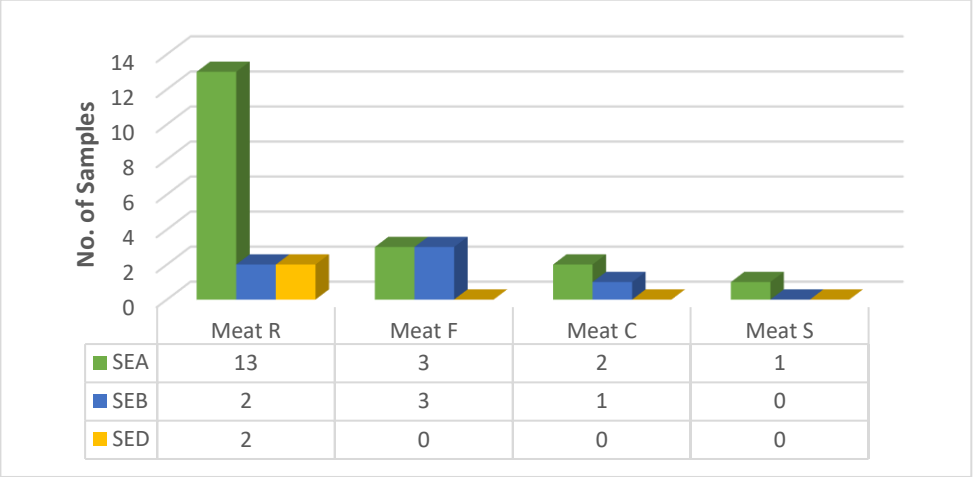
Organism Origin	VITEK 2
Selected Organism	99% Probability **Staphylococcus aureus** **Bionumber:** 010402062763231 **Confidence:** Excellent identification
SRF Organism	
Analysis Organisms and Tests to Separate:	
Analysis Messages:	
Contraindicating Typical Biopattern(s)	

Biochemical Details																			
2	AMY	-	4	PIPLC	-	5	dXYL	-	8	ADH1	+	9	BGAL	-	11	AGLU	-
13	APPA	-	14	CDEX	-	15	AspA	-	16	BGAR	-	17	AMAN	-	19	PHOS	+
20	LeuA	-	23	ProA	-	24	BGURr	-	25	AGAL	-	26	PyrA	+	27	BGUR	-
28	AlaA	-	29	TyrA	-	30	dSOR	-	31	URE	-	32	POLYB	+	37	dGAL	+
38	dRIB	-	39	ILATk	+	42	LAC	-	44	NAG	+	45	dMAL	+	46	BACI	+
47	NOVO	-	50	NC6.5	+	52	dMAN	+	53	dMNE	+	54	MBdG	+	56	PUL	-
57	dRAF	-	58	O129R	+	59	SAL	-	60	SAC	+	62	dTRE	+	63	ADH2s	-
64	OPTO	+															

Figure 3 : Vitek Compact device profile of *S. aureus*

Molecular identification of enterotoxins produced by *S. aureus*

All (37) isolates of *S. aureus* were subjected to molecular diagnosis to detect the diagnostic genes for the enterotoxins sea, seb, sed by using the PCR technique. The results showed the presence of the sea gene in (19) isolates they were distributed in different meat samples. The results of the study also showed that the seb gene appeared in (6) samples of different types of meat except for sausages, while the results of the sed gene appeared only in two samples of fresh meat, which are (R58 and R 39) as shown in Figure (4).



	Meat R	Meat F	Meat C	Meat S
SEA	13	3	2	1
SEB	2	3	1	0
SED	2	0	0	0

Figure 4 : The distribution of genes on the types of different meat samples

The results also showed the appearance of sea in isolates (R28, F7, R51, R69, F22, R8, C30, R39, R30, R26, R4, R18, F27, R45, C4, R6, R1, F40).

The results also showed the appearance of the seb gene in the isolates (F7, R51, R8, F1, C10, F40) and the appearance of the sed gene in the two isolates (R58, R39 as shown in Figure (5).

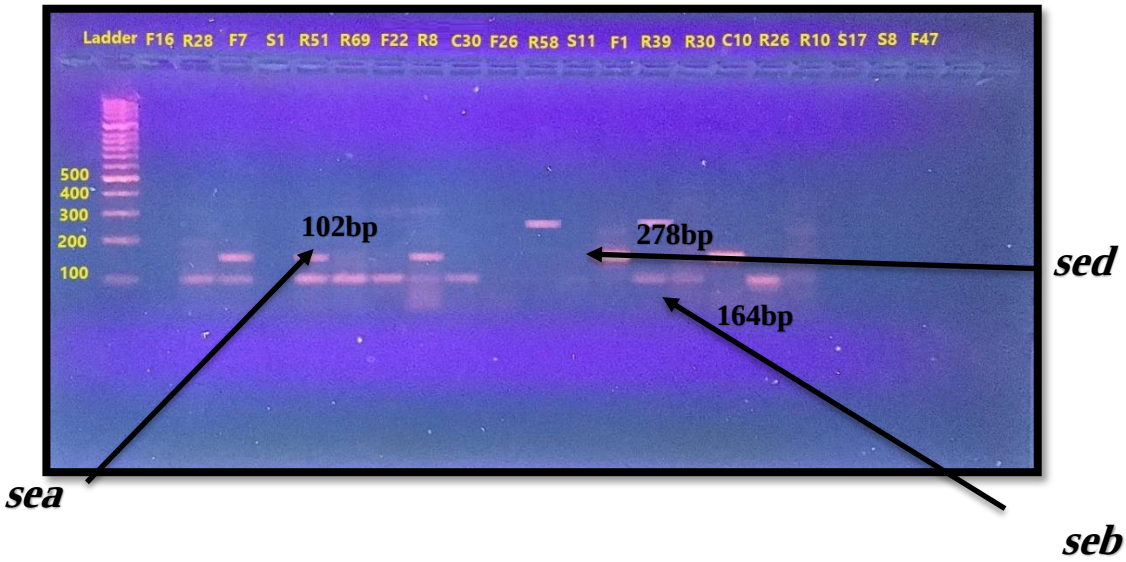


Figure (5): shows the results of Multiplex electrophoresis for the detection of *Staphylococcus aureus* toxins from different meat samples

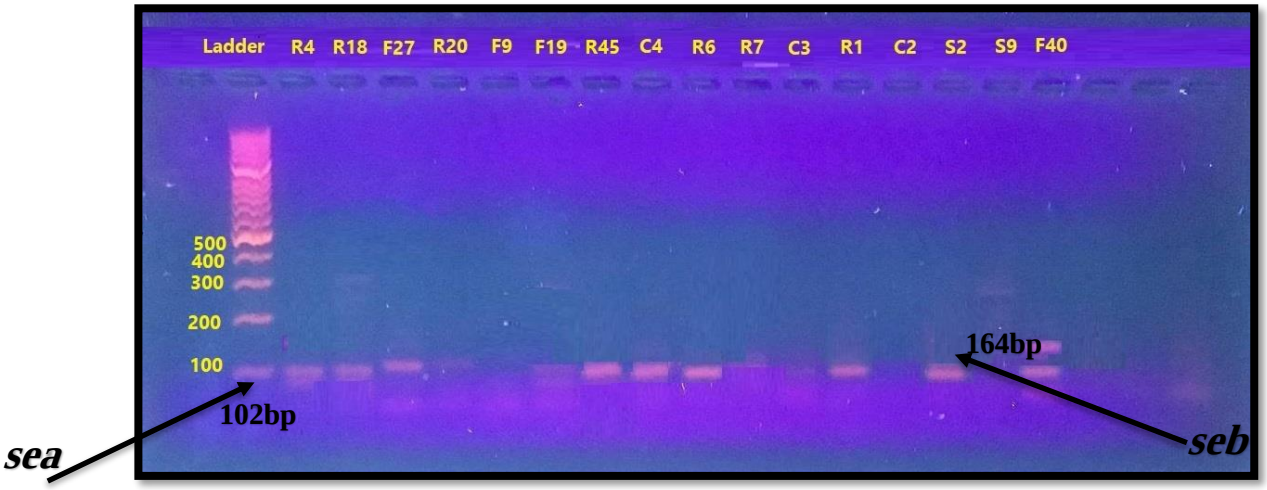


Figure 6: shows the results of Multiplex electrophoresis for the detection of *Staphylococcus aureus* toxins from different meat samples.

The results of the study also revealed that the percentage of sea in different meat samples amounted to 51% of the total number of *S. aureus* isolates. Enterobacteriaceae in chicken meat, as the results showed that the percentage of seb appearance was 18.9% of the total number of *S. aureus* isolates, and this percentage was close to what was reached by the researcher Mohieddin and his group 2017 when detecting the presence of the same gene in soft cheese samples, which amounted to 17.39%. The percentage is close to what the researcher (Ikeda *et al*., 2005) found when revealing the presence of the same gene for people dealing with food, at a rate of 18%. Also, among the results of the study, the percentage of sed appearance reached 5.4% of the total number of *S. aureus* isolates, and this result was close to what the researcher (Alhasnawi *et al*., 2018)reached. And her group 2021 reported the presence of the same gene in raw milk samples of cows, at a rate of 8.33%. The results of the research showed a large difference in the appearance of enterotoxin genes in *S. aureus* isolates of meat samples, as shown in Figure 7.

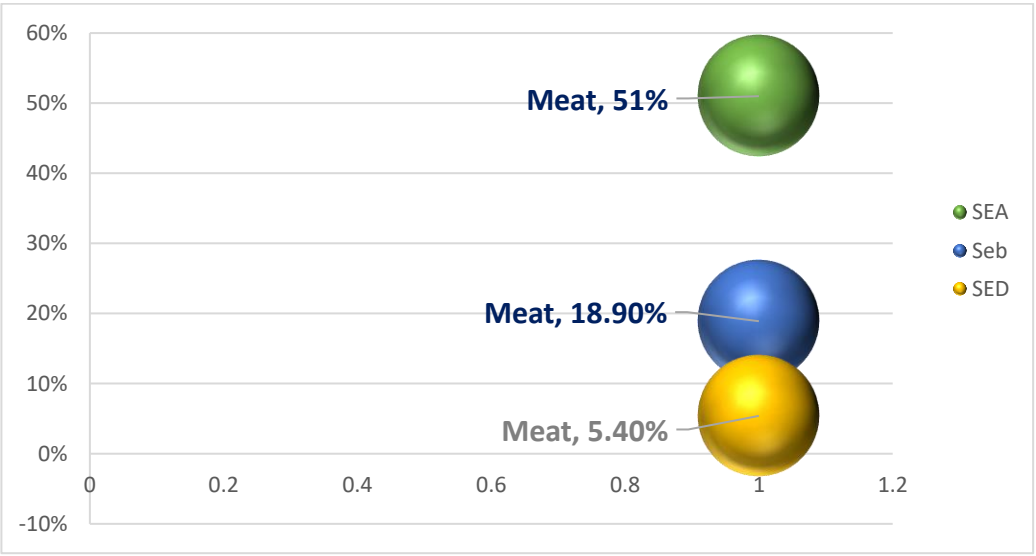
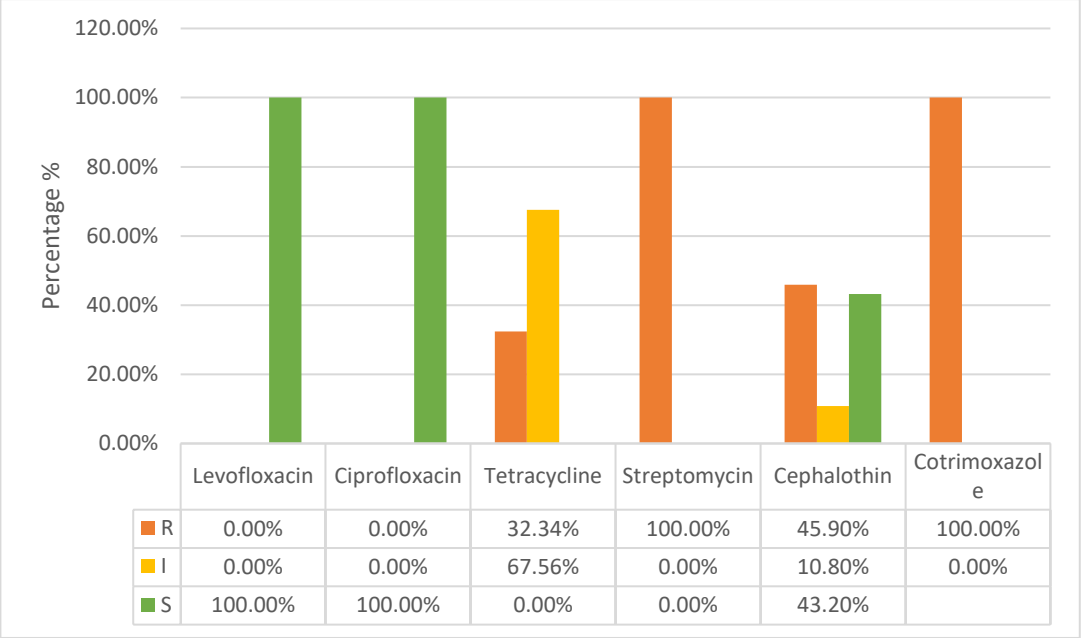


Figure 7: shows the incidence of enterotoxin genes *in S. aureus* isolates of meat samples

Antibiotic resistance of *S. aureus*

The sensitivity of bacteria to six different antibiotics was tested, and the bacteria varied in their resistance to these antibiotics Figure (8). The differences in the level of sensitivity to antibiotics are due to several reasons, including reducing the permeability of the cell wall or the production of Biofilm, which greatly helps bacteria to overcome the action of antibiotics and reduce their spread

quickly (Cheung *et al*., 2012).



	Levofloxacin	Ciprofloxacin	Tetracycline	Streptomycin	Cephalothin	Cotrimoxazole
R	0.00%	0.00%	32.34%	100.00%	45.90%	100.00%
I	0.00%	0.00%	67.56%	0.00%	10.80%	0.00%
S	100.00%	100.00%	0.00%	0.00%	43.20%	

Figure (8) Percentage of antibiotic resistance of *S. aureus* isolates under study

The results of the study showed that the isolates were 100% resistant to both anti-Streptomycin and Cotrimoxazole, and this result was completely consistent with what the researchers mentioned (Pyzik and Marek, 2012). The researcher (Evans, 1998), found that the resistance rate of these bacteria isolated from milk was 0% to the group of Fluoroquinolone antibiotics, because these antibiotics are highly effective against Gyrase and Topoisomease enzymes, which lead to stopping DNA replication and the cloning process (Silva *et al*., 2022) The results of the study also showed a difference in the resistance of the isolates to Cephalothin antibiotic, as the study showed that 45.9% of the isolates were resistant to the antibiotic and 42.2% were sensitive to the antibiotic, similar to what the researchers (Pyzik and Marek ,2012) have identified.

Conclusion

S. aureus present in the form of Normal Flora in the tissues and guts of animals makes it a high risk of food contamination. Most of the *S. aureus* isolates isolated from imported local meat showed that they contain enterotoxin genes and thus raise the alarm on food contamination with these toxins that affect the health of the consumer. These toxins have high resistance to heat, salt concentrations, and acidic media, so the possibility of getting rid of them by traditional methods may be difficult. The toxins secreted by these bacteria are of the type of exotoxin, and thus the elimination of this type of microorganisms does not reduce the risk of infection with the toxins secreted by them, as they remain effective. The most prevalent enterotoxin genes in food are Sea, with high percentages compared to Seb and Sed genes. The bacteria have a wide and varied spectrum of resistance to antibiotics, and therefore antibiotics may not be sufficient for elimination this pathogen. These findings imply that human contamination of the retail beef samples may have occurred during meat processing and that consumers and others who handle raw meat may now be at risk of contracting MRSA.

Acknowledgement

Not applicable

Reference

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