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ASSESSMENT AND EVALUATION OF BACTERIOLOGICAL HAZARDS FROM CRITICAL POINTS IN MEAT SHOPS CONCERNING SOME TOXIGENIC AND BIOFILM-FORMING BACTERIA

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ABSTRACT

To manage public health threats in meat from meat shops, the present study aimed to identify and evaluate the bacteriological hazard from critical points (meat cuts, liver, minced meat, equipment including knives and mincing machines, Tables, workers' hands, and refrigerators) in retail meat shops of different regions in Kafr El-Sheikh city, Egypt, through total bacterial count (TBC), total coliform count (TCC), and *Staphylococcus aureus* count (SC) in examined samples and swabs from each source (n =20) the mean count values in CFU/g were recorded higher ($p< 0.05$) in minced meat samples. Samples that exceeded permissible limits were also detected. *S. aureus* was isolated in descending order by a rate of 100%, 85%, 80%, 65%, 60%, 50%, and 40% from tables, workers' hands, refrigerators, minced meat, equipment, meat cuts and liver samples, respectively, this means instruments, and workers' hands are higher in isolation rate of *S. aureus* than meat products. Among10 isolates of *S. aureus* from different points, the prevalent genotypes of biofilm-forming genes were *ebps* and *eno* in all samples, while enterotoxins genes represented by *seb* and *sec*. Fifty-four out of 100 *E.coli* isolates were identified serologically as EPEC (O146: H21, O44: H18, O20: H7, O163: H2), EHEC (O111: H2, O26: H11, O91: H21, O117: H18), ETEC (O125: H21, O128: H2), and EIEC (O159). Findings revealed that meat sold in our local meat shops contains high numbers of spoilage and pathogenic organisms that could be a possible threat to meat and consumers.

***Keywords*:** Meat contamination, meat shops, biofilm-forming bacteria, *S. aureus, E.coli*

INTRODUCTION

Meat is considered one of the important sources of protein, essential amino acids, vitamins, and minerals, therefore it provokes the growth of microorganisms. Bacteriological hazard in meat is one of the potential biological hazards, the most important microbiological hazards in meat are bacteria which cause a large proportion of all foodborne illnesses (FSIS, 1999). Focus is moved from food service to the retail sector, including butcher shops selling raw meat, as several *Escherichia coli* (*E. coli*) outbreaks have been linked to butcher shops (FSAI, 2006).

As the carcass, raw cuts of meat, in general, have the same level of contamination. Plus contamination through handling and processing (Abdou *et al.*, 2015). As well as, during

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manipulation, and distribution at retail establishments (Barros *et al.,* 2007).

Staphylococcus aureus bacteria is common in the environment. It is a major cause of foodborne diseases with a short incubation period. Contamination of meat is mainly from humans. It colonizes human skin and the front nares in a substantial fraction of the human population (Castro *et al.,* 2016). Production of heat-stable toxins is the risk of their growth (Wang and Ruan, 2017). As well as, *S. aureus* is capable of colonizing processing surfaces creating biofilms that result in the persistence of such bacteria in food processing environments (Coughlan *et al.,* 2016).

The ultimate source of *E. coli* is an apparently healthy animal that may shed these bacteria in their feces. Then found in slaughtered livestock after dressing the carcass. Foodborne disease outbreaks was associated with the consumption of contaminated meat products with these pathogens (El-Gamal *et al.,* 2016).

Through the consumption of raw or undercooked contaminated beef by pathogenic *E. coli,* especially Shiga toxin-producing types (STEC), a potent toxin secreted in the intestinal tract of infected people causes diarrhea that may be bloody, fever and may result in kidney failure and death, especially in children (FSIS, 2002). The attachment of these bacteria to the food contact surfaces in form of detrimental biofilms leads to serious hygienic problems (Carpentier and Cerf, 1993), due to cross-contamination of uncontaminated carcasses (Zottola and Sasahara, 1994).

Wet food processing environments and perishable food above 0.9 water activities are suitable for microorganism multiplication and biofilm formation, so the safety of food products is threatened by biofilms that developed by toxigenic and pathogenic bacteria such as *S. aureus* and pathogenic *E. coli* (Galié *et al.,* 2018; 2020; Sanches *et al.,* 2021).

Microbial biofilms could be formed on all surfaces in the food environment such as metal, glass, plastic, wood, and cement, in which tolerance of bacteria to environmental stresses will increase (Beloin and Ghigo, 2005). Biofilm is formed through the combination of an active matrix of cells and extracellular substances (extracellular polymeric substance, EPS) with a solid conditioned surface with nutrients. Then cells grow and actively multiply to form a mass of cells that become large enough to entrap debris and other microorganisms (Kumar and Anand, 1998). This enforces the resistance of bacteria to disinfectants (Di Martino, 2018; Van Houdt and Michiels, 2005). Biofilm is also formed under strict genes essential for EPS synthesis and cell aggregation (Alvarez *et al.,* 2013).

The foodborne infections can mainly be traced to unhygienic food handling procedures (Ebied *et al.,* 2022). So contamination prevention must be taken to ensure the safety of meat (NAS, 2003). This work aimed to evaluate the microbial quality and safety of raw meat and the hygienic conditions of the surrounding environment in meat shops in Kafr El-Sheikh city, based on the estimation of indicator microorganism counts as total bacterial count (TBC), total coliform count (TCC), and *S. aureus* count (SC), besides assessing microbial hazards by detection of *S. aureus* with enterotoxin genes (*Sea, Seb, Sec*) and biofilm-forming genes (*Ebps, Cna, Eno*). As well as detection and serological identification of pathogenic *E. coli* from critical points in meat shops that include (meat cuts, liver, minced meat and environmental swabs from equipment (knives and mincing machines), tables, workers' hands, and refrigerators)

MATERIALS AND METHODS

Experiments were carried out in both laboratories of Animal Health Research Institute (AHRI), Dokki, Giza, and Kafr El-Sheikh branch.

1. Samples collection

One hundred and forty samples of raw beef meat cuts, liver, minced meat, and environmental swaps from equipment (knives and mincing machines), tables, workers' hands, and refrigerators (20 of each) were collected from different retail meat shops in Kafr El-sheikh city. Raw meat cuts, liver, and minced meat samples (200gm for each) were purchased in labeled sterile bags. Swabs were obtained from workers' hands and meat contact surfaces (equipment, tables, and refrigerators). Areas were sampled in centimeters with sterile swabs according to Köck *et al.* (2009). Samples were transported immediately in cooled ice boxes at 4^{o}C to the laboratory were prepared, and subjected to different tests. The samples were sensory

evaluated for color, odor and flavor according to Marriot (1995).

2. Preparation of Samples

Each sample of meat cut, liver, and minced meat, (25g) was prepared according to APHA (2001), while properly mixed with sterile peptone water, 225 mL (Oxoid, United Kingdom) to provide dilution of 10^{-1} after homogenization for 60 seconds using a Stomacher Lab-Blender 400 (Seward Ltd, UK). Then tenfold serial decimal dilutions were done for bacterial counts. From the hand surface of butchers and workers as well as instruments swabs tips were taken from 10 cm^2 area, and suspended in 10 mL sterile peptone water. Then serial dilution was occurred, and calculation was done per 1cm^2 (FDA, 2002).

3. Microbial count

Each count was occurred according to the specific medium and the determination technique of each microbial group. Performed serially diluted samples, enumeration of TBC using standard plate count agar according to APHA (2001) was done. TCC was done according to APHA (2001) using the most probable number (MPN) technique. *S. aureus* count (SC) on Baird-Parker agar medium was done according to FAO (1992). Detection and enumeration of bacteria in swabs, according to PHE (2017). The swabs are vortex mixed to aid the release of organisms into the diluent. Then swab diluent serially diluted. After incubation, all colonies formed on plates were counted and the results were converted to CFU/g. or CFU/cm^2.

4. Isolation and identification of *S. aureus*

Suspected colonies appear as black colonies surrounded by a clear zone with a narrow white margin were enumerated and stabbed into semisolid agar tubes for further biochemical identification (Lancette and Bennett, 2001). *S. aureus* isolates were further tested by PCR to detect Enterotoxin genes as well as biofilm-forming genes using specific primers, target genes, and cycling conditions as illustrated in Table 1.

5. Polymerase chain reaction (PCR)

- Extraction of Bacterial genomic DNA from *S. aureus* isolates was performed using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH) with modifications from the manufacturer's recommendations. Sample suspension (200 µl), proteinase K (10 µl), and lysis buffer (200 µl) were incubated for10 min. at 56^{O}C. The lysate was supplemented with 200 µl of 100% ethanol. Then washing and centrifugation according to the manufacturer's recommendations.

Oligonucleotide primers used were from Metabion, Germany; are listed in Table 1.

- Amplification of PCR:

50 µl of total PCR reaction volume were utilized containing 25 µl of Master Mix Emerald Amp Max PCR (Takara, Japan), 1 µl of each forward and reverse primer (20 pmol), 14 µl of free water, and 5 µl DNA template. Reaction in an applied bio system 2720 thermal cycler was performed.

PCR products were separated on 1.5% agarose gel (Applichem, Germany, GmbH) in gel electrophoresis, (40 µl) of PCR product were loaded in each gel slot. To determine the fragment sizes, Gene ruler 100 bp ladder (Fermentas, Germany), gel pilot 100 bp, and DNA ladder (Qiagen, Gmbh, Germany) were used. The gel was photographed by a gel documentation system (Alpha Innotech, Biometra). Data were analyzed through computer software.

6. Isolation and Identification of *E. coli*

From positive MPN tubes confirmed in brilliant green lactose broth by gassing at (35 ^{O}C for 48h), loopful of broth were streaked onto plates of Eosin Methylene Blue agar (EMB, Oxoid, England) (ICMSF, 1996). Typical colonies of *E. coli* (green with metallic sheen) were biochemically identified using IMVIC analyses (Ewing and Edwards, 1986). Isolated *E. coli* were serologically identified using rapid diagnostic antisera sets for *E. coli* (DENKA SEIKEN Co., Japan), according to Kok *et al.* (1996)

Table 1: Primers sequences with cycling conditions.

PCR type	Target gene	Primers sequences	Amplified segment (bp)	Primary denatura-tion	Amplification (35 cycles)			Final exten-tion	Reference
					Secondary denatura-tion	Annealing	Exten-tion		
		Forward(F)							
		Reverse(R)							
Multi-plex 1	***cna***	GTCAAGCAGTTA TTAACACCAGAC	423	94˚C 5 min.	94˚C 30 sec.	55˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	Tristan *et al.,* 2003
		AATCAGTAATTG CACTTTGTCCAC TG							
	ebps	AGAATGCTTTTG CAATGGAT	652						Vancraeynest *et al.,* 2004
		AATATCGCTAAT GCACCGAT							
	eno	ACGTGCAGCAG CTGACT	302						Tristan *et al.,* 2003
		CAACAGCATYCT TCAGTACCTTC							
Multi-plex 2	***Sea***	GGTTATCAATGT GCGGGTGG	102	94˚C 5 min.	94˚C 30 sec.	57˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	Mehrotra *et al.,* 2000
		CGGCACTTTTTT CTCTTCGG							
	Seb	GTATGGTGGTGT AACTGAGC	164						
		CCAAATAGTGA CGAGTTAGG							
	Sec	AGATGAAGTAGTT GATGTGTATGG	451						
		CACACTTTTAGA ATCAACCG							

RESULTS

Table 2: Mean values± SEM of *TBC*, *TCC* and *S. C* in the examined meat products and instrument samples (n=20 for each) with samples exceed the permissible limits (PLs).

Samples (20 for each)	*TBC*			*TCC*			*S. C*		
Total samples (140)	Positive samples No. (%)	Mean Count (CFU/ g.cm^2)	Samples exceed PL No. (%)	Positive samples No. (%)	Mean Count (CFU/g.cm^2)	Samples exceed PL No. (%)	Positive samples No. (%)	Mean Count (CFU/g.cm^2)	Samples exceed PL No. (%)
Meat cuts	20 (100)	$4.1\times10^4 \pm 1.1\times10^{2\,a}$	10 (50)*	13 (65)	$7.3\times10^2 \pm 1.1\times10^{a}$	6 (30)**	5 (25)	$3.3\times10^2 \pm 1.8\times10^{a}$	5 (25)*
Liver	20 (100)	$2.7\times10^4 \pm 0.6\times10^{2\,b}$	8 (40)*	18 (90)	$1.6\times10^2 \pm 0.2\times10^{b}$	9 (45)**	3 (15)	$2.3\times10^2 \pm 1.4\times10^{b}$	3 (15)*
Minced meat	20 (100)	$4.6\times10^4 \pm 1.2\times10^{2\,a}$	13 (65)*	16 (80)	$5.3\times10^2 \pm 1.1\times10^{a}$	9 (45)**	11 (55)	$4.3\times10^2 \pm 0.9\times10^{a}$	11 (55)*
Equipment	19 (95)	$2.7\times10^4 \pm 0.6\times10^{2\,b}$	4(20)***	13 (65)	$1.6\times10^2 \pm 0.2\times10^{b}$	2 (10)***	12 (60)	$3.3\times10^2 \pm 1.4\times10^{b}$	12 (60)*
Tables	20 (100)	$4.6\times10^4 \pm 1.8\times10^{2\,a}$	9(45)***	18 (90)	$6.3\times10^2 \pm 1.5\times10^{a}$	7 (35)***	20 (100)	$6.3\times10^2 \pm 1.4\times10^{a}$	20 (100)*
Workers' hands	19 (95)	$2.9\times10^4 \pm 0.9\times10^{2\,b}$	7(35)***	13 (65)	$1.7\times10^2 \pm 1.2\times10^{b}$	2 (10)***	17 (85)	$2.3\times10^2 \pm 1.1\times10^{b}$	17 (85)*
Refrigerators	20 (100)	$4.8\times10^4 \pm 1.9\times10^{2\,a}$	10(50)***	9 (45)	3.3×10^2 a $\pm 1.1\times10$	6 (30)***	16 (80)	$2.7\times10^2 \pm 1.4\times10^{b}$	16 (80)*
Total.	138(98.57)		61(43.57)	100 (71.42)		41(29.28)	84(60)		84(60)

Means carrying different superscript letters [a (highest value), b (lowest value). Values in the same column differed significantly at P<0.05.

* EOS No. 3602 (2013): TBC/cm^2 or/g limit ≤ 10^6cfu of meat surface, SC limit ≤ 10^2, E.coli/g limit = Free. ** E.O.S.Q.C No. 2079 (2005): (TCC) /cm^2 or/g limit≤ 10^2cfu. *** (GSO 2017): Indicated standard for surfaces swabs TBC ≤ 10^3cfu/cm^2, TCC were ≤ 10^1cfu/cm^2

Table 3*:* Result of multiplex PCR for enterotoxins genes represented by *Sea* (102 bp), *Seb* (164 bp), and *Sec* (451bp) and Biofilm-forming genes represented by *ebps* (652bp), *cna* (423bp), and *eno* (302bp), of *S. aureus*.

Samples types	Isolate No.	*Sea*	*Seb*	*Sec*	*ebps*	*cna*	*eno*
Meat cuts	1	-	-	-	+	-	+
	2	-	+	-	+	-	+
liver	3	-	-	-	+	-	+
	4	-	-	-	+	-	+
Minced meat	5	-	-	-	+	-	+
	6	-	-	-	+	-	+
Equipment	7	-	-	-	+	-	+
Tables	8	-	+	-	+	-	+
Workers' hands	9	-	-	-	+	-	+
Refrigerators	10	-	-	+	+	-	+
Total No. (%)	10	0	2(20)	1(10)	10(100)	0	10(100)

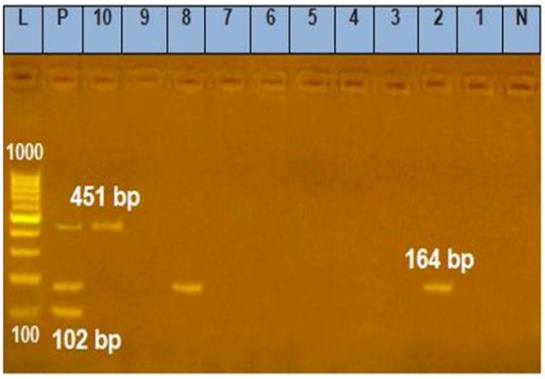

Figure 1: Agarose gel electrophoresis of multiplex PCR for enterotoxins genes *Sea* (102 bp), *Seb* (164 bp), and *Sec* (451 bp) of *S. aureus*. Lane L: 100 bp ladder as a molecular size DNA marker. Lane P: Control positive genes. Lane N: Control negative. Lane 1 to 10: Negative for *Sea* gene. Lane 2 and 8: Positive for *Seb* genes. Lane 1, 3, 4, 5, 6, 7, 9, and 10: Negative for *Seb* gene. Lane 10: Positive for *Sec* gene. Lane 1 to 9: Negative for *Sec* gene.

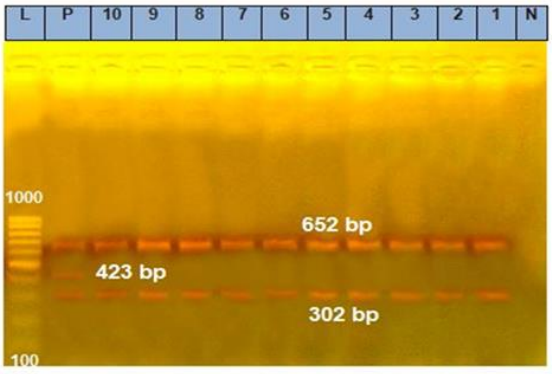

Figure 2: Agarose gel electrophoresis of multiplex PCR for biofilm-forming genes *ebps* (652bp), *cna* (423 bp), *eno* (302 bp) of *S. aureus*. Lane L: 100 bp ladder as a molecular size DNA marker. Lane P: Control positive genes. Lane N: Control negative. Lane 1 to 10: Positive for *ebps* and *eno* genes and negative for *cna* gene.

Table 4: Serological identification of isolated *E. coli* (100 isolates).

Strain characterization	Serotypes	Serotypes No. (%)	Sources
EPEC	O146 : H21	6 (6)	1 Meat cuts, 1 Liver, 1 Minced meat, 1 Equipment, 1 Workers' hands, 1 Tables
	O44 : H18	4 (4)	1 Liver, 1 Minced meat, 1 Equipment, 1 Tables
	O20 : H7	5 (5)	1 Meat cuts, 1 Liver, 1 Minced meat, 1 Equipment, 1 Refrigerators
	O163 : H2	6 (6)	1 Meat cuts, 1 Liver, 1 Minced meat, 1 Equipment, 1 Tables, 1 Refrigerators
EHEC	O111 : H2	7 (7)	1 Meat cuts, 1 Liver, 1 Minced meat, 1 Equipment, 2 Tables, 1 Refrigerators
	O26 : H11	5 (5)	1 Meat cuts, 1 Liver, 1 Minced meat, 1 Equipment, 1Tables
	O91 : H21	6 (6)	1 Meat cuts, 1 Liver, 1 Minced meat, 1 Equipment, 1 Tables, 1 Refrigerators
	O117 : H18	3 (3)	1 Meat cuts, 1 Liver, 1 Minced meat
ETEC	O125 : H21	5 (5)	1 Meat cuts, 1 Liver, 1 Minced meat, 1 Refrigerators, 1 Workers' hands
	O128 : H2	4 (4)	1 Minced meat, 1 Workers' hands, 1 Refrigerators, 1 Tables
EIEC	O159	3 (3)	1 Minced meat, 1 Workers' hands, 1 Refrigerators
Total	11	54(54)	8 (40%) Meat cuts, 9 (45%) Liver, 11 (55%) Minced meat, 7 (35%) Equipment, 8 (40) Tables, 4 (20%) Workers' hands, 7 (35%) Refrigerators

DISCUSSION

Different examined samples (n=20 for each) presented in Table 2 show that the mean TBC in CFU/g. or cm^2 was recorded higher ($p< 0.05$) in minced meat ($4.6\times10^4 \pm 1.2\times10^2$) than in meat cuts ($4.1\times10^4 \pm 1.1\times10^2$) than liver samples ($2.7\times10^4 \pm 0.6\times10^2$) and also samples exceeded PL (No., %) were in the same manner 13(65), 10(50) and 8(40), respectively. Higher results ($3.3x10^5 \pm 1.6x10^5$) were reported in minced meat by Eltanani and Arab (2021) with 28% of the unaccepted sample that was found to be exceeding the PL. Lower results ($8.20x10^2$) were reported by El- Shamy (2015). According to EOS No. 1651(2005), contamination levels over 10^5 CFU/cm^2 indicate bad hygienic conditions. EOS No. 3602 (2013) stated TBC/g limit $\leq 10^6$cfu/cm^2. However, meat contamination with 10^6 CFU/cm^2 indicates a deterioration process as set by regulatory bodies in advanced countries for meat that must not be sold to consumers (Nieto *et al.*, 2010). Moreover, the Contamination over 10^5 CFU/cm^2 reflects unsatisfactory hygienic conditions by Barros et al. (2007). In addition, contamination exceeding 10^4 CFU/cm^2 is sufficient to begin biofilm formation, which is difficult to clean (Hood and Zottola, 1995).

The mean TCC in CFU/g. was higher ($p< 0.05$) in meat cuts and minced meat ($7.3\times10^2 \pm 1.1\times10$ and $5.3\times10^2 \pm 1.1\times10$) than liver ($1.6\times10^2 \pm 0.2\times10$), lower results were detected by Barros *et al.* (2007) in meat cuts ($1.68\pm 1.28\times10$). Unaccepted samples that exceed PL in (No. %) were 6(30) in meat cuts, while (No. %) was 9(45) in both minced meat and liver. Fecal matter is the major source of contamination through contaminated carcasses, workers, equipment, and air (FSA, 2020).

The mean S. C in CFU/g. was also significantly higher in minced meat ($4.3\times10^2 \pm 0.9\times10$) than meat cuts than liver with the same descending manner in exceeding PL (No., %), as follows, 11(55%), 5(25), and 3(15) respectively. Nearly similar result was recorded in minced meat by Eltanani and Arab (2021) ($0.2x10^2\pm$ 0. $13x10^2$cfu/g) and by Ashraf *et al.* (2016) (1.1×10^3cfu/g). Contamination with *S. aureus* may occur at the slaughter or from human contact due to their poor hygiene practices through sneezing, coughing, and wounds in the hands of food handlers (Abdalhamid *et al.*, 2013). All positive samples for *S. aureus* isolation in the current study were exceed permissible limit (10^2) according to E.O.S.Q.C No. 2079 (2005).

Processing, such as grinding, distributes bacteria on the surface throughout the ground meat and raises the temperature of meat allowing bacteria to grow rapidly. (GAO, 2002)**,** that explains our higher results of minced meat count.

Concerning instrument samples and workers' hands, the samples that exceeded TBC PL No. (%) were estimated in a descending manner in refrigerators, tables, Workers' hands, and equipment with a mean count of $4.8\times10^4 \pm 1.9\times10^2$, $4.6\times10^4 \pm 1.8\times10^2$, $2.9\times10^4 \pm 0.9\times10^2$ and $2.7\times10^4 \pm 0.6\times10^2$ respectively. This agreed with that recorded by Eisel *et al.* (1997). The recorded high levels of contamination may be due to inefficient cleaning which leads to the accumulation of organic matter that encourages microbial growth and reduces sanitization procedures efficiency. Samples exceeded TCC PL No. (%) were 7(35) and 6(30) in tables and refrigerators and were equal to 2(10) in both Workers' hands and equipment. Most of the sampled tables and the traditional refrigerator boxes in local meet shops were made of wood, whose porous nature allows for the accumulation of organic matter. Nevertheless, the dirty floor of meat shops can offer an appreciative environment for microbial growth, and enforce propagation and preservation of microorganisms, also the meat is hung in the shops without any segregation or protection by glass against the outlet of the shop. Barros *et al.* (2007), identified equipment as the main contamination point in butcher shops and found that samples collected from facilities showed a logarithmic mean of 5.38 log $CFU.cm^{-2}$ for TBC and the average contamination level of TCC was 3.28 log $CFU.cm^{-2}$. Additionally for his examined products, the ground (minced) beef was the most severely contaminated product in his study, then meat cuts, just like our results. In order that exploring bacterial count can predict the presence of spoilage and pathogenic bacteria (Jay, 2005).

As shown in Table 2, *S. aureus* was isolated from 84(60%) out of a total of 140 examined samples. The lower value was detected by Telli *et al.* (2022) who isolate *S. aureus* from only17% of the total examined samples of his study. *S. aureus* was found isolated in descending order by the rate of 100%, 85%, 80%, 65%, 60%, 50%, and 40% from tables, Workers' hands, Refrigerators, Minced meat, Equipment, Meat cuts, and liver samples, respectively. This is means instruments and workers' hands are higher in isolation rate of *S. aureus* than meat products. This nearly parallel to results detected by Bughti *et al.* (2017) who detected *S. aureus* in 30% of cattle beef samples and in 66.6% of the equipment used in meat shops. *S.* C that exceeded PL, No. (%) was highly recorded in Tables, Workers' hands, refrigerators, and equipment in a descending manner of 20(100), 17(85), 16(80), and 12(60), respectively. This reflects bad hygienic practices in the place that affect the hygiene of meat.

Table 3 shows that three classical *S. aureus* enterotoxins genes (SEs *genes*) represented by *sea, seb,* and *sec* (Fig 1) and biofilm-forming genes represented by *ebps*, *cna*, and *eno* were targeted to detected by PCR (Fig 2), and illustrated that, from 10 out of 84 *S. aureus* isolates (2 meat cuts (1, 2), 2 liver (3,4), 2 minced meat (5,6), 1equipment (7), 1tables (8), 1 workers' hands (9) and 1from refrigerators (10). *Sea* gene was not found in any of the isolates tested. Two isolates (20%) harbored the *seb* genes recovered from meat cuts and tables, and only one isolate harbored the *sec* gene which recovered from refrigerators. *Seb* and *sec* genes are classical antigens identified to be including staphylococcal enterotoxins B and C which are the most commonly related to food poisoning cases worldwide (Sezer *et al.,* 2015).

Biofilms are important virulence factors in the development of pathogens (Tahaei *et al.,* 2021), *ebps*, *cna*, and *eno* genes involved in the biofilm formation of *S. aureus* that play important role in adhesion (Vergara *et al.,* 2017). Several outbreaks were caused by biofilm-forming *S. aureus* in the EU (EFSA, 2015). In the United States, 241,994 cases of this food poisoning type were reported, with 1,067 hospitalizations and 6 deaths (CDC, 2016). Toxins can be secreted by biofilm and can contaminate food, causing multiple intoxications in the case of an outbreak (Galié *et al.,* 2018). A study on retail foods in China (Wang and Ruan, 2017) detected more than 90% of the isolates carrying *S. aureus* with enterotoxins genes with the ability to form biofilm.

In our study *cna* gene was not found in any of the isolates tested, while *ebps* and *eno* genes were detected in 100% of *S .aureus* isolates. Isolates 2, 8, and 10 that harbored enterotoxin genes harbored also biofilm-forming genes, which amplify the risk of foodborne outbreaks.

Kowalska *et al.* (2020) found that bacterial pathogens isolated from retail food samples including *S. aureus* in his study formed biofilms. Biofilm-forming serovars are the most persistent in food processing environments (Meyer, 2015). *S. aureus*, was detected with *E. coli* in meat samples of cattle beef and meat-cutting equipment samples in butcher's shops by Bughti *et al.* (2017).

One hundred isolates of *E.coli* from positive MPN tubes after phenotypic and biochemical examination were subjected to serological identification, and resulted in 54 out of 100 isolates being serotyped pathogenic as illustrated in table 4 which indicated that the rate of isolation of pathogenic *E. coli* from each source (No. =20) were 40% Meat cuts, 45% Liver, 55% Minced meat, 35% Equipment, 40% Tables, 20% Workers' hands, and 35% Refrigerators. This means the higher percentage was in minced meat followed by the liver then meat cuts. Tables were the most contaminated instrument followed by workers' hands. EHEC including (O111, O91, O26, and O117) is considered as Shiga toxin-producing *E. coli* (STEC) these were detected in raw beef samples and instruments samples obtained from 25 retail markets in Kafr El-sheikh governorate by El-Gamal *et al.* (2016**)** that agreed with our results. Conversely, with us, the prevalence of (STEC) strains in instrumental samples was more than in raw ground beef samples in his results.

Different *E. coli* serotypes have been reported to form biofilms through their Flagella, pili, and membrane proteins that are used to initiate attachment on inanimate surfaces **(**Vogeleer *et al.*, 2016**).** *E. coli* strains, such as O111 and O26 displayed a biofilm formation ability (Chagnot *et al.*, 2014). Additional 13 cases were belonged to serotype O157 which developing hemolytic uremic syndrome (HUS) foodborne outbreak was reported in Romania and Italy in 2016, with an overall of 19 cases and 3 deaths, the majority of the cases were due to serotype O26 (EFSA, 2016).

Bacteria *E. coli* is usually present in high numbers than other pathogens (FSA, 2020). So, to achieve the lowest microbial load, Food safety management systems including hazard analysis and critical Control points systems (HACCP) should be applied strictly. In developing countries like Egypt, the meat shops' environment, is not only contaminated but also enhances the growth of bacteria (WHO, 2011; EFSA, 2015).

CONCLUSIONS AND RECOMENDATIONS

The current study evaluated the bacterial contamination in raw meat, liver, butchers' instruments, and workers' hands in Kafr El-Sheikh city, Egypt**,** and it was found that meat sold in the shops contain high numbers of viable bacteria that could be a potential threat to consumers' health since pathogens such as toxigenic -forming *S. aureus* and pathogenic *E. coli* could be detected in the raw meat cuts, minced meat, liver, and processing environment.

This work is expected to aid butchers in meat shops to improve their hygienic procedures to reduce microbial contamination. For consumers' health. The findings of this research will provide the necessary information for relevant authorities to manage public health threats associated with the consumption of meat and offal from meat shops that consider a part of the meat production chain. Compliance with hazard analysis and controlling CCPs for ensuring the safety of meat for consumers was recommended in this study.

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تعيين وتقييم الخطر الميكروبيولوجي من النقاط الحرجه فى محلات بيع اللحوم وخاصة بعض البكتيريا المنتجه للسموم و المكونه للبيوفيلم

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نظرا لحدوث مشاكل صحيه مثل حالات النزلات المعويه والتسمم الغذائى وما يترتب على ذلك من خسائر اقتصاديه وذلك لتاثير بيئة العمل والعاملين انفسهم على اللحوم لذا تم تحديد اماكن الخطر الميكروبى عامة وبالأخص بعض البكتيريا المنتجه للسموم والمكونه للبيوفيلم فى محلات بيع اللحوم بمدينة كفر الشيخ - مصر على مستوى المحل ككل وذلك بالفحص الميكروبي الشامل للحوم وبيئة العمل اولا، ثم أخذ عينات من قطعيات اللحم والكبد، واللحم المفروم، ومسحات من المعدات والمناضد،وأيدي العمال والثلاجات ثم عد الميكروبات المختلفه، وعزل ميكروب المكور العنقودى الذهبى المنتج للسموم المعويه وميكروب القولون النموذجى (الايشيرشيا القولونيه) و باستخدام انزيم البلمره المتسلسل تم الكشف عن تواجد بعض الجينات المسؤله عن حدوث مخاطر التسمم وهى (*seb, sec*)، وخاصة ان السموم المنتجه غير قابله للتكسير بالحراره، وكذلك تم الكشف عن بعض الجينات المسؤوله عن تكوين البيوفيلم في تلك العترات (*ebps, eno*) والتى تمثل خطر كبير في انتشار التلوث البكتيري بهذه السلالات الخطيره فى المنتج والمكان المحيط حيث وجد ان أعلي معدل تلوث بكتيري(العد الكلى للبكتيريا الهوائيه، وبكتيريا الايشرشيا القولونيه،وبكتيريا استافيلوكوكس) كان في اللحم المفروم وأعلى نسبة عزل لميكروب المكور العنقودى الذهبى كانت في الأدوات وأيدى العمال، كما تم عزل وتصنيف ميكروب القولون النموذجى (الايشيريشيا القولونيه) سيرولوجيا الى EPEC, EHEC, ETEC, EIEC وتم مناقشة سبل السيطره على الخطر والتوصيه بعمل الاجراءات اللازمه عند كل نقطه لضمان جودة وسلامة اللحم ورفع كفاءة العمل بالمكان بالاضافه الى ان نتائج الدراسه تدعم تطبيق نظام تحليل المخاطر ونقاط التحكم الحرجه في محلات بيع اللحوم وتوصى بتطبيقه لضمان سلامة المنتج.